Utilisation of waste organic matter by selected earthworm species in agroecosystems.

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Abstract

The addition of waste organic matter (WOM) treatments into agroecosystem soils may improve crop growth, alleviate negative soil properties resulting from intensive farming and provide an effective waste management solution. WOM addition within agroecosystems may also assist the establishment of sustainable earthworm populations, which can enhance WOM decomposition and nutrient cycling through their burrowing and feeding behaviour. Previous research has indicated the positive effects of WOM additions on earthworm population development, however there is uncertainty towards the effects of WOM properties (application type, particle size and decomposition rate) on the utilisation by earthworm populations within these systems. The aim of this thesis was to investigate how earthworm populations utilise WOM and determine how this impacts decomposition, soil organic matter formation and nutrient cycling in no-till agroecosystems. Initial field surveys determined that WOM additions increased earthworm density and species richness compared with standard practice fertiliser, where increased anecic densities were determined. Laboratory-based research indicated the effects of 7 common WOM applications on the growth and reproduction of 4 common species of earthworm (*Lumbricus terrestris, Aporrectodea longa, Aporrectodea caliginosa* and *Allolobophora chlorotica*), where horse manure, wheat straw residues and anaerobic digestate (dry fraction) were effective treatments. WOM incorporation experiments of adult *A. longa*, *A. caliginosa* and *L. terrestris* populations determined that *L. terrestris* had the highest rate of incorporation, where WOM was utilised at an increased soil depth. Presence of *L. terrestris* middens in the field increased the density of epigeic populations, indicating a facilitation of *L. terrestris* on epigeic populations in agroecosystems. Increased WOM incorporation by earthworm populations had no effect on the soil macro- and micronutrient concentration in the short-term, however evidence suggested a higher available nutrient concentration was contained within *L. terrestris* casts. Behavioural investigations explored the selection and utilisation of cereal residue by *L. terrestris*. Observations indicated a specific resource utilisation based upon particle size, where larger fragments of straw were used for midden construction and smaller fragments were utilised within the burrow. This highlighted that potential increased decomposition and incorporation of WOM by *L. terrestris* under homogenous milled particle size may compromise their midden-burrow complex and cause a population reduction. The impact of *L. terrestris* population density on the burial and incorporation of surface applied cereal straw over a period of harvest to spring sowing indicated a significant effect of high density on the rate of incorporation. This thesis demonstrates the advantages of a high *L. terrestris* population density in no till agroecosystems and how altering application methods of WOM can impact effects on soil functioning.

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I would like to dedicate my PhD to the loving memory of my cousin, Darren Macfarlane (1976-2015). A passionate advocate for environmental issues, his enthusiasm will continue to be a great inspiration to me.

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Glossary of Terms

Term	**Definition**
Aestivation	A state of animal dormancy, similar to hibernation, which takes place in the summer months. When an earthworm enters aestivation it becomes inactive and lowers its metabolic rate, often as a response to increased temperature or arid conditions.
Anecic	Deep burrowing earthworms, which develop near-vertical burrows connected to the soil surface. They predominantly feed on surface organic matter, but also ingest large quantities of soil which they redistribute in the soil profile.
Bioturbation	The reworking of soils and sediments by animals and plants. In earthworm ecology, bioturbation activities include burrowing, ingestion and casting.
Diapause	A period of suspended development when placed under adverse environmental conditions. Earthworms enter diapause by curling up in a knot within a mucus lined cavity to retain moisture until conditions improve.
Detritivore	An animal which feeds on dead organic material, such as leaf litter.
Drilosphere	Soil that has been influenced by earthworm secretion, burrowing and casting.
Endogeic	Earthworms which develop horizontally orientated burrows, often shallow in depth. These earthworms feed on a mixture of inorganic soil and deposited organic matter and produce casts both on the soil surface and within the subsurface layer.
Epigeic	Earthworms which inhabit and feed on the leaf litter, comminuting organic matter into smaller particle sizes.
Geophagus	Organisms which feed on soil.
Hibernation	A state of animal dormancy which takes place in the winter months. During this time earthworms become inactive and curl up in a knot within a small mucus lined cavity within their burrow until temperature conditions improve.
Midden	A collection of organic and inorganic materials, plus castings, around the surface of a burrow entrance.
Nutrient Cycling	The movement and exchange of organic and inorganic matter back into the production of matter, which passively and mechanically alters the soil environment.
Waste Organic Matter	Biodegradable material that consists of organic matter. Examples include anaerobic digestate, municipal solid waste and crop residues.

1. INTRODUCTION

1.1. General Introduction

Over the last century, agricultural intensification has increased to meet the provisioning ecosystem service demands of a growing global human population, currently exceeding 9 billion people. This has created challenges for soil ecosystem services, where arable management practices such as inorganic fertilisation, deep ploughing and mono-cropping have degraded soil health. An increase in agricultural intensification has caused a global population decline in soil biodiversity (FAO, 2019b), where the complexity of soil food webs and mean soil fauna biomass has been reduced (Tsiafouli et al, 2015). A degradation in soil health is considered one of the biggest threats to human population survival, where modelled estimations of global soil erosion have suggested that one third of conventionally managed top soil (< 30 cm depth) could have less than 200 years of harvests left if current practices remain (Evans et al, 2020). To ameliorate these threats, sustainable arable management practices have been adopted, with reduced tillage management and the utilisation of organic fertilisation being common methods used.

Although there are many positive impacts of reduced/no-tillage and organic fertilization on soil health, the transition from conventional farming methods to more sustainable practice poses a number of challenges (Soane et al, 2012), such as soil compaction, weed control and a reduction in yield. A key soil faunal group which may mitigate some of the negative impacts of no-till farming are earthworms, where their physical, biological and chemical interactions in soil increase supporting services such as soil formation and nutrient cycling (Blouin et al, 2013). The increased abundance and biodiversity of certain earthworm species under sustainable arable management practices is well documented, where reduced tillage can increase abundances of anecic populations and organic matter applications can sustain population growth (Bertrand et al, 2015a). However, accurate quantification of the effects of earthworm interactions with waste organic additions on soil processes is still to be fully determined, where the difference in the application type, method and particle size can influence earthworm utilisation of organic matter (Lowe and Butt, 2003; Leroy et al, 2007, 2008; Butt, 2011; Le Couteulx et al, 2015). Earthworm species inter- and intra-specific interactions in soil and their burrowing behaviour (Bouché, 1977) can also have significant effects on organic matter decomposition and movement within a soil profile.

A range of waste organic matter types can be used as amendments or soil conditioners to improve crop growth, including animal manures, anaerobic digestate, cereal and legume residues and composted green waste. Differences in the effects of these waste organic matter applications on the growth rate and fecundity of earthworm populations have been reported, where the effects of C:N ratio, particle size, lignin content and carbohydrate concentration can be limiting factors (Satchell, 1967; Löfs-Holmin, 1983; Boström and Löfs-Holmin, 1986; Moody et al, 1995; Curry and Schmidt, 2007; Blouin et al, 2013). Although the effects of positive interactions between earthworm populations and waste organic matter is acknowledged, the utilisation of organic matter differs between earthworm populations and few studies have intrinsically investigated the effects of waste organic matter types on the utilisation by earthworm species. Based on the positive effects of earthworm populations on nutrient mineralization (van Groenigen et al, 2019) and crop growth (van Groenigen et al, 2014), further information on the responses of earthworm populations to organic matter applications could be utilised as a bio-indicator for sustainable land management. This thesis investigates the effects of earthworm species interactions with waste organic matter applications on the decomposition and bioturbation within a soil profile. The information gained within this thesis could be utilised to inform land managers of the effects of waste organic matter types on earthworm populations.

1.2. Problem Statement

The addition of waste organic matter is an effective way of improving soil quality of no-till agroecosystems, subsequently benefitting crop growth and reducing negative effects of conventional farming management. It has been indicated that the increased bioturbation effects caused by earthworm interactions with waste organic matter in no-till agroecosystems can increase decomposition, however, there is currently little knowledge regarding the effects of different wastes on earthworm population dynamics and responses waste addition in no-till sites, nor the influence of application type on the rate of bioturbation and earthworm population development. Addressing these knowledge gaps will enable more informed future waste organic matter applications in no-till agroecosystems; providing more effective crop growth, a reduction in soil erosion and soil ecosystem service provision in no-till agroecosystems.

1.3. Research Aims

Based on the gaps in the literature defined in Chapter 2, the overall aims of this research project were:

- To investigate the utilisation of waste organic matter by earthworms in agroecosystems.
- To determine the use of cereal straw residues, with a focus on *Lumbricus terrestris*.

These aims are addressed by specific objectives, which are provided at the outset of each experimental Chapter.

1.4. Thesis Structure

This first Chapter positions this research project within the wider scope of sustainable agricultural management and soil ecology and provides the main aims of the thesis. Chapter two is a review of the relevant background and literature to this thesis, concluding with a summary of the research gaps identified during the review process. The methodological considerations adopted within the research project are presented in chapter three. Chapters four to eight fully describe the experimental work conducted to address the research aims presented in Chapter one. Finally, chapter nine provides a critical discussion of the assimilated findings from all experiments, in the context of the thesis objectives and the wider field in which this research exists. This final Chapter also presents the conclusions of this thesis, highlights the contributions to knowledge and provides recommendations for future research.

2. LITERATURE REVIEW

2.1. Introduction

This chapter provides an overview and discussion of the literature relevant to the scope of the thesis. The concept of ecosystem services is described within the context of soils and agroecosystems, followed by a discussion of the role of earthworms as ecosystem engineers, with an overview of their ecology. Agroecosystem management practices are introduced, where the environmental impacts of conventional farming are discussed, followed by a review of sustainable management practices, the current challenges faced and how earthworms are impacted upon in these systems. Utilisation of waste organic matter in agroecosystems is then discussed, with reference to the effect of increased applications on soil ecosystem services. The interactions between earthworm species and waste organic matter applications used in agriculture are then described, including the role that earthworms may play in increasing soil organic matter concentration and nutrient cycling within no-till agroecosystems. This chapter concludes with a summary of the main gaps identified in the literature, as relevant to the scope of this thesis.

2.2. ECOSYSTEM SERVICES AND EARTHWORMS AS ECOSYSTEM ENGINEERS

2.2.1. Soil and agricultural ecosystem services

Ecosystem services have been defined as the benefits to humankind and all other species by the provision of ecosystems (Millennium Ecosystem Assessment, 2005). To identify economic values of ecosystem services, they have been categorised into four categories: Provisioning services are those that provide material goods such as food, fuel, water and medicine; Regulating services relate to ecosystem processes such as climate regulation and flood regulation; Cultural services are the educational, recreational an aesthetic values provided to humans; and Supporting services are the key processes which support biodiversity and ecosystems, such as nutrient cycling and soil formation (Millennium Ecosystem Assessment, 2005; Figure 2.1.). Although soil ecosystem functions are well defined (Figure 2.2.), the value and quantification of some supporting ecosystem services, such as biodiversity and soil formation are still to be determined and implemented into management targets (Schmidt et al, 2011; Blouin et al, 2013; Bertrand et al, 2015a). To reach management objectives set by the Sustainable Development Goals, (UN, 2015) there is a need to identify the relationships between ecological entities and ecosystem functions or services and develop techniques for their manipulation and utilisation in sustainable agricultural practices (Bertrand et al, 2015a).

The focus of agricultural ecosystem services is provisioning, where food, medicine and fuel supply are vital for human survival. Production increases in crops and livestock, to meet a growing global demand, has negatively impacted regulating and supporting ecosystem services, which has caused a decline in soil health. This is a threat to future food security, where soil degradation, declining water quality and a reduction in soil biodiversity can all reduce future yields (Godfray et al, 2010). It is currently estimated that 12 million hectares of agricultural land are annually lost to soil degradation (Rickson et. al, 2015). To ensure food production continues to meet demands of a growing population, further work is required by agroecosystem managers to protect regulating and supporting services to reduce the negative soil impacts caused by intensive farming. Numerous soil security measures have been introduced to develop a framework to support global environmental sustainability, where the inter-connected measures include: Capability, Condition, Capital, Connectivity and Codification (McBratney et al, 2014). Capability is the measurement of soil to determine its functions (CEC, 2006); Condition is a measurement of soil quality based on the current use; Capital is the economic value of the soil based on its natural stocks (Constanza et al, 1997); Connectivity is the relationship between society with soil, where improved connections between land managers and soil science awareness could improve soil protection; and Codification is producing effective policy to support soil security measures. The implementation of soil security measures is a major focus of the UN, where one of the

17 defined Sustainable Development Goal targets (UN, 2015) is reducing world hunger whilst protecting agricultural ecosystems.

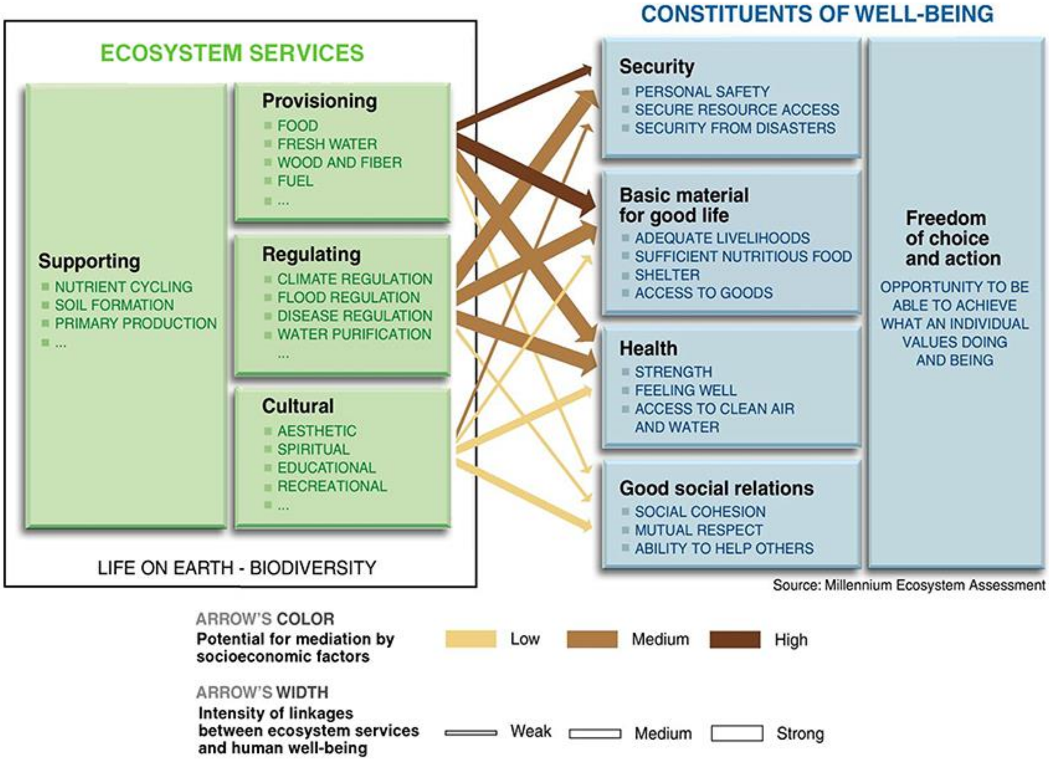


Figure 2.1. Ecosystem services, as defined by the Millennium Ecosystem Assessment (MEA, 2005).

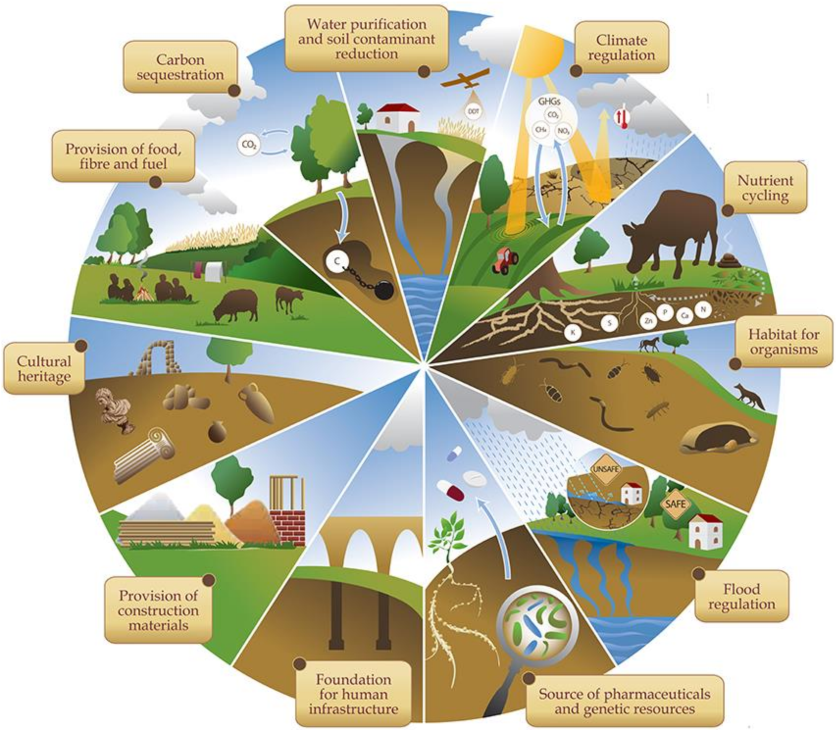


Figure 2.2. Schematic diagram of soil functions developed by the FAO (2015).

It is becoming increasingly recognised by ecologists that the biological diversity of soil is an important factor in the regulation of ecosystem processes, where soil fauna has a key role (Bardgett, 2005; Blouin et al, 2013; Creamer et al, 2016). The provision of soil functions and supporting ecosystem services which are vital for processes, such as soil formation and nutrient cycling, are often based on the functional outputs of biological soil processes (Millennium Ecosystem Assessment, 2005; Bardgett et al, 2011; Pulleman et al, 2012). Ecosystem services are delivered by different functions of the soil system (Figure 2.3), where specific functional groups of soil biota influence specific ecosystem processes (Wall et al, 2012). Agricultural services depend on all four aggregate ecosystem functions, so therefore depend on a network of soil biota to operate successfully. To target specific areas of the soil ecosystem, managers could focus on particular ecological entities which have high functional importance, known as “Ecosystem Engineers” (Jones et al, 1994; Lavelle et al, 1997; Wall et al, 2012; Blouin et al, 2013; Bertrand et al, 2015a). In the soil, the main ecosystem engineers are earthworms, termites and ants (Wall et al, 2012). Biotic interactions of keystone soil biota, such as earthworms, could provide future indicators of healthy soil and be implemented into adaptive management cycles for soil ecosystem services (Birgé et al, 2016). The monitoring of soil fauna as a biological indicator of ecosystem function has been assessed across Europe, where it was recommended that earthworms could be used as a bio indicator, alongside functional genes and bait lamina tests (Griffiths et al, 2016). However, currently there is no agreed framework between policymakers towards the biological assessment of soils in agroecosystems.

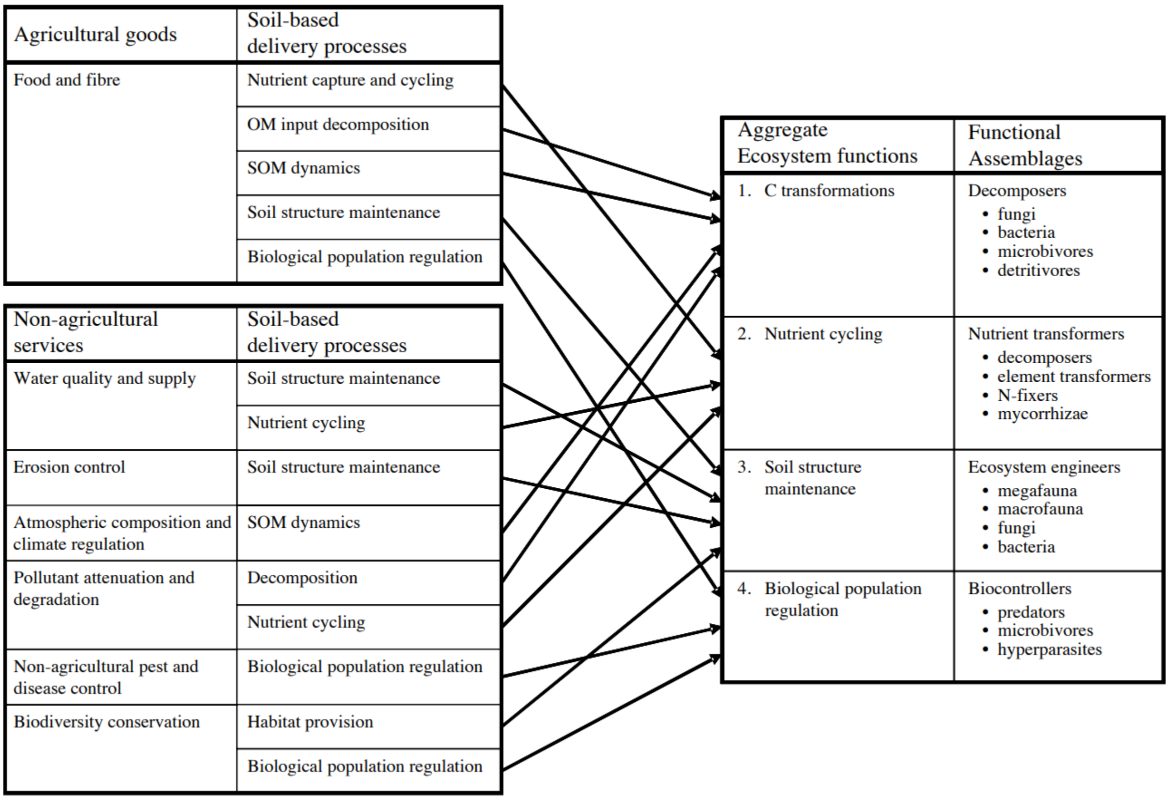


Figure 2.3. Relationships between activities of the soil biological community and a range of ecosystem goods and services that society might expect from agricultural soils. OM = organic matter; SOM = soil organic matter. Modified from Kibblewhite et al (2008).

2.2.2. Earthworms as ecosystem engineers

Earthworms are recognised as ‘Ecosystem Engineers’ by ecologists (Blouin et al, 2013), where they play a number of crucial roles in soil development (Edwards and Bohlen, 1996). An ecosystem engineer is classified as an organism which modifies its environment by changing the distribution of materials or energy by non-trophic interactions with abiotic and biotic components of its respective ecosystem (Jones et al, 1997). The physical, biological and chemical interactions of earthworm populations influence soil formation and nutrient cycling (Eisenhauer et al, 2010). Soil formation and nutrient cycling are classified as supporting services (Millennium Ecosystem Assessment, 2005) and are vital for the stability of ecosystem processes because they affect plant growth, soil drainage and aeration (Blouin et al, 2013). Through bioturbation (reworking of soils), earthworms influence soil physical properties such as nutrient cycling and soil fertility. Earthworms enhance bioturbation through burrowing activities, castings and mixing leaf litter and organic matter through the soil (Eisenhauer et al, 2010).

There are many positive effects of earthworm populations on agricultural ecosystem services, particularly in no-till agroecosystems (Bertrand et al, 2015a). Plant and vegetation health are largely affected by the physical, chemical and biological parameters in soil (Edwards, 2004; Edwards and Bohlen, 1996; Blouin et al, 2013). Key processes which support soil quality are nutrient cycling and soil formation, both of which are influenced by earthworms (Blouin et al, 2013). Utilisation of earthworm populations in cropping systems differs dependent upon the requirements of the ecosystem and the resources required (Figure 2.4), where earthworms can be used as a crop management resource (e.g. vermicomposting or earthworm inoculation) or arable management techniques can be altered to benefit earthworm populations and utilise their functioning properties (e.g. reduced tillage practices). Large scale meta-analysis of the effects of earthworm populations on soil ecosystem processes have widely indicated the positive influences of increased abundance and species diversity on plant production and soil fertility (van Groenigen et al, 2014; Bertrand et al, 2015a; van Groenigen et al, 2019). A review of the effects of earthworm populations in sustainable agroecosystems is discussed in section 2.5.

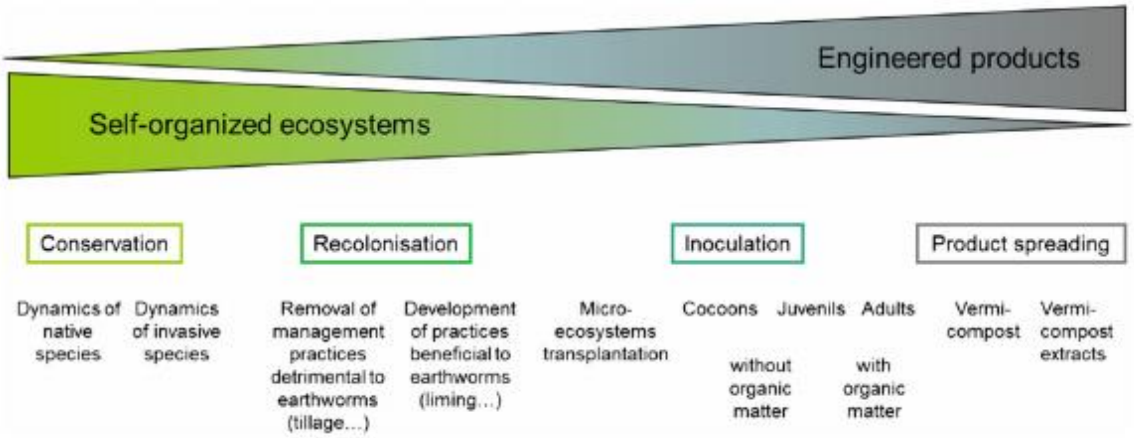


Figure 2.4. Diagram distinguishing the approaches used to increase earthworm beneficial effects on crops. Figure sourced from Bertrand et al, (2015a); adapted from Blouin et al, (2013).

2.2.3. *Earthworm ecology*

Earthworms have been a focus of scientific investigation for centuries, where the impact of earthworm populations on organic matter decomposition was first discovered by Charles Darwin (1881). Earthworms are soil organisms belonging to the phylum Annelida, (class Clitellata, subclass Oligochaeta) and are found in most soil ecosystems globally, except for climatic extremes such as deserts and polar regions (Edwards, 2004). Globally, earthworm abundance ranges from 1-50 individuals m^{-2} (20.59 individuals m^{-2} ± 24.84 SD; Phillips et al, 2019), where there is an increased species richness and abundance at mid latitudes (Figure 2.5.). Earthworm behaviour, species richness and fecundity are affected by a number of factors including resource availability, temperature, soil water saturation, organic matter content, pH, land use and rate of predation (Edwards and Bohlen, 1996; Butt et al, 2003; Edwards, 2004; Curry, 2004; Curry and Schmidt, 2007; Blouin et al, 2013). In temperate regions, earthworms are dominated by Lumbricid species (Lavelle et al, 1999; Edwards, 2004), where activity is seasonal, with most occurring in Spring and Autumn (Edwards, 2004). Earthworms can adapt their behaviour to survive extreme temperatures and weather conditions often experienced in summer and winter periods, such as aestivation in mucus-lined cells to survive drought conditions (Edwards, 2004).

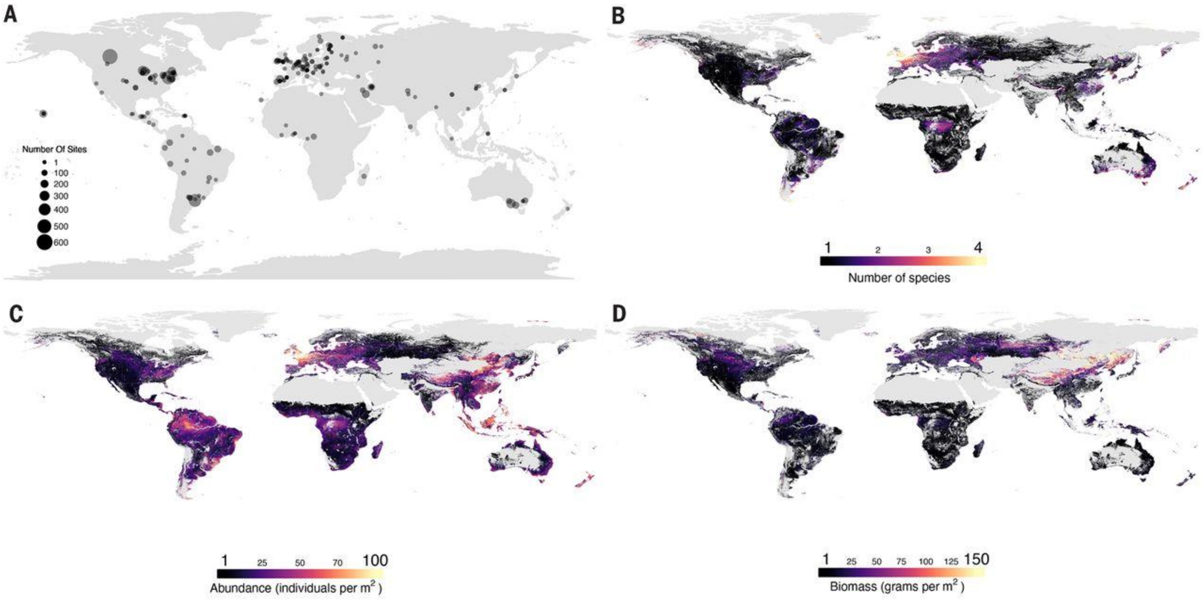


Figure 2.5. Global distribution of earthworm populations. A = Site locations and number; B = Number of species found; C = Earthworm abundance; D = Distribution of biomass. Figure sourced from Phillips et al (2019).

Earthworm species can be separated into three ecological groupings: anecic, epigeic and endogeic (Bouché, 1977). Each category expresses specific burrowing and feeding behaviour (Edwards, 2004); this difference in behaviour within the soil ecosystem could cause unique contributions to ecosystem services (Blouin et al, 2013). Epigeic earthworms (e.g. *Lumbricus castanaeus* and *Lumbricus rubellus*) dwell and feed in the leaf litter above the soil surface, enhancing the decomposition of fresh organic matter; Endogeic earthworms (e.g. *Aporrectodea caliginosa* and *Alollobophora chlorotica*) are geophagus and make temporary horizontal burrows in the soil which are often shallow below the soil surface; and anecic species (e.g. *Lumbricus terrestris* and *Aporrectodea longa*) create vertical burrows connected to the soil surface, which can be inhabited for long periods of time (Edwards and Bohlen, 1996). Anecic earthworms feed on organic matter, however they also ingest large amounts of inorganic soil and vertically redistribute this through the soil profile, creating a drilosphere micro-environment. With growing research into earthworm behaviour, it has been identified that some species express behaviours of two ecological groupings, e.g. *L. terrestris* has been identified as epi-anecic (Hoeffner et al, 2018; 2019). Following a re-visitation of Bouché’s ecological triangle, the behaviours of earthworm species have been quantified to determine how their behaviour fits within these ecological groupings (Botinelli et al, 2020a; Figure 2.6.). Although this research is in its early stages, further evidence of earthworm species behaviour, particularly in under-represented groups, will inform ecologists and land managers of the effects of earthworm behaviour on soil ecosystem processes and organic matter incorporation.

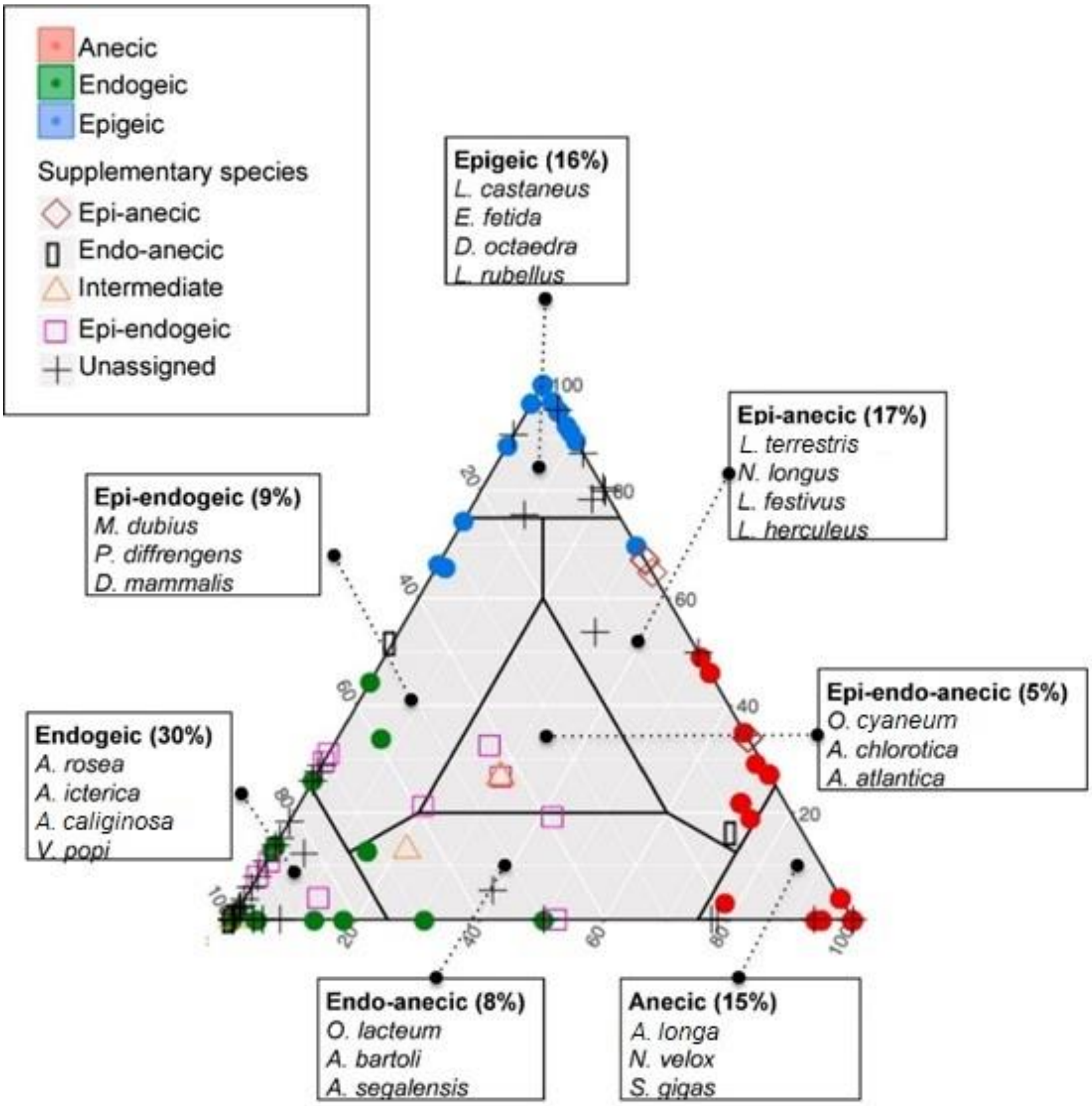


Figure 2.6. Adaptation of a ternary plot of earthworm ecological groupings defined by the three main categories (anecic, endogeic, epigeic) and sub-cateogries (Epi-endogeic, Epi-anecic, Epi-endo-anecic, Endo-anecic). Seven zones were defined to assign each species to one ecological category, some typical species of each category are listed. Developed by Botinelli et al, (2020a), based on the initial ecological groupings compiled by Bouché (1977).

The different burrowing activities and cast production of these groups influences a number of soil processes, such as an increase in water infiltration and soil water holding capacity (Bouché and al Addan, 1996; Hallam and Hodson, 2020), organic matter incorporation (Bohlen et al, 1996), and soil pore structure (Joquet et al, 2008); all of which could have a beneficial influence on vegetation health (van Groenigen et al, 2014) and soil fertility (Blouin et al, 2013; van Groenigen et al, 2019). Added to the effects of earthworm behavioural networks on organic matter cycling in soil ecosystems (Bouché, 1977), studies of intra- and inter-specific earthworm relationships have recognized that there are limiting factors on the positive effects of earthworms on soil health, such as the density of earthworms in soil (Eriksen-Hamel and Whalen, 2007a), food supply (Curry and Schmidt, 2007), particle size (Lowe and Butt, 2003), climate (Nordström and Rundgren, 1974; Singh et al, 2020) and vegetation (Lee, 1985). Controlled experiments into single and paired species of earthworms indicated some positive inter-specific interactions, however competition between anecics was also noted (Butt et al, 1998) and reflected in field conditions (Edwards and Lofty, 1982). More research is required to enhance knowledge on the behavioural relationships between earthworm species and the impact of earthworm populations on soil properties and plant health.

2.3. SUSTAINABLE AGROECOSYSTEMS

2.3.1. Introduction

Intensification of conventional arable systems through improved management and biotechnology has increased global production by 150% over the last century (MEA, 2005). However, food insecurity still exists in the world, where a growing global population (expected to reach 9.8 billion by 2050) has placed increasing demands on food production (UN, 2017). Agricultural land populates 11% (1.5 billion Ha) of the world's terrestrial ecosystems and is a key provisioning service to maintain human life (FAO, 2019a). Alongside fossil fuel generation, global warming has increased, due to increased CO_2 emissions caused by the heavy machinery used during ploughing, which is currently estimated at 25% of global greenhouse gas emissions (Smith et al, 2014; Bennetzen et al, 2016). To reduce the effect of agricultural production on the rate of greenhouse gas emissions, sustainable agricultural intensification will be a key driver in the future of food production, with the aim of enhancing yields and reducing the environmental impact simultaneously (Parry and Hawesford, 2010; UN, 2015). However, agroecosystems differ across spatio-temporal scales and a number of environmental (e.g. soil type, climate) and socio-economic factors (e.g. farmer income, governmental policy) influence management decisions. Integrated management planning, which considers the economic effects of regulating and supporting ecosystem services, may increase agricultural sustainability. However, the development of legislative framework is complex due to trade-offs between interlinking ecosystem factors (Smith et al, 2013). The development of supporting services within agroecosystems may be crucial in the future to mitigate soil erosion losses, where increased soil biota activity within soil may enhance organic matter decomposition and restore soil health (Blouin et al, 2013; Bertrand et al, 2015a). This section will indicate the damages caused by conventional farming and define sustainable management methods. The future challenges of sustainable agricultural management will then be discussed and how the effects of earthworm species on soil properties may impact agroecosystems.

2.3.2. Environmental impacts of conventional farming

There are four key management methods of intensive conventional farming which have a significant impact on the global decline in soil health: tillage, fertiliser usage, pesticide and herbicide applications and mono-cropping. This section will discuss these management systems and the negative effects they have on soil properties.

2.3.2.1. Tillage

Tillage (the mechanical over-turning of soil) is conducted in conventional farming to create a uniform aggregate size soil structure and to improve soil porosity, which enhances seed germination and root growth (Blevins and Frye, 1993; Kraska, 2012). There are different types of tillage management, varying by depth of plough. Mouldboard ploughing is the most common form of tillage, where the top layer (30 cm) of soil is overturned. A significant problem affecting global agricultural ecosystems is an increase in soil erosion, caused by regular ploughing. After ploughing, the soil is loose and often left fallow, therefore under exposure to wind and heavy rain, the soil erodes. Soil erosion leads to a reduction in available nutrients for crop uptake and alters the soil structure, which reduces crop yields (Biot and Liu, 1995; Broadman et al, 2009). It is widely accepted that an increase in ploughing events decreases macro and micro fauna populations in agroecosystems, in particular larger soil faunal species such as earthworms (Gerard and Hay, 1979; Chan, 2001; Crittenden et al, 2014; Briones and Schmidt, 2017). Tillage events reduce earthworm populations by physical destruction, habitat destruction and increasing exposure to predation. Earthworms are ecosystem engineers and have significant impacts on supporting services such as soil formation and nutrient cycling (Blouin et al, 2013), therefore their destruction will enhance the soil degradation effects caused by increased erosion.

A common negative effect of the heavy tillage machinery used in mouldboard ploughing is soil compaction. Soil compaction is where an element of stress causes an increase in density and a reduction in soil porosity. An increase in soil density can cause a reduction in the absorption of water, potentially resulting in an increased surface runoff, flooding risk and further erosion (Alaoui et al, 2018). A reduction in soil permeability can negatively impact nutrient availability to plants (Shah et al, 2017) and increase the risk of convective flow during heavy rainfall (Berisso et al, 2012), increasing the risk of fertiliser loss. The use of heavy machinery in conventional agriculture also has a significant cost to global warming, accounting for 25% of global greenhouse gas emissions (MEA, 2005). The impact of heavy machinery on CO_2 emissions differs dependent upon the bulk density of the soil, where a higher bulk density soil type such as clays will use more energy to plough (Koeller, 1989). Alongside protecting soil health, there is a need to reduce or alter ploughing management to mitigate the losses of CO_2 into the atmosphere to reduce the impact of climate change and global warming.

2.3.2.2. *Fertiliser Usage*

Inorganic fertilisers are added to increase the nutrient availability of macronutrients such as nitrogen, phosphorus and potassium to crops in areas where these nutrients are limiting. Common fertilisers used by farmers include ammonium nitrate, urea and potassium sulphate. Inorganic fertiliser applications on agroecosystems were initially developed to enhance yields and subsidize nutrient losses, creating a green revolution in the 1960 and 70s. Although current EU regulations ban a number of substances to be used in agricultural farming to protect human health and the soil ecosystem (Fertilisers Regulation, 2003), consistent poor application management has created a number of environmental problems, such as eutrophication of nearby aquatic environments and increased global N_2O emissions (Schlerbak et al, 2014). Over application of inorganic N is a significant problem in arable farming and cumulative applications of inorganic fertiliser can decrease soil pH (Fox and Hoffman, 1981; Moffatt, 1998) which can impact nutrient availability to crops (Bardgett, 2005). Leaching of nitrates into surface and groundwater systems is more prevalent in applications of over 100 N kg/Ha and following heavy rainfall events (Bergström and Brink, 1986). Applications of fertiliser post-ploughing often increases the rate of leaching, as increased soil aeration alongside increased rainfall experienced during sowing seasons can increase fertiliser transport through soil. Also, if soil is compacted from heavy machinery use, surface applied fertiliser will erode away. It has been suggested that N leaching increases at a higher rate under inorganic N additions than applications of manure and composted manure (Fan et al, 2017). Effects of climate change have increased the complexity of fertiliser application, where timely applications are required to prevent fertiliser runoff following increased rainfall events (Lavalle et al, 2009; Trnka et al, 2011; Gramig et al, 2017).

More recently, it has been indicated that increased applications of inorganic fertilisers have negatively impacted soil fauna species and plant pollinators, where there are toxic effects (Edwards and Bohlen, 1996; Godfray et al, 2014). A reduction in pollinators such as bees and butterflies could negatively affect key regulating ecosystem services and reduce crop fertility (Godfray et al, 2014). Applications of inorganic fertiliser can alter microbial community composition, by increasing bacterial biomass (de Vries et al, 2006; Chen et al, 2016). A reduction in soil fauna and alteration of the microbial community composition could reduce the rate of soil formation and nutrient cycling, which could further increase the rate of decline of supporting services in soil ecosystems. Applications of organic fertilisation may mitigate the biodiversity and species density loss of soil organisms (Lazcarno et al, 2013; Bertrand et al, 2015a), however whether this could replicate the nutrient availability of inorganic fertiliser applications is uncertain.

Farmers in the UK are required to make fertilisation management plans, which aims to reduce the impact to the environment and to ensure that nutrients depleted by crop cycles are restored to support future yields. This has reduced the rate of fertiliser over-application by targeting fertilisation to crop nutrient requirements. For example, the concentration of fertiliser added to agroecosystems can be assessed via crop root depth, where crops that have lower root depths, such as sugar beet, required increased fertilisation to improve nutrient availability, and crops with higher root depths (such as wheat) less fertilisation is needed (Williams et al, 2013). Future challenges for farmers and policymakers will be the balance of reducing the negative environmental impact of fertiliser use whilst ensuring crop nutrient requirements are met whilst adapting to climate change conditions (Tubiello et al, 2007; Macholdt and Honermeier, 2017). Current global projections of N fertilisation requirements suggests that demand will increase by 1.5% over the period of 2015-2020 (FAO, 2017).

2.3.2.3. Pesticide Usage

Pesticides are applied in agroecosystem management to maximise crop yields. Pesticides used in agriculture are categorized into different groups: insecticides, herbicides and fungicides. Insecticides kill target organisms that feed on crops, reducing crop destruction. Herbicides kill target weeds which compete for the same nutrients as crops, maximising the available nutrients in the soil for crop growth. Fungicides prevent the growth of fungi and development of fungi spores, which reduces crop damage such as mildew growth on barley crops. Pesticides can be chemically inorganic (e.g. copper sulfate) or organic (e.g. neonicotinoids). Initially, synthetic pesticide applications were developed to target specific pests without negatively impacting other soil organisms and plants (Düring and Hummel, 1997). However, recent research has indicated that over increased rates of application, toxic effects on non-target organisms have occurred (Duah-Yetumi and Johnson, 1986; Thiour-Mauprivez et al, 2019). Repeated applications of herbicides, such as glyphosate, have decreased the rate of reproduction and growth of soil fauna such as earthworms (Gaupp-Berghausen et al, 2015). Soil micro and macrofauna are vital for ecosystem regulation (Millennium Ecosystem Assessment, 2005; Zhang et al, 2017; Kardol et al, 2016); the limiting of these organisms through pesticide applications could have negative impacts on soil fertility. This indicates that although pesticide application may not be harmful to human health, the pesticide applied can reduce populations of other organisms, which are beneficial to the soil ecosystem.

Pesticides are applied to crops via different methods, they can be sprayed on the aboveground plant (contact pesticides) or onto the seeds before sowing (systemic pesticides). There are different impacts on invertebrates dependent upon application method. Applications of pesticides via plant contact may have a lower impact on soil invertebrates. Investigations of the insecticide fipronil indicated no effect of contact application on arthropod community structure, where community development was influenced by field climatic and environmental conditions (Maute et al, 2017). However, neonicotinoids, systemic insecticides that are sprayed on seeds, have a negative effect on invertebrates. Neonicotinoids were developed to kill invertebrates without causing bioaccumulation to higher organisms. There are 5 types historically applied, clothiamidin, imidacloprid, thiameoxam, acetamiprid and thiadoprid. Due to high risk to bee populations, Clothiamidin, imidacloprid, and thiameoxam have now been banned by the EU (EU485/2013). Research tends to focus on the negative impact of neonicotinoid applications on a reduction in pollinator population density, which negatively impacts plant fertility (Godfray et al, 2014; Godfray et al, 2015). However, the damage of neonicotinoids on belowground soil fauna has recently been discovered (Atwood et al, 2018). Applications of insecticides such as imidacloprid (neonicotinioid) reduced earthworm burrowing activity, where *A. caliginosa's* activity reduced after 0.2 mg mg kg^{-1} applications, a low dosage (Dittbrenner et al, 2011). A reduction in belowground invertebrate populations such as earthworms could have negative impacts on crop growth (Bertrand et al, 2015a). Further investigations on the effects of synthetic pesticide applications on belowground soil fauna and the impact on crop growth is required.

2.3.2.4. Mono-cropping

Large scale conventional cropping systems intensively farm the same monocultures of crops, where crops have the same nutrient requirements between seasons, making agricultural management decisions, such as fertilisation, simpler. Plant homogenisation within conventional agroecosystems can negatively impact soil properties by depleting soil nutrients and reducing the density of soil macro- and microorganisms (Li et al, 2007; Wang et al, 2014; Wang et al, 2015). The repetition of one cultivar of a crop species can increase disease spread, where it has been determined that an increased concentration of crop-specific soil pathogens are populated within monocultures, compared with crop rotations (Bullock, 1992), due to increased risk of carryover (Bockus and Shroyer, 1998). This could be caused by a homogenization of bacterial communities experienced in monoculture environments (Figuerola et al, 2015). Wheat monocropping can increase plant exposure to pests such as parasitic nematodes (Zatón et al, 2017). Monocropping is a significant threat to future food security, where the increase of disease could reduce yields.

Alongside increased risk of disease spread, monocultures have negative impacts on soil physical properties, where increased levels of soil compaction from repetitive wheel loading can reduce soil hydraulic activity (Götze et al, 2016). A change in soil properties caused by monocropping has caused gradual yield reductions. Under the comparison of agroecosystems, wheat monocultures yielded significantly less C and N than wheat grown on previously pastured land (Magarida et al, 2010). In no-till farming, maize monoculture with direct drilling yielded 17-27% less than no-till with crop rotations applied (Księżak et al, 2018). Increased nutrient losses from repetitive crop monocropping could be mitigated by organic matter applications within these systems (Cai et al, 2017).

2.3.3. Sustainable management methods in agroecosystems

The negative impacts of intensive conventional farming systems (defined in Section 2.3.2) has increased the development and implementation of sustainable management methods. To reduce the environmental impact of conventional farming (defined in Section 2.3.2.), key initiatives have been outlined by international governments to regulate agricultural production whilst protecting the environment and future human health (Global Sustainable Development Goals; UN, 2015). The integration of organic matter addition, cover cropping, reduced tillage and renewable energy generation has encouraged a synergistic approach to farming with lower environmental footprint. Integrated management systems which assess soil health and nutrient status are conducted by farmers to encourage specific crop selection and maintain yields without reducing soil fertility. It is widely accepted that integrated management of nutrients from organic matter application, cover cropping and crop rotations can increase C sequestration and maintain soil fertility (Fortuna et al, 2003). The future of agroecosystems depends on increased development of this approach and the implementation of soil fauna testing alongside nutrient condition (Piron et al, 2017). This section will define some of the sustainable agricultural techniques used in replacement or alongside conventional methods and indicate how they encourage crop growth whilst sustaining or improving soil properties.

2.3.3.1. Reduced Tillage Management

Across Europe, tillage reduction (conservation tillage) or elimination (no-till farming) has been adopted. There are a number of different types of conservation tillage farming methods: no-tillage, mulch tillage, strip tillage, ridge till and non-inversion tillage. The impacts of reducing tillage depth and rotation on soil properties has been demonstrated in a number of research studies. Compared with mouldboard ploughing, non-inversion tillage (NIT) increased soil aggregate stability and soil water retention in both conventional and organic farming systems (Morris et al, 2010; Crittenden et al, 2015) without compromising crop yield. A reduction in tillage can increase soil macrofauna biodiversity and abundance, which are vital organisms for ecosystem processes within cropping systems (Blouin et al, 2013; Bertrand et al, 2015a). Reduced tillage soils have a pronounced vertical orientation of macroporosity, which enhances the penetration of roots and water (Soane et al, 2012; Palm et al, 2014). The positive effect of reduced tillage on increased earthworm populations could have caused this increase in hydraulic activity (Andriuzzi et al, 2015a), where an increase in vertical burrow presence from a resurgence of anecic species could regulate water flow (Pelosi et al, 2017). This thesis will investigate anecic earthworm behaviour within no-till systems and the effects on soil properties.

It has been calculated that if no-till farming is adopted on 40% of the EU farmed area, the reduction of fuel emissions alone could reduce CO_2 emissions by 4.2 Mty^{-1} and increase the rate of carbon sequestration within soil (Tebrügge, 2003). The amount of carbon stored in soil is important for the balance of carbon in the environment (Lal, 2007), where there is a need to increase carbon sequestration in soils to reduce global warming. Yang et al, (2008) found that the concentration of soil organic carbon (SOC) in no-till soils exceeded that of ploughing, particularly at the upper soil profile depths (0-30 cm). The increase in SOC content can improve yields for autumn sown cereals, which are more profitable for farmers. Added to a lower fuel consumption, omitting autumn ploughing can reduce nitrate losses, lowering the need for fertiliser and increasing nutrient availability to crops (Hansen et al, 2010). These three economic advantages of no-till has increased the conversion to no-till farming by European farmers (Derpsch et al, 2009; Soane et al, 2012).

2.3.3.2. Waste organic matter addition

Waste organic matter (WOM) addition can provide many benefits to the biological, chemical and physical soil processes, enhancing soil quality encouraging crop growth. WOM is added within both conventional and no-till arable systems to increase the soil organic matter (SOM) content lost under intensive farming, which improves yields (Griffiths et al, 2010). In organic arable systems, addition of WOM is a key source of nutrients for crop growth, where the application type and rate of decomposition have significant effects on the nutrient mineralization and nitrogen availability for crops (Karami et al, 2012; Menšík et al, 2018). Findings from a research project by Hu et al, (2015) determined that by applying sequential amounts of crop residue, the derived mineral N in soil increased by 32.9%. A recent meta-analysis on the impacts of crop residue application on the N cycle indicated that soil, crop and microbial N concentration increased by 10.7 %, 20.8 % and 34.9 % respectively, where the impacts of increased N by crop residue application was suggested to be cumulative (Wang et al, 2018). In addition to nitrate, WOM addition can increase available macro and micronutrient concentration, increasing plant growth (Wood et al, 2018). Combining no-tillage with WOM addition can alter the distribution of soil nutrients, causing stratification. Stratification is the layering of nutrients within soil depths. This can increase nutrient availability at the soil surface, enhancing seed germination (Peigné et al, 2007). In an 84 year old study, it was indicated that in the upper layers of soil under crop residue the micronutrient concentration was highest under farmyard manure applications for extractable Mn, B and Zn compared with wheat plots with inorganic nitrogen applied. Over time, the overall nutrient concentration of the experimental fields decreased compared to the control, however the addition of WOM with fertiliser mitigated cultivation losses (Shiwakoti et al, 2019). The increase in nutrients from organic matter additions can regulate soil nutrient status.

In addition to the regulation of nutrient mineralization, applications of WOM within agroecosystems can provide to soil structural properties and soil organic carbon (SOC) storage. The transition from conventional management with no organic matter addition to no-tillage farming with larger scale WOM addition can have significant benefits, where surface applications of organic matter can regulate soil moisture content and reduce erosion (Shaver et al, 2002) and increase SOC content (Pulleman et al, 2000) and carbon storage, where macroaggregate concentration is increased (Annabi et al, 2015; Singh et al, 2015). An increase in the SOC content improves soil fertility, which can increase crop productivity (Tiessen et al, 1994). Increased carbon sequestration in the formation of SOC through decomposition of crop residues can also reduce greenhouse gas emissions and lowering the carbon footprint agroecosystems (Liu, 2011; Brankatsch and Finkbeiner, 2017). The addition of organic matter such as farmyard manure in conventional arable systems can increase the SOC pool by 4.3 Mg ha^{-1} (Brar et al, 2015). This indicates that the utilisation of farm waste in agroecosystem management could have a great impact on the mitigation of greenhouse gas emissions whilst improving crop yields.

WOM applications can increase the population size and biodiversity of macro- and microorganisms in soil, by increased supply of a food source and habitat provision for soil fauna (Curry and Schmidt, 2007), and arthropods (Gill, 2011), whilst increasing bacterial richness (Kavamura et al, 2018). Soil biota stimulate organic matter decomposition, which can enhance the rate of nutrient mineralization in soil, vital for crop growth (Bertrand et al, 2015a). The application of organic matter and organic matter type used is different across farms. There is a wide range of WOM types used within farming, where applications can be untreated, such as animal manures or crop residues, and treated, such as anaerobic digestate or composted green waste. Application method can differ between farms, where organic matter can be surface applied, injected or mixed. There is a significant effect of organic matter type and its application method on the selection and utilisation by earthworms (Pulleman et al, 2005). This thesis will assess the effects of organic matter type on the rate of selection and utilisation by selected earthworm species. Differences between organic matter types and their impact on soil properties will be discussed in Section 2.4.

2.3.3.3. Cover Cropping

Cover cropping is the application of crops to cover the soil in between growing seasons. There are a number of benefits to soil provided by cover cropping. Cover cropping can reduce the impacts of soil erosion between growing seasons by providing an organic layer, which compacts the soil surface and absorbing moisture from heavy rainfall events (De Baets et al, 2011; Basche et al, 2016). Cover crops can reduce fertiliser loss by minimising leaching and runoff effects and providing future nutrients within their biomass (Poudel et al, 2001; Couëdel et al, 2018). The implementation of cover cropping added with non-inversion tillage can increase the net nitrogen gain in soil of 1.7 g N m^{-2} yr^{-1}, which reduces fertilisation requirements (Walmsley et al, 2018). When used with organic matter amendments, wheat-clover rotations can significantly increase organic %N in the soil (Sanchez et al, 2001). Selected cover crops can act as natural pesticides and herbicides and are grown in-situ with crops during the growing season, supressing plant diseases in subsequent crops and improving yields (Duval et al, 2016; Monteiro et al, 2012). Brassicaceae cover crops have been found to act as biofumigants for a number of soil-borne pests such as plant parasitic nematodes and soil insects, reducing the need for synthetic pesticide use (Zasada and Ferris, 2004; Vig et al, 2009; Brown et al, 1994; Brown and Morra, 1997). The projected reduction in soil erosion and pesticide use provided by cover crops, alongside a regulation of soil nutrients, could significantly reduce farming costs, whilst improving the environment.

There are a wide number of cover crops used such as rye, oat, rape and mustard. Implementation of cover crops generally has a positive effect on soil biodiversity and species density, where cover crop applications increased fungal and bacterial populations, earthworms and arbuscular mycorrhizal fungi (AMF) populations in soil (Reddell and Spain, 1991; Zarea et al, 2009; Lehman et al, 2012; Brennan et al, 2017). AMF have mutualistic benefits with plants, providing roots with water, nutrients and protection of pathogens (Berruti et al, 2016). Introducing cover cropping into a conventional system may mitigate soil biodiversity losses, however considerations into the cover crop type and its effects on soil properties are required before implementation. The cover crop type, frequency of application and effects on soil moisture can have significant impacts on soil biological activity (Roarty et al, 2017; Stroud et al, 2017; Martínez-García et al, 2018; Euteneuer et al, 2020). Impacts on earthworm populations could cause indirect effects on the rate of organic matter decomposition and nutrient mineralization, effecting future growing seasons. Further analysis of the effects of organic matter decomposition is required to quantify how these effects may impact the decomposition and transport of nutrients within soil.

2.3.3.4. Energy Generation

Farming generates significant amounts of organic waste per season, which requires energy investments for farmers. One method in offsetting these energy costs and utilising bio-waste is by energy generation. Many farms in Europe generate their own energy through anaerobic digestion (Searle and Malins, 2013). Anaerobic digestion is the decomposition of organic wastes (animal manures mixed with crop residues) by micro-organisms in the absence of oxygen. During this process, methane biogas is emitted, which can be used to generate fuel (Figure 2.7.). The energy output of anaerobic digestate differs depending on the inputs, where mixtures of animal manure, organic municipal waste and crop residues can be added.

Figure 2.7. The process of anaerobic digestion (A) and its two by-products: biogas (B) and anaerobic digestate (C). [A adapted from Nkoa, (2013); B and C are images from Riverford Organic farms.]

Added to increased energy output, anaerobic digestion produces a nutrient rich by-product (anaerobic digestate; Figure 2.7.), which can be reapplied to soil as a fertiliser. To be reapplied on UK agricultural soils, digestate must be PAS 110 verified, which regulates the nutrients going into soil and protects human health. Reapplication of anaerobic digestate on farm reduces the cost of fertilisation and regulates soil nutrient status by re-inputting the losses from harvest. Replacing manure additions with anaerobic digestate may enhance the response of soils to drought conditions by regulating soil moisture conditions (Smith et al, 2019). The reapplication of an organic layer can also enhance the physical and biological properties of soil, where it can regulate soil moisture and increase N mineralization within soil (Colombani et al, 2017). Digestate can be in a solid or a liquid form, dependent upon digester inputs. The differences in consistency and nutrient quality of digester outputs ëld have an effect on organic matter utilisation by soil organisms. The effects of anaerobic digestate on earthworm populations is to be determined, but high metal content of some applications could have a toxic effect (Nkoa, 2013; Peng et al, 2020). This research will explore the effects of anaerobic digestate on earthworm species diversity and growth alongside other organic matter applications.

2.3.4. Sustainable management challenges

There are numerous challenges faced by agricultural managers when adapting conventional farming practices to include more sustainable methodological approaches (discussed in Section 2.3.3.). Maintaining high yields during the transition of conventional practices to sustainable practices under challenging effects of climate change will be a significant challenge for farmers in future and a multi-faceted approach must be applied. This section discusses the limitations faced by sustainable management methods and how these challenges are currently being addressed.

2.3.4.1. Reduced yields

The transition from traditional mouldboard ploughing practices to reduced or no-tillage methods can cause a significant reduction in yield (Alakukku et al, 2009; Riley et al, 2008), e.g. a 9.8% reduction was calculated from Swedish farms (Arvidsson et al, 2014). Soil type and moisture content are significant factors in determining the success of no-till, where compacted clays with high moisture content negatively impact crop yields (Alakukku et al, 2009). Soils in the UK with poor drainage and a weak structure can lead to nitrate losses and lower yields under no till compared with ploughing (Cannell et al, 1978; Soane et al, 2012; Daryanto et al, 2017). Added to soil type, the success of no-till practices is limited by crop type. For some crops, such as winter wheat, yields are increased (Schlegel et al, 2018), yet for root vegetables such as potatoes and sugar beet, yields are less successful (Koch et al, 2008). This has limited the adoption of no-tillage or reduced tillage practices in the UK, where the majority of NT is under wheat and rape cultivations in SE England (Townsend et al, 2016). Increasing limitations on herbicide use set by the EU Water Directive and growing weed resistance to herbicides causes further challenges in sustaining crop yields (Davies and Finney, 2002). No-tillage can increase the number of seeds returned to the soil surface, encouraging a faster weed germination and reduces weed dormancy caused by ploughing (Christian and Ball, 1994). The negative responses of crop yields to no-tillage practices could result in a high short-term economic cost for farmers adapting from conventional systems, which could discourage farmers from changing their management systems.

Long-term adoption of no-tillage practices can cause the stratification of nutrients within soil. This can provide some benefits, where a high nutrient availability at the soil surface can enhance seed germination and crop establishment (Peigné et al, 2018). However, the stratification of nutrients under no-till can cause a reduction in soil pH (e.g. no-till pH = 6.25; ploughed fields pH = 6.48; Ekeberg and Riley, 1997), impacting nutrient availability. Without an added cost of liming, a reduction in pH can decrease the NPK availability for plants, reducing yield. The element most affected by the stratification under no-till soils is P, due to its lack of chemical mobility within soil (Wright et al, 2007). Radford and Cowie (2011) found that the combination of no-till farming with chemical fertilisation can increase crop yields by 29-74% when compared with, disk tillage and conventional farming methods. However, following a fully sustainable management approach, a reduction in chemical fertilisation use is preferred. Comparisons of the effects of tillage farming and no-tillage on soil properties have often been conducted over a short period of time (<3 years). A review of no-tillage farming over a period of 175 years indicated similar yields when compared with mouldboard ploughing systems (Christian and Ball, 1994). It is considered that there may be a transitional period when converting between tillage and no tillage systems (Riley et al, 1994) where after 3 years the soil conditions in no-till improve. Increased earthworm populations under no-till systems may mitigate the effects of ploughing, particularly in soils high in anecic species due to their bioturbation effects (Briones and Schmidt, 2017). This research project will investigate the effects of *L. terrestris* populations on the bioturbation of organic matter within no-tillage soils.

2.3.4.2. Soil compaction

No-till farming can increase the rate of soil compaction, which can cause, such as reduced infiltration, surface runoff and poor seed germination. Effects of compaction on soil properties differs between soil type, where it is most common in clay soils, where soil porosity and water flow is already low (Turtola et al, 2007). Sandy soils are also susceptible to compaction under no till, recorded in Danish fields (Soane et al, 2012). Soil compaction can occur at different depths, influencing different stages of crop growth. In the lower depths of a profile (30 cm) can limit root growth and seed germination, decreasing yields. Long term effects of soil compaction can result in a residual compaction layer (Peigné et al, 2018; Allison, 2015). Under the conversion of conventional farming to no-till, the residual plough pan may be present in the soil for greater than 5 years (Capowiez et al, 2009) where lifecycle assessment has indicated that compaction in the sub soil layers (25-40 cm and > 40 cm depth) is not easily reversible (Stoessel et al, 2018). However, recent long-term field investigations have outlined that negative effects of soil compaction reduce temporally and benefits to soil physical properties outweigh initial compaction effects (Blanco-Canqui and Rius, 2018).

In the UK, there is a negative water deficit and often heavy periods of rainfall. This added to soils with poor drainage (clay soils with no ploughing applied) can cause anaerobiosis in soils, reducing microbial activity (Soane et al, 2012). In this particular system, limited periods of tillage maybe accepted to alleviate potential compaction. Climatic effects of soil compaction are different between regions (Berisso et al, 2012), where large amounts of rainfall in Scandinavian soils are impacted differently to Mediterranean soils with long arid regions. The impact of climate change can increase phosphate erosion in clay soils, where warmer climate conditions can increase the availability of surface P and increase runoff during heavy rainfall events. The increased nutrient stratification seen in no-till soils could increase the risk of dissolved reactive phosphate run off (Soane et al, 2012). This indicates that a spatio-temporal management approach is required when applying sustainable methods.

The physical effects of soil compaction can reduce biotic activity, particularly in macrofauna and plant roots, whilst increasing anaerobic microbial activity (Whalley et al, 1995; Beylich et al, 2010). Anoxic environments in soil can increase N_2O emissions during gas transport (Berisso et al, 2012), increasing greenhouse gas emissions. Although greenhouse gas emissions are lower in no-tillage due to the reduced usage of heavy machinery, in some instances there are still negative impacts caused by no-tillage farming. N_2O emissions are influenced by soil wetness, compaction, climate and type of fertilization (Soane et al, 2012). If the soil type is not suitable for no-tillage management, in some instances this can cause poor drainage, which increases soil moisture and compaction, leading to increased N_2O emissions (Regina and Aklukkuku, 2010). Stimulating biological activity through the addition of organic matter may reduce anoxic effects within soils through increasing soil aeration (Capowiez et al, 2009).

2.3.4.3. Pest management

Sustainable management practices do not apply synthetic pesticides and herbicides to soil and use alternative methods such as crop rotations, biological control and cover cropping. Although these methods provide significant benefits to soil ecosystem services (defined in Section 2.3.2.3.), developing a sustainable system is complex, where often several pest management challenges arise simultaneously.

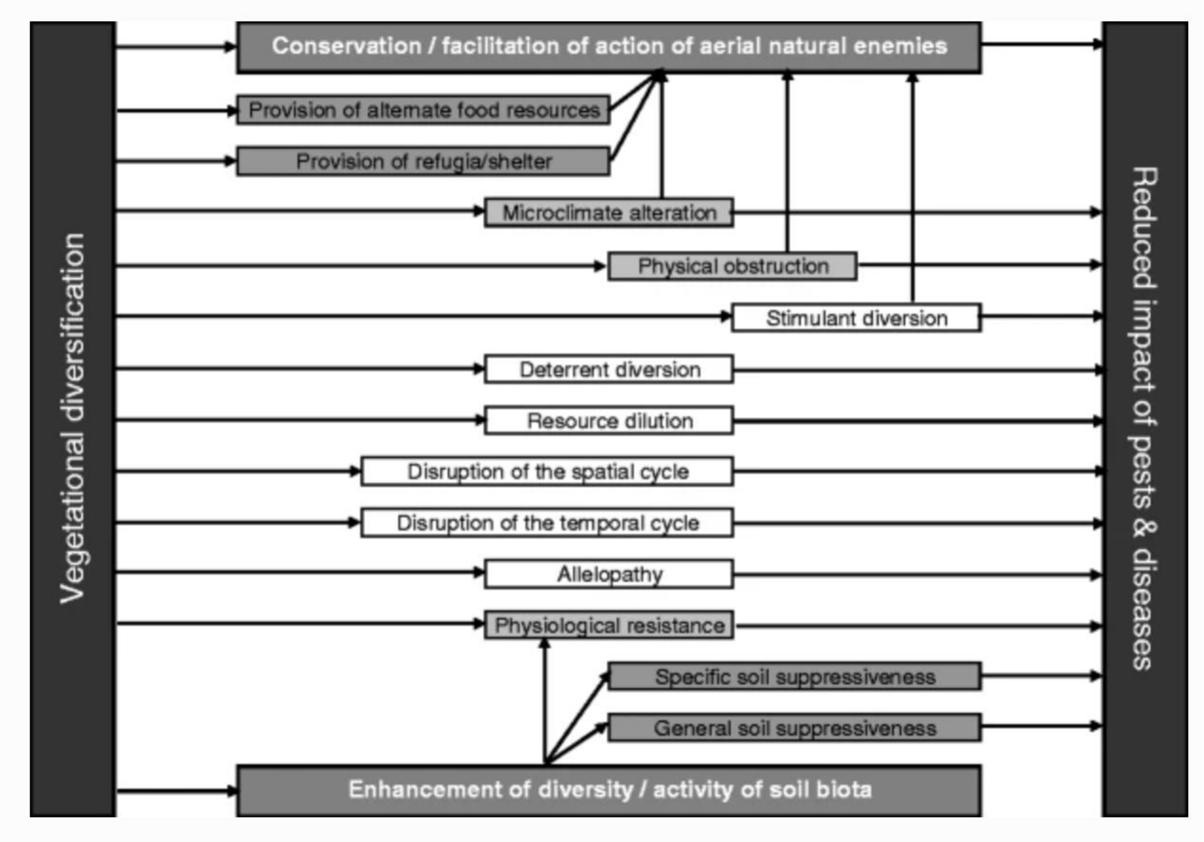


Figure 2.8. Major pathways for reducing the impact of pests and diseases via the introduction of plant species diversity in agroecosystems. Figure sourced from Ratnadass et al, (2012).

A reduction in tillage can cause an increase in weed growth, which can limit yields by competing with plants. An introduction of cover crops to these systems can supress weed growth and provide other benefits such as retaining soil nutrients and storing soil moisture (see Section 2.3.3.3.). The benefits of cover crops to reducing weeds is limited by crop type and application timing (Mirsky et al, 2011), where in wheat rotations, applications of red clover can significantly reduce downey borne infestations in winter wheat without a reduction in yield, whereas in spring yield there was a significant yield reduction (17%; Andersson, 2016). Crop rotations may have a greater impact on the weed flora through reducing resistance (Blackshaw et al, 2007; Bohan et al, 2011).

No-tillage farming can also increase the number of pests within soil, where mechanical disturbance (alongside herbicide application) in conventional systems can significantly reduce many aboveground pests, such as molluscs. Ecologically based strategies for removing common pests such as molluscs include biological control, where living organisms such as the parasitic nematode *Phasmarhabditis hermaphrodita* are purchased and applied to soil to feed on molluscs, reducing the population size and damage to crops (Kozlowski et al, 2014). Practical application of biological controls is not always effective, where they are temperature sensitive and have a low shelf life (Speiser and Kistler, 2002).

Applications of alternative plant flora (e.g. cover crops) along with the main crop may reduce damage to crops by providing an alternative food source to pests (Cook et al, 1997). Increasing plant diversification within cropping systems may also reduce pests through increasing natural enemy populations (Douglas and Tooker, 2012; Schipanski et al, 2014). However, crop residues from alternative plant flora may provide a better habitat for pests (Vernavà et al, 2012), therefore accurate plant selection in multiple cropping systems is essential to manage pests effectively.

Continued development of integrated pest and weed management based on cropping systems at a regional scale will increase soil resilience (Dainese et al, 2017), enhancing crop yields sustainably. Further modelling of cropping systems under the agroecological methods described above may develop accurate forecasting of weed and pest suppression.

2.3.4.4. Organic Matter Addition

Although there are many benefits to soil quality and yield from the addition of organic matter, there are also some potential problems that need consideration. Organic matter types have significantly different chemical properties, which e.g. impact the rate of nitrogen mineralization within soil (Iritani and Arnold, 1960; Abbasi and Khaliq, 2016; Bhogal et al, 2016). The decomposition rate of organic matter differs between its chemical (e.g. C:N ratio) (Wang et al, 2017a) and physical (e.g. particle size) composition (Rinkes et al, 2014), soil type and temperature (Conant et al, 2011); accurate assessment of organic matter properties and environmental conditions before application is essential. The method of organic matter application can also cause practical issues. Surface application can create crusting, which can increase runoff and erosion of nutrients during heavy rain fall (Armand et al, 2009). In North West European soils, an issue with crop residue application can occur in the drilling of new crops when residues are already applied (Soane et al, 2012). Drilling is negatively impacted by increased surface residue, stubble height, root presence and the amount of solar radiation reaching new seedlings, which can cause a reduction in the rate of crop germination and development (Mikkola et al, 2005). In monoculture systems with no-till, the application of crop residues can cause a carryover of pathogens such as leaf spot and root diseases (Duczek et al, 1999; Wolfarth et al, 2011). However, the impact of increased carbon and nitrogen concentration from organic matter often outweighs these factors.

Even though anaerobic digestion is considered a sustainable management system for farms, often farming systems are not fully optimized to maximise the environmental benefits. For example, pastoral farmers must often purchase or transport agricultural waste over large distances, which increases CO_2 emissions. Pierie et al, (2017) have suggested that improving the cooperation between neighbouring farms can minimise transport costs by sharing waste resources. Added to this, renewable energy developments have increased the rate of cash crop farming. This limits space for food generation, which threatens food security, particularly in large scale systems (Auer et al, 2016). Although further development is required to improve the sustainability of the system, anaerobic digestion may form a significant part of farm management in the future, particularly in OECDs (Nayal et al, 2016). The effects of organic matter utilisation within agroecosystems on soil ecosystem services are discussed in Section 2.4.

2.3.5. Earthworms in agroecosystems

The effects of sustainable agricultural practices have a positive impact on earthworm populations (Ball et al, 1999; Blouin et al, 2013; Moos et al, 2016). Due to the impact of earthworms on supporting ecosystem services (defined in Section 2.2), an increase in earthworm populations following a transition from conventional farming practices may mitigate the negative outcomes on soil properties, such as soil erosion, where rate of soil loss outweighs soil formation by a ratio of 17:1 (Troeh and Thomson, 1993). This section discusses earthworm populations in agroecosystems, referring to the effect of management type on population dynamics and the resulting impacts on soil ecosystem services.

2.3.5.1. Tillage

The usage of tillage in agroecosystems has a negative impact on earthworms, where mechanical disturbance can increase mortality rate and increase their exposure to predators (Briones and Schmidt, 2017). A field experiment analysing the impact of low (20 cm) and high (35 cm) depth mechanical loosening and power harrow cultivation on earthworm populations indicated that at both depths mechanical loosening (which involves the overturning of soil) reduced the abundance and biomass of earthworms (Lees et al, 2016). Earthworms can survive conventional farming methods, however the species richness within conventional systems is different to no-tillage, where predominantly small endogeic species can survive within these conditions (Crittenden et al, 2015). A lack of an organic layer and a destruction of the soil habitat has negative consequences for anecic species, which are larger in biomass and feed on the soil surface (Blouin et al, 2013). The functional differences between endogeic and anecic species could impact the rate of organic matter decomposition and transport through soil. This research project will assess the organic matter utilisation by anecic and endogeic earthworm populations. Further information on earthworm utilisation of organic matter is discussed in Section 2.5.

Some of the challenges faced following conversion from conventional to no-tillage farming could be mitigated by the projected increase in earthworm population density and species biodiversity. Two notable physical interactions of earthworms which positively impact soil are increased water infiltration and soil aeration (Andriuzzi et al, 2015a). An 8 year field study by Pelosi et al (2009), indicated that increased populations of earthworms present in no-till farming caused a higher hydraulic conductivity compared with mouldboard ploughing. Positive effects of earthworm populations on the mitigation of soil compaction was experimented by Capowiez et al, (2012). Earthworms were assessed under compacted soil (under wheel tracks) and non-compacted soil (above wheel tracks) over a period of two years following a compaction event. The increased bulk density within compacted soil resulted in lower earthworm biomass and abundance, which agreed with Lees et al (2016). However, earthworm populations regenerated quickly after the compaction event, where at three months post compaction there were no significant differences in earthworm biomass and abundance between compacted and uncompact zones. The recovery of soil functional properties caused by earthworm behaviour (water infiltration and macroporosity) was slower and there were no observed effects until 18 months later. Therefore, potential remediation of soil by earthworm populations under the conversion of arable tilled soils to no-tillage management may occur over a longer time.

Soil compaction under no-till systems may limit the potential of earthworms to remediate soil. A long-term experiment was conducted by Schlüter et al, (2018) to determine whether the expected increased earthworm and soil fauna abundance in reduced tillage systems mitigated the effects of mouldboard ploughing. Results from this experiment indicated that there were no differences in the soil structure or porosity between tillage systems in the lower soil pan structure (28-38 cm) after 25 years and a more compacted surface structure in the upper soil layer (13-23 cm) in reduced tillage systems. This indicated that the expected effects of increased bioturbation by earthworm species did not imitate the effects of ploughing. Similar effects of soil compaction on earthworm populations were observed in a compaction field experiment conducted by Peigné et al, (2009). There were a number of suggested reasons why the earthworm populations did not improve long term soil conditions. Low abundances of anecic earthworms, such as *L. terrestris*, were present. Anecic populations have different effects on bioturbation and the removal of organic matter compared with endogeic species, which do not make permanent burrows (Bouché, 1977). Within these experiments, the climatic and soil conditions of the field site was not favourable for anecic species, with frequent dry periods and low penetrability of the upper soil layer. Thirdly, it could be that earthworm population development after tillage management takes longer to mediate soils than 25 years. This could be enhanced by earthworm inoculation, a technique developed in the regeneration of restored soils (Butt et al, 1997). Although no tillage can increase earthworm populations, which can increase WOM decomposition and reduce nutrient stratification effects through bioturbation (Milleret et al, 2009; Bityutskii et al, 2016), specific impacts on crop growth is undetermined and requires further research.

2.3.5.2. Organic matter

In conventional agricultural systems, nutrient supply is maintained through the addition of inorganic fertiliser. Often, there is no surface organic matter applied. Applications of organic waste such as animal manures, crop residues, composted green waste and anaerobic digestate are added for a number of reasons dependent upon their properties (Peltre et al, 2017), where they can increase soil nutrient inputs, soil organic matter concentration and maintain soil moisture (Wang et al, 2018; Menšík et al, 2018). The impact of biodynamic production systems under organic matter applications has increased earthworm abundances (Pfiffner and Mäder, 1997) when compared with conventional and organic systems. Applications of organic waste can predominantly benefit earthworm populations as they are a food source, regulate soil moisture and provide a habitat (Edwards and Bohlen, 1996; Curry and Byrne, 1997; Curry and Schmidt, 2007; Stroud et al, 2016a). However, the method of organic matter application (surface applied or mixed) can impact earthworm population dynamics, due to species-specific feeding behaviour and inter- and intra-specific relationships (Bouché, 1977; Edwards and Bohlen, 1996). Laboratory experiments have indicated that the particle size of organic matter applications influence earthworm feeding and growth (Boström and Löfs-Holmin 1986; Boyle, 1990; Lowe and Butt, 2003; Sizmur et al, 2017), where maximum particle size ingested is relative to body mass (Piearce, 1978). Surface applied organic matter generally benefits anecic and epigeic earthworm populations and is detrimental to endogeic earthworm populations, whereas incorporated organic matter benefits endogeic populations (Lowe and Butt, 2002). Therefore, the application of crop residues can significantly affect organic matter transport through a soil profile and its decomposition. Alongside the method of organic matter application, the type of organic matter can influence earthworm selection. A long-term field experiment conducted by Ashworth et al, (2017) indicated that earthworms have a preference of bio-covers in the field, where poultry litter was preferred, and are influenced by crop sequences, where cotton decreased earthworm species by 55-68%. Therefore, in order to utilise specific earthworm populations and their impacts on soil physical properties, organic matter application type and application method needs to be carefully managed. More details on organic matter amendments and earthworm interactions are explained in Section 2.4.

In turn, increased populations of earthworms can have positive impacts on the degradation of organic matter and mineralization of nutrients in soil. The increase of N mineralization by earthworm populations can have a critical role in plant production, where earthworms can increase crop yields by 25% and aboveground biomass by 23% (van Groenigen et al, 2014). Earthworm casting has positive impacts on soil properties, such as an increased aggregate stability (Botinelli et al, 2010a, 15, 20b), available nutrient content (Ros et al, 2017) and SOC storage (Sheehy et al, 2019). Combining reduced tillage farming with organic matter application can increase the rate of nutrient mineralisation and hydraulic activity in soils by stabilised earthworm and plant root channels (Vogeler et al, 2009).

Although it is concluded in most of research investigations that high concentrations of earthworms within soil have positive effects on soil properties, earthworms also can be considered as a dis-service to agricultural ecosystems. Like many organisms, earthworms produce emissions of CO_2 and N_2O, which accumulates in their gut, which contributes to global warming effects (Horn et al, 2003). It is predicted that earthworm populations can increase CO_2 and N_2O by 33% and 42% respectively (Lubbers et al, 2013); increased earthworm populations in reduced tillage systems could increase climate change effects. Alongside the accumulation in the gut, habitats within earthworm casts and drilosphere promotes N_2O production (Majeed et al, 2013). Earthworms can ingest and transport seeds in the soil (Grant, 1983; Kirchberger et al, 2015), which can increase the spread of weeds, which compete with crop yields (Smith et al, 2005). Findings indicated that earthworms have a preference of seeds, which is particle size limiting. The trade-off between the impact on earthworms on ecosystem service and dis-service was investigated by Wu et al, (2015) where the impact of two earthworm species (*Eisenia fetida* and *Metaphire guillemi*) on the N_2O production and crop growth of fields applied with mulched and incorporated organic residues was investigated. Findings indicated that, while both species increased N_2O emissions (19-25%), this was outperformed by the increase in crop productivity (18-47%) by earthworm activity. This investigation analysed two factors of ecosystem services; further research is required to increase understanding of ecosystem service trade-offs of soil macrofauna.

2.3.5.3. Cover Cropping

The implementation of cover cropping has a significant impact on earthworm population biodiversity and abundance, which increases supporting services. Cover crops form an increased coverage of organic matter, which provides an extra food source for earthworm populations when land would normally be fallow. It is predicted that an increased food diversity aboveground encourages an increased diversity in belowground fauna (Wardle, 1995). In a research study comparing earthworm populations in wheat-clover rotations compared with conventional wheat monocropping, total earthworm biomass increased by 101 gm^{-2} and earthworm abundance increased by 354 individuals m^{-2} (Schmidt et al, 2001). The earthworm biodiversity was similar to that found in pasture grasslands. Earthworm abundance and biomass is influenced by cover crop type, where Reeleeder et al. (2006) indicated that the presence of rye cover crops increased *A. turgida* populations. However, it was also highlighted that the impact of cover crop additions on earthworm populations was superseded by the impact of poor-quality soils. Therefore, other environmental factors may displace the benefits provided by cover cropping.

Food preference within cover cropping has been reported in several studies. Findings by Valckx et al, (2011) highlighted a food preference of cover crop by *L. terrestris*, where ryegrass residues where preferred. This correlated with a higher C:N ratio of ryegrass, which disagreed with other studies referring to organic matter content with a low C:N ratio preferred by earthworms (Bohlen et al, 1997; Curry and Schmidt, 2007; Blouin et al, 2013). However, the impact of particle size and glucosinolate concentration of the cover crops and earthworm preference was not studied. Field investigations into cover crop preference by Roarty et al, (2017), highlighted an overall higher earthworm population abundance in fields with cover crops, where pea cover crop had the highest abundance (221 ± 55 ind. m^{-2}). In this experiment there was preferences of pea, oat and rye cover crops with lower earthworm abundances under brassicacea species. There was no correlation between cover crop biomass and earthworm abundance, indicating a possible cover crop preference. Also, species diversity altered dependent upon cover crop, where rye increased *A. rosea* and oat increased *A. chlorotica* populations. This change in earthworm biodiversity could impact soil functioning and nutrient mineralisation in soils. Further research into food selection of organic matter sources by earthworm species is required to understand the impact on soil properties in agroecosystems.

2.4. UTILISATION OF WASTE ORGANIC MATTER IN SUSTAINABLE AGROECOSYSTEMS.

2.4.1. Organic waste applications in sustainable agroecosystems

There are a number of benefits from the utilisation of waste organic matter within agroecosystems. Organic waste is a material that is biodegradable originating from a plant or animal. It is degraded through soil mineral changes and aerobic and anaerobic digestion processes by macro and micro soil organisms. Some examples of organic matter applied in agroecosystems are: crop residues, anaerobic digestate, animal manure, cow slurry, composted green waste. Organic wastes differ in their nutrient properties (Zikeli et al, 2017); it is important that the nutrient requirements of the crop and nutrient deficiencies in the soil are considered when choosing the application type. WOM can be applied in addition to or in replacement of inorganic fertiliser, where applications could reduce negative environmental impacts of inorganic fertilizer pollution (discussed in Section 2.3.5.2). Utilising on farm waste could significantly reduce farmer costs in fertilisation and waste disposal. Addition of organic matter can increase soil organic matter (SOM) content, regulate soil moisture, increase soil fauna activity and stimulate microbial activity (Médiène et al, 2011). Waste organic matter applied on farms differs between agroecosystem, where the waste organic matter can have significant impacts on soil properties. This Section defines selected waste organic matter types used within no-till agroecosystems and their impacts on soil properties.

2.4.1.1. Manure

Animal manure applications can increase soil properties beneficial to crop growth, such as SOM content, N mineralisation, soil porosity and water holding capacity, whilst decreasing bulk density and surface crusting (Marinari et al, 2000; van Kessell et al, 2000; Maillard and Angers, 2014; Kidd et al, 2017). However, there is variability between the quality of manure provided within and between pastoral species (Omara et al, 2017) and cattle diet can influence slurry nutrient outputs (Paschold et al, 2007). Liquid and solid manure treatments also have differing effects on soil properties and the application method, where liquid manure is often injected and manure incorporated. Solid manure treatments increase the SOM content and rate of macroaggregate formation, whereas liquid treatments have a more timely effect on nutrient release (Webb et al, 2014). Manure is high in nitrate and application rates in Europe are limited to 170 kg ha^{-1} yr^{-1} (EU, 2019). In areas where nitrate is not the limiting nutrient in soil, manure may be replaced with another fertiliser.

Compared with other treatments, cattle manure was determined to be a better treatment than sewage sludge and control soil (with NPK fertilisation), where manure provided higher thermal stability, lignin content and bacterial amino-sugars (Peltre et al, 2017). This resulted in accumulations of different C compounds within organic waste types, which was altered by decomposition and microbiological activity. Cow manure applications can increase the yield of wheat-maize rotations and water use efficiency compared with straw and biogas slurry (Zhen et al, 2019), where nitrogen was increased in the upper soil layer (0 - 20cm). The responses of crops to manure applications is limited by crop nutrient requirements and soil type. A comparative study between the effects of liquid swine manure (LSM), liquid dairy manure (LDM) and solid poultry manure (SPM) on corn and wheat cropping systems indicated that yields were more responsive under sandy loam soil than silty clays and had an increased effect on yields within wheat systems, where LSM was the most effective fertiliser (Samson et al, 2019). Future applications should assess the soil type and crop nutrient requirements before application.

There are negative properties of manure application which need to be considered. Increasing use of antibiotics within pastoral agriculture could be transmitted into soil and food pathways, a risk to human health and soil functioning (Campagnolo et al, 2002; Aga et al, 2016). The use of manure over treated waste (composted manure or anaerobic digestate) has also been contested, where treated waste can enhance macroaggregate stability and prevent P losses common in swine manure (Grande et al, 2005; Wortmann and Shapiro, 2007). Treated manures can also reduce the risk of antibiotic leaching into soil (Urra et al, 2019). The ratio of available N:P in swine manure can often be limiting for crop requirements, resulting in P losses (Sims et al, 1998). Long-term field investigations of the effect of FYM, inorganic fertiliser and control applications indicated the positive effects of FYM on nitrogen concentration and SOM content, but did not result in increased yields over time (Liang et al, 2011). A mixed fertilisation management may be more effective, using FYM as a soil conditioner.

2.4.1.2. Anaerobic digestate

Anaerobic digestate is a soil conditioner resulting from the process of anaerobic digestion (as discussed in Section 2.3.3.4). Digestate applications utilise on-farm waste and recycle organic matter back into the agroecosystem, replenishing N concentration and regulating SOM concentration (Möller and Muller, 2012). There are two types of anaerobic digestate waste: a dry and a liquid fraction. Both are used as soil conditioners, where the dry fraction increases SOM and stimulates soil microbial activity and the liquid fraction enhances nutrient concentration and soil moisture (Möller and Muller, 2012; Nkoa, 2013). Liquid anaerobic digestate can be injected into the soil to reduce ammonia emissions (Webb et al, 2004; Nicholson et al, 2018), stimulate the bacterial decomposer community (Walsh et al, 2012b) and reduce odours (Riva et al, 2016; Orzi et al, 2018). Dry anaerobic digestate is often applied incorporated into the upper soil layer. Alongside the difference in state of matter, anaerobic digestate quality differs depending on its inputs, which is normally a ratio of animal manure and crop waste.

Anaerobic digestate produced from municipal solid waste (MSW) inputs is utilised and processed differently dependent upon several factors, such as governmental regulations, the biogas method used and the waste produced. The inputs determine whether it can be applied to agricultural land and used as a soil conditioner and meets BSI PAS 110 regulations (WRAP, 2016). The limit value for digestate VFAs (Volatile Fatty Acids) in the UK is 0.43 g COD g VS^{-1} (BSI, 2010). The differences between source segregated domestic food waste, organic municipal solid waste and waste activated sludge with vegetable waste was analysed by Tampio et al (2016). Findings indicated that although MSW digestates provided a 5-30% increase in ryegrass yields, there was significant differences in the nutrient availability of treatments, where mixtures of waste activated sludge with vegetable waste increased metal loading. By meeting the nitrogen requirements for the crop (170 kg TKN ha^{-1} yr^{-1}), waste activated sludge also supplied 626.5 mg kg^{-1} TS Cu and 1.9 mg kg^{-1} TS Hg, above legislative levels (BSI, 2010). Optimization of MSW inputs into a digester to meet BSI PAS 110 requirements could enhance the use of digestates within agroecosystems.

Applications of anaerobic digestate can increase yields compared with other fertilisation treatments (Walsh et al, 2012a), where the rate of N mineralization is influenced by inputs, application time and temperature (Øvsthus *et* al, 2017; de Notaris et al, 2019). The rate at which anaerobic digestate is more successful than raw manure differs between experiments, where some suggestion that anaerobic digestate increases available plant nitrogen compared to aerobic digestion (Singh, 2015) and some have no effects on yield (Loria and Sawyer, 2005; Chiyoka et al, 2014). Both outcomes suggest that anaerobic digestate is a useful soil fertiliser. The elimination of pathogens in manures is more rapid and effective under anaerobic digestion compared with the traditional practice of manure storage (Costa et al, 2017). Anaerobic digestate of green manures (the biomass of cover crops) is more effective than fresh application, but N mineralization is variable dependent upon silage conditions (Liebman et al, 2011; de Notaris et al, 2019).

The focus of anaerobic digestion is often on the energy valorisation and how to increase energy profits (De Vries et al, 2012; Styles et al, 2015; Burg et al, 2018; Parmar and Ross, 2019). The difference in digester inputs can have significant effects on the digestate and its effect as a soil conditioner, where increased crop residue inputs can increase plant available nutrients, yet manure can inhibit P availability (Möller and Muller, 2012). Market valuation of anaerobic digestate as a fertiliser may increase the economic focus of anaerobic digestion towards developing a fertiliser that is more targeted to improving crop production and soil health alongside energy outputs. The UK provides guidance on good management practice for digestate use in agriculture (WRAP, 2016), however current research highlights increased optimization of anaerobic digestion management is required to balance financial biogas production outputs with anaerobic digestate quality to maximise the positive impacts on soil properties (Vaneeckhaute et al, 2016; Logan and Visvanathan, 2019).

2.4.1.3. Crop residues

The application of crop residues to arable soils is considered to mitigate global climate change, by sequestering SOC, reducing nutrient leaching and inorganic fertiliser usage, which results in the offsetting of CO_2 emissions and improvement of soil quality (Singh and Singh, 1994; Downing, 1993; Moran et al, 2005; Lal, 2008; Lavalle et al, 2009). Residue applications have differing impacts on soil properties dependent upon their decomposition rate, where legumes degrade more quickly compared with corn or wheat, due to higher C:N ratios and higher lignin and polysaccharide content (Johnson et al, 2004; Hoyle and Murphy, 2011; Zhao et al, 2016). Another significant factor influencing the rate of residue decomposition is microbial activity and community composition, which is influenced by crop residue quality (Bending et al, 2002; Hoyle and Murphy, 2011) and application depth (van den Bossche et al, 2009; Turner et al, 2017). Keeping crop stubble intact in addition to residue application enhances the amount of C available to microorganisms in the rhizosphere, further stimulating microbial activity and enhancing SOM decomposition (Kätterer et al, 2011; Finzi et al, 2015; Adamczyk et al, 2019).

The method of crop residue application has specific impacts on the rate of decomposition, which may influence the mineralization of nutrients for crops and the timing of nutrient release. For example, wheat residues that are mixed in the soil profile decompose at a faster rate than when surface applied, which could be due to increased fungal activity on the soil surface (Holland and Coleman, 1987) and increased microbial activity when incorporated (Hoyle and Murphy, 2011, Turner et al, 2017). The increased exposure to UV radiation under surface applications of crop residues enhances phytodegradation, which can cause changes in litter chemistry, such as a reduction in hemicellulose (Lin and King, 2014). The impact of crop residue degradation into SOM is influenced by crop rotation complexity and farm management history, where increased crop rotations can increase the rate of decomposition by 0.2-8.3% compared with a monocropping management system (McDaniel et al, 2014). Crop rotation complexity can increase soil organism biodiversity, which has a positive effect on SOM formation, pollination and pest regulation (Isbell et al, 2017; Barnes et al, 2020). This has been reflected in grassland systems where an increase in plant species increased microbial biomass and function (Eisenhauer et al, 2010), which could enhance organic matter degradation. An increase in the number of crops grown in a rotation system and improved application methods could enhance the rate of organic matter degradation and in turn increase the rate of N mineralization.

Crop residue applications can have negative impacts on soil properties. For example, surface applications of residue mulches increased waterlogging under wetter climates, which reduced maize yields (Rusinamhodzi et al, 2011). Crop residues as a sole application does not always increase crop yields but improves soil conditions to encourage crop growth. Comparisons with anaerobic digestate and cow manure indicated that cow manure increased yields and C:N concentrations at a faster rate than residue application and standard practice fertiliser (Zhen et al, 2014). This results in crop residues being utilised for other resources, such as animal bedding and bioethanol production. Removal of crop residues removes nutrients from the soil system and causes a 12-19% reduction in SOM content (Warren Raffa et al, 2015). In sustainable systems where residues are removed, nutrients and SOM are replenished with other organic amendments such as manure (Blanco-Canqui and Lal, 2009). Applications of straw residues mixed with other organic amendments (such as manure) could utilise the positive attributes of both applications on soil properties and regulate nutrient mineralization (Blanco-Canqui and Lal, 2009; Soane et al, 2012; Bertrand et al, 2015a).

Crop residue applications can increase soil biodiversity, where surface applications can provide habitats for a number of fauna species. In comparison with other agroecosystems, arthropod communities were higher in no-tillage cereal farms with residue applications, where there were significant differences in community diversity between crop type (Menta et al, 2020). Crop residues can provide a habitat for epigeic earthworms, which enhance the decomposition of WOM (Pang et al, 2012; Bertrand et al, 2015a). Interactions between soil fauna such as earthworms and their movement throughout soil may be an important process in enhancing degradation of slow decomposing organic matter (Blouin et al, 2013) and reducing fungal pathogens (Wolfarth et al, 2016). This research project will investigate the utilisation of crop residues by earthworm populations in no-till agroecosystems.

2.4.1.4. Composted Green Waste

Composted Green Waste (CGW) is any organic (biological) waste that has been composted, normally consisting of a mixture of garden waste and domestic and industrial kitchen waste. CGW is applied in land reclamation projects to improve degraded soil, where it provides a source of SOM and nutrients to encourage plant growth and ecosystem regeneration (Foot et al, 2003; Nason et al, 2007; Ashwood et al 2017a). Within agroecosystems, strict regulations limit whether it can be applied to protect human health (BSI, 2010). Trials of CGW addition to agroecosystems have had differing results.

Alongside organic components of municipal waste, CGW can also consist of farm waste, which can then be reapplied on-farm in a similar method to anaerobic digestate. CGW with manures and crop residues can reduce N losses and increase crop yields over a longer term (more than one cropping season) compared with inorganic fertiliser (Petrik and Mustavić, 2015; Pinto et al, 2017), encouraging long-term sustainable intensification of agriculture. There is no evidence to suggest that CGW addition can increase soil available nutrient concentration to the same rate as inorganic mineral fertiliser, however it has been suggested that CGW addition can improve plant uptake of the nutrients, where increased P and K of wheat grains have been recorded (Gopinath et al, 2008). Differences between the composter inputs can influence the nutrient uptake of plants within soil. Composting sheep manure can increase the nutrient uptake and seed yield of triticale (Naderi et al, 2018) whereas applications of composting poultry waste increased SOM storage and soil C and N concentration but had no effect on crop nutrient uptake or yield (Blair et al, 2014). Compared with other WOM applications, CGW had a reduced effect on yield and decreased shoot length of triticale than sewage sludge amendments (Kchaou et al, 2018). Increased nutrient supply from CGW can increase weed growth (Little et al, 2015), therefore in sustainable systems CGW inputs should be assessed based on crop requirements to avoid over-fertilisation.

The positive impacts of CGW on soil fauna is uncertain. The positive effects of SOM addition may improve food availability within poorly resourced soils, however high metal concentration may have toxic effects (Wierzbowska et al, 2016). Within forestry and land reclaimation ecosystems, earthworm populations increased which improved tree development and CGW decomposition rate (Lowe and Butt, 2004; Ashwood et al 2017a). However, additions to no-till wheat agroecosystems determined a population collapse of *L. terrestris* populations (Stroud et al, 2016c). The production of CGW can be accelerated by the utilisation of earthworms through vermicomposting (Frederickson et al, 1997). Vermiculture can be used in composting and can increase microbial abundance and nutrient quality of CGW additions (Cai et al, 2018). Earthworms used in the vermicomposting process (e.g. *Eisenia fetida*) are not applied to agroecosystems and are not soil-dwelling (Bouché, 1977; Sims and Gerard, 1999). Further research is required to determine the effect of CGW additions on earthworm population dynamics within agroecosystems.

2.4.1.5. Sewage Sludge

Sewage sludge is the residual matter produced during industrial and municipal wastewater treatment. It is a semi-solid material and can be applied to soil in agroecosystems as a soil conditioner. In the EU, there are a number of regulations which limit the application of sewage sludge to agroecosystems to ensure public safety and reduce soil contamination, which is defined in the Sewage Sludge Directive 86/278/EEC (EU, 1986).

Applications of sewage sludge to soil can have many benefits to soil properties and plant growth (Börgesson et al, 2014). In agroecosystems, it is often used in replacement of inorganic P fertilisation (Andriamananjara et al, 2016; Houben et al, 2019). Commonly used inorganic P fertilisers are derived from phosphate found in rocks, which is a non-renewable resource (Reijnders, 2014). The utilisation of sewage sludge as a renewable source of P fertilisation could reduce the threat to food security posed by a lack of global sources of phosphate rock (Dawson and Hilton, 2011). Sewage sludge also increases the N concentration within soil, where the amount of mineralizable N from organic N in sewage sludge ranges between 8-40% dependant upon the pre-treatment (Parker and Sommers, 1983). Alongside soil nutrient concentration, sewage sludge improves soil structure by increasing aggregate stability and soil porosity (Pagliai et al, 1981). Sewage sludge also improves C sequestration in agroecosystem soils, where carbon retention was increased by 18-20% by long-term (30 year) applications of sewage sludge (Börgesson and Kätterer, 2018). The positive attributes of sewage sludge application to soil has resulted in it being a widely used organic fertiliser in agroecosystems.

Added to the changes is physio-chemical conditions of soil, sewage sludge addition can have positive implications on biological communities within agroecosystem soils. Long term applications of sewage sludge can increase microbial biomass, which enhances organic matter decomposition (Börgesson et al, 2014). However, repeated applications of sewage sludge over long-term periods can decrease pH, which could influence community structure (Börgesson et al, 2014). Within fungal communities, sewage sludge application influences species diversity differently dependant on belowground location, where fungal communities in barley roots was different to the rhizosphere (Ondreičková et al, 2021). Research analysing the effect of sewage sludge application on soil meso- and macrofauna population density and species diversity has had mixed results. Sewage sludge applications in the field had a positive effect on earthworm populations, increasing population size by 320 ind. m^{-2} at 75 g Kg^{-1} application rate (Barrera et al, 2001). Findings by Andrés et al (2011) indicated that applications of fresh sewage sludge decreased the density of oribatid mites, where negative effects were seen 3 years after field application. Anaerobically treated sewage sludge had lesser effects on the soil invertebrate community. Further research is required to determine how the positive effects of treated waste on soil faunal communities influence soil properties and crop growth.

2.4.2. Impacts of organic waste applications on soil ecosystem services

2.4.2.1. Soil formation

There are several benefits to soil from a high organic matter content including increased soil quality, turnover and productivity (Manlay et al, 2007). Preservation of organic matter in soil is vital to ensure long-term soil stability and maintaining arable productivity (Janzen et al, 1998). Organic matter is preserved by inherent recalcitrance and mineral protection (Christensen, 1987; Six et al, 2002). Inherent recalcitrance is the resistance of organic compounds to degradation due to their chemical structural properties (Lorenz et al, 2007), where organic matter containing more aliphatic structures (lipids and waxes) have more resistance to chemical decomposition than labile structures (carbohydrates and lignin) (Mikutta et al, 2006). Mineral protection is the development of OM-mineral complexes, where organic matter chemically binds to mineral (clay) fractions of soil, increasing resistance to microbial decay (Six et al, 2002). The effects of organic matter preservation were investigated by Clement et al, (2011), where comparisons were made between organic matter types and preservation by different soil fractions. Findings from this study indicated that longer chain aliphatic compounds (organic acids) accumulated in the finer fractions of soil, whereas lignins were protected in silt-size fractions. Therefore, soil type and consistency are significant factors in the preservation of organic matter and should be considered before choosing organic matter application in arable soils.

The rate of soil organic matter (SOM) formation depends on the rate of WOM degradation, which is influenced by residue quality, litter chemistry, application method (surface applied, mixed, injected, pre-treated), environment, soil type, soil fauna activity and microbial activity (Lehmann and Kleber, 2015; McDaniel et al, 2014). In experimental studies, decomposition of organic matter is calculated by CO_2 respiration and microbial biomass. Soil organic carbon (SOC) is the carbon occurring in SOM (Milne, 2012). SOM can increase the amount of SOC in the soil, however at different rates dependant on the ratio of carbon to other elements (such as N and P) in the soil. Therefore, the effect of soil carbon sequestration is only partially influenced by organic waste type. Interactions between litter chemistry and soil type have significant impacts on the decomposition of organic waste and soil quality. An experiment by Candemir and Gülser (2010) determined the impacts of different organic waste applications (manure (M), hazelnut husk (H), tea waste (TE) and tobacco waste (TO)) on the soil quality of a sandy loam and clay fields. Although all waste treatments increased aggregate stability and reduced bulk density, H and TE provided a higher soil quality index for loamy sand fields, where M and TE were more effective in clay soils. Further investigation of the C structural properties in WOM changes during decomposition may provide insight to the impact of applications on the physical properties of soil.

The rate of WOM decomposition is determined by a number of biotic (interactions of macro and micro soil organisms, bacteria and fungi) and abiotic factors (pH, moisture content, C:N ratio, S and P concentration) (van Eekeren et al, 2010; Caricasole et al, 2018). Inputs of WOM provide a food source for soil fauna such as earthworms, which increases the transformation of organic matter through microbial gut processes and bioturbation (Devliegher and Verstraete, 1995; Schrader and Zhang, 1997; Aira et al, 2008). Soil fauna and microbial activity consume organic matter in different stages of decay dependent upon species type. Interactions between soil fauna and microbes with different organic matter types can significantly influence the rate of soil formation, where organic matter degradation is slower in organic matter types with higher C:N ratios (Blouin et al, 2013; McDaniel et al, 2014). Added to the impacts of soil fauna, organic matter inputs can stimulate SOM decomposition through a microbial priming effect (Bingemann et al, 1953; Kuzyakov et al, 2000). Different organic matter types influence microbial growth, which in turn impacts the rate of organic matter decomposition (Zhang et al, 2017).

2.4.2.2. Soil structure

Organic matter decomposition can significantly enhance soil physical properties such as aeration, aggregate stability, soil fertility and SOC protection (Pagliai and Vittori Antisari, 1993; Annabi et al, 2011). SOC content can enhance the amelioration of soil structure (Mulumba and Lal, 2008) and influence aggregate stability (Wright and Hons, 2005), which improves water infiltration and protects against erosion and compaction. An indicator of good soil structure is aggregate stability. Soil aggregation is used widely as an indicator of soil structure (Six et al, 2000), where the percentage of macroaggregates formed in soil is increased by surface applied organic matter (Pagliai et al, 1981). Macroaggregates physically protect soil organic matter, which increase soil carbon stabilization. The decomposition of macroaggregates within the soil can alter the rate of carbon storage, where WOM type and application rate have significant effects. Repeated applications of fresh WOM can increase macroaggregate retention in soil, where the younger C content binds the macroaggregate together (Puget et al, 2000). The impacts of organic matter application on macroaggregate formation was assessed by Andruschkewitsch et al, (2014). In this study it was indicated macroaggregate formation increased within the first 7 days of application, followed by a slow decline as SOM contained within the macroaggregates was decomposed by microorganisms. Under wetting and drying cycles, incorporation of wheat residues to field soil can enhance macroaggregate turnover compared with no organic matter additions (Denef et al, 2001). The release of humic acids during WOM decomposition can also increase microaggregate stability, further increasing carbon sequestration and improving soil fertility (Piccolo and Mbagwu, 1990). Increased earthworm casting following WOM applications may contribute to the overall macroaggregate concentration, however the rate of macroaggregate contribution differs between species type and the fate of macroaggregates from casts alters dependent upon species type and environmental conditions (van Groenigen et al, 2019). Further information on earthworm utilisation of waste is discussed in Section 2.5.

Applying mixed applications of organic matter may increase SOC content. Recent findings by Zhao et al (2018) indicate that mixing maize with wheat residues increased the number of small and large macroaggregates in the upper soil layer (0-20 cm). Although applications of organic matter increase C inputs much consideration is required when assessing its ability to increase carbon sequestration. For example, even though anaerobic digestate application contributes significantly to SOM formation and increases N concentration for plant growth, the rate of carbon mineralization following application outweighs total storage (Coban et al, 2015). There are also differences between the type of crop residue on the impact of soil organic carbon sequestration. Crops that have slower decomposition rates such as maize can increase SOC, however more decomposable crop residues such as legumes have less influence (Chen et al, 2018). Future application management must consider organic matter impacts on aggregation effects to maximise C sequestration in arable soils.

Crop residue mulching provides positive impacts on soil structure properties, such as an increase in SOC content, increase in water conservation, protection from erosion, a reduction in bulk density by reducing compaction effects and enhancing soil aggregation (Wilhelm et al, 1986; 2007). By acting as a buffer between the aboveground and belowground soil, crop residues can also improve air and water quality by reducing soil erosion and diffuse source pollution, whilst absorbing agrichemicals and filtering run-off (Lindstrom, 1986; Mickelson et al, 2001). Under the assessment of cover crop residue applications on no-till soils, it was discovered that there may be a threshold of application where the maximum impacts of crop residues (8 Mg ha^{-1} yr^{-1}) on soil physical properties can be seen (Mulumba and Lal, 2008). In areas of low mulching resources, residues could be managed more effectively to maximise effects on soil structure. Crops that produce larger amounts of residues result in greater soil aggregation than those with lower biomass (Skidmore et al, 1986).

2.4.2.3. Water regulation

The effects of WOM applications on water regulation in agroecosystems is dependent upon many factors, including geographical location, soil physical properties and climate. In areas of drought, surface applications can insulate water from loss through evaporation. The maintenance of moisture at the soil surface could help crops absorb moisture, improving water use efficiency in more arid regions (Wang et al, 2017b). The amount of moisture retained in soil and the length of this retention is determined by soil type, WOM application and particle size (Hollis et al, 1977; Eden et al, 2017). For example, in a study comparing crop residue applications, water retention of rapeseed residue was higher than that under wheat straw, where the water content increased by 17.84% after infiltration (Xing et al, 2017). However, consideration must be taken to ensure that nutrients gained from organic matter decomposition are not lost through runoff during heavy rainfall events by incorporation of organic matter into soil.

The impact of incorporating organic matter into soil on the water regulation in agroecosystems is different between farming systems. Incorporating organic matter into soil can reduce nitrate losses, where in no-till soybean agroecosystems the reduction of TKN (total Kjeldahl nitrogen) was 50% in soils with incorporated poultry manure than surface applied (Ruiz Diaz et al, 2010). In this field experiment, increasing the concentration of surface applied N also increased the rate of run off. Although liquid swine manure saw benefits from reduced runoff of P and N, under other WOM types runoff increased. This was evident with farmyard manure slurry, where five different tillage applications were compared: no tillage, chisel ploughing, shallow disk injection, pressure injection and aeration with banded application (Johnson et al, 2011). P losses from ploughing were higher than no-tillage, however there was variation between the two years of application attributed to changes in soil moisture from a dry and wet season. Long-term experiments investigating WOM applications under different tillage systems is required to determine impacts on water regulation.

However, this depends on the organic matter consistency. Analysis of organic wastes have indicated that liquid fractions cause a reduction in soil hydraulic properties and the rate of aggregate formation (Tlustoš et al, 2017). However, manure addition can increase water holding capacity by 14.2% in arid regions under water stress (Tahir et al, 2012). Added to organic matter type, the application rate also effects soil porosity, where there is a threshold of mulch application on increased available water capacity (8 Mg ha^{-1}) (Mulumba and Lal, 2008). Not only does surface applied WOM increase the amount of water stable aggregates in soil, it also insulates soil so during warmer periods there is a reduction in water loss via evapotranspiration (Aguilera et al, 2013). Therefore, although there are differences between organic matter type and age on soil water retention, in general organic matter addition improves and regulates soil moisture content.

Increased soil fauna populations following organic matter addition may influence the rate of water infiltration into soil through increased activity enhancing soil aeration (Lavelle and Spain, 2001;

Capowiez et al, 2009; Botinelli et al, 2010b; Andriuzzi et al, 2015a). Interactions between earthworms and organic matter is discussed in Section 2.5.

2.4.2.4. Nutrient cycling

Cultivation of arable land places stress on soil by removing nutrients and increasing soil erosion. For example, a rice-wheat rotation that yields 7 tons ha^{-1} rice and 4 tons ha^{-1} wheat can remove up to 300 kg N, 30 kg P and 300 kg K ha^{-1} from the soil (Akhtar et al, 2015). Reapplication of organic matter can replenish the nutrients lost during a growing season. There are a number of different properties of waste organic matter (WOM) which impacts the decomposition and rate of nutrient mineralization, which needs to be carefully managed to ensure crop nutrient requirements are met. This section will explore the impacts of organic wastes on nutrient availability and the agroecosystem management challenges faced.

The differences in nutrient composition of WOM influences the application selected by farmers, based on the nutrient requirements of the crop to be cultivated. With the associated mineral differences between WOM applications, some applications may be more effective than others. This was highlighted in a study by Grüter et al, (2017), where the applications of farmyard manure (FYM), green manure and mineral fertilizer were analysed for their impact on zinc and cadmium availability in wheat crops and soil. Findings indicated that FYM provided significantly more available zinc than green manure, which had no effect. WOM can increase the biofortification of zinc, which is a significant nutrient for human health (Zhou et al, 2012; McGrath et al, 2012). WOM can also increase some nutrients and limit the availability of others. For example, the application of composted FYM to wheat crops over a two year period increased grain P, Fe and Zn, but reduced N, K, Cu and Mn (Wang et al, 2014). Adequate soil assessment before application is crucial to ensure crop requirements are met. This thesis will explore the effects of WOM applications on soil nutrient properties.

Alongside adding nutrients to the soil, the physical structure changes caused by organic amendment application can have unique effects on N availability in soils. For example, decomposition of organic matter causes a reduction in viscosity, resulting in improved infiltration characteristics. This could enhance the short-term N availability to plants (Möller and Stinner, 2009). SOM decomposition can impact metal bioavailability, where organic matter solubility is positively correlated with metal bioavailability (Hernandez-Soriano and Jimenez-Lopez, 2012). However, dependent upon the physical structure of organic matter, applications can increase N leaching. Under the comparison of anaerobically digested (AD) manure with fresh slurry application, there were no significant differences between treatments on N leaching, where leaching increased with increased concentrations of N application (Svoboda et al, 2013). This was conducted using maize monocultures, cultivating different crops could also impact N leaching, alongside other factors such as soil type and climate. Comparisons between the impacts of green manure (manure from the cultivation of cover crops) and crop residue application on N fixation was further explored by Råberg et al, (2018), who indicated that applications of digestate and silage caused a positive N balance (9.9 – 24 kg N ha^{-1}) compared with fresh crop residue application. It was highlighted that temporal application was important to reduce N losses in winter, where N is not utilised by a crop. Silage and AD may assist with this. Further research of organic waste applications on N leaching is required to indicate the impact of waste type on N leaching. Overall, this indicates that with consideration of decomposition characteristics and soil nutrient requirements, organic matter can become a nutrient source for soils, and inhibit leaching pathways.

Cereal residue applications biodegrade at a slower rate than other WOM treatments, where cereal crops residues such as wheat and corn (high C:N ratio) are decomposed at a slower rate than other crops such as legumes, and manures (lower C:N ratio) (McDaniel et al, 2014). It is widely indicated that C:N ratio correlates with N mineralization in soils (Jensen, 1994; Janssen, 1996). MBC (microbial biomass carbon) is considered as an indicator for changes in SOM (Powlson et al, 1987). Although cereal residues do not alter the physiochemical properties of soil in the short-term, they still have a significant impact on the availability of nutrient pools and MBC, which enhances the rate of N mineralization (Singh, 1995). Applications of WOM mixtures may regulate nutrient release within soil, where it has been suggested that a mixture of crop residues with 17% chicken manure improves plant available N by increasing the MBC (Zhao et al, 2016). The effect of WOM mixtures on earthworm population growth and diversity will be explored within this research.

Long-term monitoring of the effects of farmyard manure (FYM) application on the soil nutrient status has indicated that there are overriding environmental conditions which limit the impact of FYM on nutrient conditions. For example, Tlustoš et al, (2017) analysed the soil nutrient concentration of wheat monocultures with applied FYM over four different geographical locations in the Czech Republic for a period of 60 years. Findings indicated that differences in soil type and climatic conditions between the sites had a larger impact on soil nutrient concentrations than FYM application. Although FYM can mitigate nutrient losses, over the long term there were no significant changes or remediation effects. This experiment was conducted under wheat monocultures and with only one type of organic waste; more diverse cropping systems may see different effects.

The positive impacts of utilising anaerobic digestate as an effective nutrient addition to arable soils is inconclusive. Many suggest that it is a useful soil conditioner which increases the amount of N mineralization in soil, improving crop growth (Tambone et al, 2010). Anaerobic digestate can recover essential macronutrients from a variety of different organic wastes, which reduces agricultural costs from replacing inorganic fertilisers (Alburquerque et al, 2012; Nkoa, 2013). N recovery from green manure digestate has been found to be 9% higher than that of mulched waste (Frøseth et al, 2014). However, there are also negative reviews of digestate. For example, anaerobic digestate from wastewater treatment plants can be a source of concentrated heavy metals (Fu and Wang, 2011). Analysis of the liquid fraction of anaerobic digestate indicated significant variation in nutrient concentrations (Coelho et al, 2018), making it difficult for farmers to make application management decisions based on crop nutrient requirements. Under the comparison of digestate application with digester inputs (fresh organic waste) it was emphasized that there were no significant differences between the applications of digestate and digester inputs on available nutrients (Bachmann et al, 2014). However, there were significant increases in soil enzymatic activity under anaerobic digestate; the activation of microbial organisms could speed up the mineralisation over time.

Alongside organic matter application type, soil management has significant impacts on soil chemical properties. Long-term field experiments have suggested that increased crop rotation and incorporation of organic matter can increase nutrient turnover in soils (Sainju et al, 2015). However, incorporating residual biomass may result in N losses if mineralisation and crop acquisition of N is not synchronised (Pang and Letey, 1998; Möller et al, 2008). In areas with periods of heavy rainfall, surface applications of organic manure can lose nutrients through surface run off. This is particularly experienced in applications of manure fractions, where surface application of liquid swine manure can increase the run-off rate of Phosphorus (P) compared with incorporation (Allen and Mallarino, 2008). The positive effect of incorporation on P run-off was reduced over time, where P concentrations in soil were similar. Considerations should be applied to the geographic location of the agroecosystem and the temporal impacts of organic matter management on nitrogen fixation (Råberg et al, 2018).

2.4.2.5. Primary production

Arable production has significant impacts on Net Primary Production (NPP). NPP is quantified and used in ecological research to assess the impact on ecosystem services (Costanza et al, 2007). Croplands cover 37% of global land area (FAOSTAT, 2016) and produce approximately 14% of potential NPP (Medková et al, 2017). Differences within WOM applications can have significant impacts on NPP, where wastes such as composted green waste (CGW) and sewage sludge can reduce the ratio between soil respiration rates and plant production, compared with other wastes such as crop residues (Ryals et al, 2015). A study by Ryals and Silver (2013) investigated the impact of CGW applications on soil respiration, plant production and carbon concentration to determine impacts on NPP. Increased soil respiration results in enhances CO_2 and N_2O emissions, contributing to global warming and C losses from soil. CGW increased soil respiration by 18 ± 4%, however this was offset by an increase in plant production and soil carbon concentration enhancing net ecosystem C storage by 25-70%. Overall, it was concluded that CGW had a positive impact on NPP.

The ability of a terrestrial ecosystem to sequester C (increase NPP) has been linked to fungal:bacterial ratios, which influence organic matter decomposition rates (Strickland and Rousk, 2010). Animal-based organic matter applications generally increase the bacterial community over fungal species. This was highlighted by Walsh et al, (2012b) where the liquid fraction of anaerobic digestate and undigested slurry applications increased bacterial growth at a higher rate than mineral fertiliser applications. This could be caused by an increase in alkalinity, experienced in pastoral inputs compared to plant-based residues, which reduces the competitive ability of the fungal community (Rousk et al, 2009). This experiment showed positive correlation between increased bacterial community positively and plant productivity.

Waste organic matter (WOM) type and application amount could have significant impacts on fungal and bacterial communities, which may influence NPP. WOM application can cause rapid growth of soil microorganisms, however it can also suppress mycorrhiza species and an alteration of community structure could impact nutrient uptake by plant roots (Harrison, 1999; Eggerton-Warbuton et al, 2007). For example, applications of biosolids to wheat-fallow cropping systems changed the abundance of fungal communities in the rhizosphere (Schaltter et al, 2017). Excessive organic matter application can lead to imbalanced microbial function and nitrogen immobilization (Alburquerque et al, 2012; Gutser et al, 2005), negatively impacting yields. This highlights that although there are clear links between WOM application and increased crop growth, to maintain NPP microbial and fungal ratios need to be assessed.

Increase in plant NPP in agroecosystems is vital to secure future food security and support provisioning ecosystem service demands. Applications of WOM may enhance NPP sustainably without negatively influencing regulating services and damaging the environment. This thesis will investigate the effects of earthworm populations on the decomposition of WOM, which may enhance the rate of NPP by increasing plant production (Blouin et al, 2013). Interactions between WOM and earthworm populations are discussed in Section 2.5.

2.5. EARTHWORMS AND ORGANIC WASTE APPLICATIONS IN SUSTAINABLE AGROECOSYSTEMS.

Earthworm populations have been used as a bioindicator of soil health in agricultural systems, where they increase the rate of organic matter decomposition through enhanced microbial activity within their gut and by increased bioturbation of surface applied organic matter into soil, which is further decomposed by the activity of soil fauna and microbial activity within the upper soil layers (Pérès et al, 2011; Aira et al, 2008). This could enhance the nutrient mineralization of organic matter and soil fertility (Blouin et al, 2013). Earthworm populations may be of significant benefit to no-till agroecosystems, where their burrowing behaviour increases soil aeration and aggregate stability, which could mitigate the positive effects of ploughing on crop yields (Bertrand et al, 2015a). The fertility of earthworm casts may enhance nutrient availability of residues within the soil, where enhanced N mineralization could increase crop yields (van Groenigen et al, 2019). However, earthworm species have different responses to waste organic matter (WOM) applications, where community dynamics could have a significant effect on the utilisation of waste organic matter and the resulting improvement of soil properties. Section 2.4 defined WOM application in agroecosystems and how the type of waste, application method, crop nutrient requirements and soil type effect the impact on soil properties. All these variables may influence earthworm behaviour and subsequent organic matter selection. This section explores further organic matter selection, earthworm species behaviour under organic matter applications, and utilisation in no-till agricultural settings.

2.5.1. Organic matter selection by earthworm species

Earthworms feed on organic material in many stages of decay (Lee, 1985), alongside living microorganisms, nematodes and mineral soil. Earthworms feeding behaviour is species-specific and dependent upon whether they are detritivores (surface feeders of organic matter on the soil surface) or geophages (feed deeper in the soil horizon on nutrients from soil organic matter)**.** More specifically, these groups are split into three ecotypes; anecic, epigeic and endogeic (Bouché, 1977) based on their burrowing behaviour, where anecic species make permanent horizontal burrows, endogeics make semi-permanent horizontal burrows in the upper soil layers, and epigeics do not burrow and live in leaf litter. Alongside ecotype, earthworm age also influences consumption rates, for example endogeic juveniles have a higher rate of soil consumption than adults due to a higher energy requirement (Scheu, 1987; Curry, 1997; Curry and Schmidt, 2007). Choice chamber experiments have demonstrated that earthworm species preferentially select different organic matter types (Satchell, 1967; Doube et al, 1997; Butt et al, 2020; Rajapaksha et al, 2013; Ashwood et al, 2017b; Euteneuer et al, 2020). Studies e.g. Satchell (1967) identified that earthworms have a preference for plant material with a high soluble carbohydrate content. Organic matter preference could influence earthworm population dynamics and dispersal. Organic matter preference can depend factors such as the quality of organic matter, the amount applied, the size of organic matter, where it is applied upon a soil profile, its C:N ratio, the cellulose/lignin content and soil type. This section will discuss the effects of WOM applications on earthworm populations and how this influences selection and decomposition.

Earthworm preference and avoidance tests are important experiments to understand whether species will utilise WOM as a food resource, which could be used to predict how earthworm populations will respond to applications in the field. There have been many studies of earthworm preference of organic wastes such as slurry, boardmill sludge waste and anaerobic digestate (Butt et al, 2005; Li et al, 2016; Rolette et al, 2020). The responses of earthworms to WOM is different between species, where it has been acknowledged that *L. terrestris* prefer plant material such as leaf litter and barley straw to boardmill sludge waste (Piearce and Boone, 1998; Butt et al, 2005), however endogeics such as *A. caliginosa* shown no preference. The different responses by earthworm ecological groups to WOM applications, such as boardmill sludge, could have significant effects on soil properties within agroecosystems and the decomposition rate of the WOM. WOM applications that do not support anecic populations may reduce the rate of decomposition, where *L. terrestris* has the highest litter consumption, ranging from 6-27 mg DM g^{-1} FM d^{-1} (Curry and Schmidt, 2007). Preference testing will be used in this thesis to determine the effects of organic matter particle size on anecic and endogeic earthworms.

Earthworm responses to organic matter applications differs depending on the application method. Anecic and epigeic species feed on organic matter within the soil surface (Bouché, 1977; Lee, 1985) and have higher growth rates under surface applied organic amendments (Lowe and Butt, 2002). However, endogeic earthworms, such as *A. caliginosa*, feed on decomposed WOM held within the upper soil layers. Therefore, the placement of WOM in the soil (surface applied, mixed or injected) could significantly alter earthworm species richness within soil and the associated behavioural effects (such as burrowing behaviour) on soil functioning (Frazão et al, 2019). Earthworm burrowing behaviour can increase the transfer of water, air and solutes down a soil profile (Lavelle and Spain, 2001; Jarvis, 2007), where the vertical burrowing behaviour of anecic species such as *L. terrestris* can increase surface infiltration (Shipitalo and Le Bayon, 2004; Zaller et al, 2011) and the extensive burrowing behaviour of endogeic earthworms is considered to have a larger impact on water transfer (Ernst et al, 2009). Earthworm density can have a significant impact on transfer properties in soils, where mean geodesic distance between burrows and the spatial arrangement of the burrows change with population size (Capowiez et al, 2014a). An alteration of species richness and density following a

WOM application could influence soil hydraulic activity and nutrient transport through the soil system (Andriuzzi et al, 2015a), which has potential implications for crop growth.

Alongside the placement of organic matter within a soil profile, the impact of organic matter particle size on earthworm growth rates and fecundity has had a noted effect, where lower particle size increases growth rates and the incorporation of surface applied organic matter into soil (Boström and Löfs-Holmin, 1986, Lowe and Butt, 2002, Sizmur et al, 2017). Applications of a uniform milled particle size (benefitting all species) implied that milled wheat straw increased *L. terrestris* growth at a faster rate than horse manure (Sizmur et al, 2017), a known effective earthworm feed (Butt, 2011). This highlights that a homogenous application of milled particle size may reduce the effects of WOM C:N ratio on earthworm populations. In the field, surface applied crop residues are unlikely to be surface applied milled due to dust implications and the energy required to mill large quantities of material. Incorporating milled straw into soil would require ploughing, which would negatively impact earthworm populations (specifically anecic species) (Briones and Schmidt, 2017).

Numerous studies of earthworm feeding preferences have highlighted that organic matter with a high C:N ratio is a low quality feed for earthworm species (e.g. Ernst et al, 2009; Frouz et al, 2014), where a high C:N ratio organic application can cause N immobilization, which reduces food availability for earthworms (Aitken et al, 1998). For example, Ernst et al (2009) studied the interactions of anecic *L. terrestris* with endogeics *A. caliginosa* and *O. tyrtaeum* under energy crop miscanthus and maize residues. They found that the higher N content of maize (C:N ratio 34.8) decomposed and was selected earlier than miscanthus, which has a significantly lower N content (C:N ratio 134.4). However, the impact of C:N ratio on earthworm organic matter selection requires further study, because some results have indicated no impact of C:N ratio on selection, where palatability from organic decomposition has had a larger effect (Valckx et al, 2011). The mixture of high C:N ratio crop residues and low C:N ratio WOM may regulate earthworm populations and soil properties in no-till agroecosystems (Delgado et al, 2012). The degradation of WOM high in C:N ratio may also increase palatability for earthworms. A study by Abhail and Whalen (2018) indicated that after 11 months, corn residue (which has a high C:N ratio) was palatable and sustained earthworm populations over a year. The degradation of organic residues with high C:N ratios could be an important addition for population survival over longer periods of time. This research project will investigate the impact of WOM type with different C:N ratio and residues at different stages of decay on the growth and reproduction of earthworms. This may provide information on best application practice to increase the rate of earthworm activity in the soil.

Although many studies have emphasized the negative impact of tillage on earthworm survival (Kennedy et al, 2013; Kuntz et al, 2013; Pelosi et al, 2016; Briones and Schmidt, 2017; Giannitsopoulos et al, 2019), endogeic species have been unaffected or even benefitted from tillage management (Crittenden et al, 2015). Endogeics predominantly feed on degraded SOM in mineral soils (Edwards and Bohlen, 1996). The introduction of WOM into soil through ploughing increases SOM content, which improves nutrient uptake for crops and maintains aggregate stability. Increased SOM content is hypothesized to benefit endogeics, because it increases food availability (Capowiez et al, 2009; Ernst and Emmerling, 2009). However, other factors may also benefit endogeic species in conventional tillage management. They are smaller in size than anecic species, therefore less likely to be injured during mechanical disturbance, and have a faster reproductive rate so can regenerate populations more rapidly (Edwards and Bohlen, 1996). The burrowing and casting behaviour of endogeic populations may have significant impacts on soil functioning, where the lifespan of casts is predicted to be higher amongst belowground endogeic communities and increasing the rate of soil porosity (Bottinelli et al, 2010).

Tillage may provide some growth opportunities for endogeics in agroecosystems however the quality of organic matter additions and soil can still limit their survival (Baldivieso-Freitas et al, 2018). Organic matter applications high in nutrient concentration influence the rate of endogeic growth at a higher rate under low quality soils, where the effect of leaf litter Ca concentration increased *A. caliginosa* growth and Mg concentration increased *O. tyrtaeum* growth (Cesarz et al, 2016). As endogeic populations feed predominantly on soil, effects of organic matter in high quality soils had less of an effect on endogeic growth. Within poor quality soil, the effect of organic matter type, tree canopy, and increasing time influenced the growth and development of *A. caliginosa* populations (Roubíčková and Frouz, 2014). The change in soil properties that occur when transitioning from conventional to sustainable agricultural systems may negatively impact endogeics, however addition of WOM may retain populations and over time increase population development. Surface applications under no-till systems may limit *A. caliginosa* access to WOM, however in high quality agricultural soils this might not influence populations.

Decomposition of organic matter by soil fauna is dependent upon the relationship between earthworms, decomposer fungi, fungal feeding fauna (nematodes and microarthropods) and decomposer bacteria and bacteria feeding fauna (protozoa and bacterial feeding nematodes). The trophic relationships between the decomposer communities influences organic matter decomposition (Beare et al, 1992; Aira et al, 2008; Gómez-Brandón et al 2011; Gómez-Brandón et al, 2012), where low moisture and low pH benefits fungi and water rich environments with anaerobic conditions benefit bacteria. The interaction between epigeic *Eisenia fetida* with soil arthropods on the decomposition of pig manure was investigated by Monroy et al (2011). It was suggested that regardless of the amount of pig manure present (1.5 or 3 kg), there was a significant increase in soil arthropod abundance under *E. fetida* populations. This indicates how, in the early stages of decomposition, the presence of earthworms increases the arthropod population, which enhances the decomposition rate. Agroecosystems are normally low in epigeic populations due to a lack of an organic layer; introduction of WOM may increase epigeic populations which could further accelerate the decomposition process. Interactions between endogeics and anecics with microarthropods may indicate further how decomposition works in agroecosystems.

2.5.2. Interactions between earthworm populations and organic matter

Earthworm species have complex interactions. There are a number of environmental conditions which impact earthworm interactions, regardless of the species or ecotype. For instance, food availability (Butt et al, 2003; Grigoropoulou and Butt, 2010; Bacher et al, 2018), soil moisture content (Eriksen-Hamel and Whalen, 2006), temperature (Berry and Jordan, 2001; Uvarov et al, 2011) earthworm density (Eriksen-Hamel and Whalen, 2007a; McTavish et al, 2013) and climate (Eriksen-Hamel and Whalen, 2007b) are all factors which influence earthworm behaviour. Utilising sustainable intensification practices, such as no-tillage and waste organic matter (WOM) addition, may result in different responses by earthworm populations based on the species-specific benefits provided by the new management system. For example, findings from a field study by Irmler (2010) indicated that under conversion from conventional to organic arable farming systems, the ratio of *A. caliginosa* to *L. terrestris* species changed from 140:1 to 10:1 as organic farming management increased *L. terrestris* populations. This research also indicated that the effect of rainfall had a higher impact on *A. rosea* and *A. caliginosa* than farming management. As earthworm species have different impacts on soil processes, it is important that the impact of environmental change and farming management on earthworm biodiversity is monitored to understand any impact on ecosystem services.

Earthworm interactions can be intraspecific or interspecific. In no-till or reduced till agroecosystems, interactions of anecic species are of wider interest, due to their ability to incorporate organic matter into their burrows, replicating a form of ploughing. Inter- and intraspecific relationships between earthworms can be mutualistic or antagonistic. A common research hypothesis indicates that earthworm species interactions may possibly facilitate one another (Lowe and Butt, 2002; Simberloff, 2006; Uvarov, 2009, 2019). Facilitation is the encounter of organisms that benefits at least one of the organisms and does not harm neither (Bruno et al, 2005). The action of anecic earthworms may facilitate endogeic populations through the degradation and removal of waste organic matter down a soil profile, increasing food availability (Uvarov, 2009). However, the effects of facilitative and competitive interactions between earthworm species is still uncertain, where some state positive facilitation (Lowe and Butt, 1999, 2002, 2008) and others competition or neutral interactions (Cameron and Bayne, 2011; Eriksen-Hamel and Whalen, 2007a). A meta-analysis investigation into inter and intraspecific interactions of lumbricid earthworms was constructed by Uvarov (2009), where 60 interaction laboratory studies were compared. Findings emphasized unique positive effects of anecic species *L. terrestris* and *A. longa* on epi- and endogeic species growth and development, however *L. terrestris* and *A. longa* suffered in the presence of increased epi- and endogeic populations. However, this study indicated a specific focal point that most intra- and interspecific interactions were conducted in species pairs and experiments were often conducted in the short term. Recent investigations of interactions between three earthworm species of different ecotypes on organic matter decomposition were inconclusive (Frazão et al, 2019).

The theory of earthworm ecotype facilitation is supported by a number of studies. Inter- and intra-specific relationships have mainly been studied using microcosm laboratory analysis, where earthworm behaviour can be monitored under controlled conditions. The positive relationship between *L. terrestris* and *A. chlorotica* species was explored by Lowe and Butt (2002). In this experiment, *A. chlorotica* hatchlings matured at a faster rate under unmilled applications of separated cattle solids when paired with adult *L. terrestris* samples than under adult *A. chlorotica* conspecifics. However, although maturation and mean biomass of *A. chlorotica* increased, the biomass of adult *L. terrestris* decreased compared with monocultures. Interactions between *L. rubellus and O. lacteum* indicated that there were synergistic effects on the decomposition of tulip poplar (*Liriodendron tulipfera*) between the two species, where decomposition increased under mixed species cultures (Xia et al, 2011). Although tulip poplar is not a common WOM application in agroecosystems, this experiment highlights how facilitative actions of earthworms can impact WOM degradation, which may be useful in no-till settings. In this experiment, intra-specific competition of *L. rubellus* was highlighted under high population densities, possibly caused by reduced food availability. Facilitative effects between earthworms could be limited to life stage, where *L. terrestris* population's midden habitat can support the juveniles of other species (Lowe and Butt, 2007). The support of early life stage earthworms by *L. terrestris* may enhance populations in no-till agroecosystems (Stroud et al, 2016a). The positive effects of an *L. terrestris* midden-complex is discussed in 2.5.4.

In an analysis of all three ecotypes, Frazão et. al (2019) confirmed that there were no facilitation effects between *L. terrestris* and *A. caliginosa*, two species which are commonly found in UK agroecosystems. This was also determined in experiments by Eriksen-Hamel and Whalen, (2007a), where presence of *L. terrestris* inhibited the growth rate of *A. caliginosa* with increasing earthworm density. Both experiments were conducted over a short time period (Eriksen-Hamel and Whalen = 28 days; Frazão et. al = 61 days); due to the life cycles of these earthworm species being longer than this (Edwards, 2004), it is possible that facilitation effects may occur at later life stages. Recent research by Vidal et al (2019) analysed cast formation of *L. terrestris* under ryegrass applications and indicated that organic material in the casts changed over time. Following ryegrass applications, the organic carbon contained within *L. terrestris* casts consisted predominantly of decomposed ryegrass, whereas after 1 year, 85% of organic carbon was found to be mineral organic carbon. Mineral organic carbon may be a more useful feeding resource for endogeic populations, which predominantly feed on large quantities of mineral soil (Curry and Schmidt, 2007; Blouin et al, 2013). The facilitation of endogeics by anecic casting over time requires more investigation under different WOM types. Further information on the consistency and mineral content of anecic casts may provide unique information on the development of earthworm populations over time in sustainable agroecosystems, where organic matter is applied yearly or in between crop rotations. When considering multi-species experiments, the quality and amount of organic matter applied should be considered. The crops analysed by Frazão et. al (2019) had a high C:N ratio, which may be difficult to feed upon or not nutritious enough to sustain one species over the length of the experimental period.

There have been numerous inter-specific earthworm laboratory investigations that suggest negative interactions between earthworm species of different ecotypes. Under surface application of organic matter, *L. rubellus* was shown to inhibit growth of *L. terrestris* (Lowe and Butt, 2002). Mesocosm analysis of *L. rubellus* (epigeic) and *A. caliginosa* (endogeic) interactions under the surface application of maize resulted in 100% *L. rubellus* mortality over a period of 750 days and no cocoon production (Lubbers et al, 2017), whereas *A. caliginosa* had a lower mortality and produced cocoons. A mesocosm experiment by Andriuzzi et al, (2016) indicated that interactions between *L. terrestris*, *A. longa* and *A. chlorotica* found more species-level effects on aboveground and belowground plant properties than interspecific effects, where *A. longa* and *A. chlorotica* increased shoot biomass under cow manure applications and *L. terrestris* increased root biomass. Earthworms all had similar responses to organic matter treatments, but differing effects on plant properties. Therefore, the interactions between earthworm species may be mediated by plant and soil type. The inconsistency in earthworm interactions by ecotype indicates that more research into interactions under controlled conditions is required. Also, it highlights that earthworm functional traits should be analysed at a species level and not just as an ecotype, as recent research suggest some earthworm species demonstrate traits from more than one ecological group (Hoeffner et al, 2018; Hoeffner et al, 2019; Section 2.2), which has led to the introduction of sub-groups such as epi-anecic.

In no-till agroecosystems, the interactions between anecic and endogeic populations may determine the transport and decomposition of organic matter within the soil profile. Inter and intra-specific interactions between earthworm species can influence their dispersal strategy, which impacts soil engineering processes (Caro et al, 2014; Chatelein and Mathieu, 2017). The influence of earthworms on soil structure is still misunderstood and collaboration between soil physicists and soil ecologists is required to address the challenges faced (Bottinelli et al, 2015). Further research on earthworm interactions and their burrowing behaviour will indicate how organic matter is decomposed and transported through the soil profile and how this changes soil structure such as bulk density and pore structure.

2.5.3. Types of organic waste and applications in sustainable agroecosystems, with examples of earthworm utilisation.

As discussed in Section 2.4, waste organic matter application management depends on a number of factors, such as crop type, soil type, nutrient requirements, temporal and physical scales, pest control requirements and climate. All of these environmental and anthropogenic factors influence earthworm behaviour and population dynamics, which influence supporting services in terrestrial ecosystems. Analysis of earthworm population size and biodiversity in agricultural management systems across Europe indicated that there is increased variance within countries than between countries, indicating that land management may have an impact on abundance (Dinter et al, 2013). In arable systems, land management on a field scale has a larger impact on lumbricid communities than the surrounding landscape and soil properties (Frazão et al, 2017). Therefore, the impact of organic matter applications in arable systems may be of increasing importance in supporting earthworm communities in no-till arable ecosystems. This section discusses different sustainable land management systems within agroecosystems and how earthworm populations within these systems influence supporting ecosystem services.

The role of earthworms in the decomposition of organic matter in agricultural soils differs between land management type, as soil cultivation, fertilization and crop rotation influence earthworm species abundance, biodiversity and behaviour. Previous research by Curry and Byrne (1992) highlighted the impact of earthworms on decomposition of straw, where the presence of earthworms increased decomposition rates by 26-47% over an 8-10 month period. It was calculated that 3.2 g N was mineralized annually through excretion and tissue turnover and 3.3 g N through enhanced mineralization in faeces. This study was designed to investigate earthworm activity under crop residue application in a conventional system (mouldboard ploughing), with low anecic population density, therefore incorporation rate may be increased by no-till or reduced till management (Pelosi et al, 2014). This research was followed by a further field study analysing incorporation of wheat straw by earthworms in conventional farming systems (Curry and Byrne, 1997), which found that although earthworm species increased the rate of decomposition of straw over a 3-year period, the effect of earthworms on the rate of straw decomposition decreased after the first year. This could have been due to mouldboard ploughing decreasing the anecic populations in the soil, which feed on fresh organic matter, compared to the high concentrations of endogeic species, which feed on degraded soil organic matter.

The implementation of leys and planting cover crops generally improves soil fertility and reduces soil erosion. Earthworm populations benefit from the regulation in soil moisture and increased food availability in times where land would normally be fallow (Schmidt and Curry, 2001). The cover crops used in agroecosystems are chosen based on the soil functioning requirements and the temporal implications of the previous harvest (i.e. winter wheat or spring barley). Cover crop type can have significant impacts on earthworm population abundances and species diversity. Field investigations of cover crop effects on earthworm abundances indicated that pea legumes had the largest impact on earthworm abundance and biomass (221 ind. m^{-2}; 67.4 g m^{-2}) and brassicaceaes such as mustard had the lowest impact (89 ind. g m^{-2}; 23.1 g m^{-2}) (Roarty et al, 2017). Choice chamber and field mesocosm experiments confirmed a cover crop preference by earthworm species, where the C:N ratio and soil moisture have significant influences (Euteneuer et al, 2020). Potential negative impacts of cover cropping (reduction soil aggregate stability and increased soil compaction) can cause a reduction in endogeic populations (Wyss and Glasstetter, 1992). An introduction of anecic populations could address the negative impacts of compaction (Capowiez et al, 2009, 2012), where they have previously been inoculated into agroecosystems to improve soil conditions (Butt et al, 1997; Frazão et al, 2019). However, implementation of cover crops into a land management system often incurs some shallow cultivation to replenish the SOM (green manure), which may negatively impact anecic populations. Further investigation into the effects of cover crop management on earthworm populations is required.

Introducing ley periods into agricultural management systems has increased earthworm abundances and soil properties. Scullion et al, (2002) investigated the impact of ley periods on earthworm populations under conventional and organic crop rotations. They found that leys increased the biomass of earthworm populations regardless of farming management treatment. This could be due to a reduction in ploughing and clover being a food resource for earthworms during fallow management. The impact of leys had a larger impact on earthworm biomass in conventional systems rather than organic arable systems, however there was a larger difference in biomass in organic cereal farms, where nutrient release from crop residues is slower than other systems. The positive impact of organic amendments with leys on soil structure, organic matter and earthworm activity in 6 different farming management systems was investigated by Riley et al, (2008) over a period of 10 years. The impact of organic matter addition and leys into cropping systems with ploughing significantly increased soil porosity and aggregate stability, reduced over-compaction and increased earthworm density (from 33 g m^{-2} in organic farming no ley or slurry addition to 108 g m^{-2} in organic farming with ley and slurry addition). This indicates that even with ploughing management, the influence of organic matter addition can maintain earthworm populations by increasing food resource availability. By implementing a ley period with a cover crop, earthworm populations can recover from collapse caused by mechanical disruption of soils.

Applications of WOM alongside cover cropping could further enhance positive earthworm effects on soil properties within no-till agroecosystems. An American agricultural research study analysing the effects of bio-covers and crop rotations in no-till agroecosystems indicated that the nutrient quality of bio-covers had a larger impact on the earthworm species *D. caroliniana* and *L. rubellus*, where poultry litter applications increased earthworm species by 2.2 times (Ashworth et al, 2017). This was also reflected in a no-till corn-soybean crop rotation where high nutrient quality residue (crop) was applied and compared with low quality residue (silage) where there were 2.3 times more earthworms in the high quality residue treatment (Abail and Whalen, 2018). The impact of cover crops and organic matter addition in these no-till systems was investigated by Korucu et al, (2018) to investigate the impacts on nitrate loss and soil properties following extreme rainfall events in between cropping seasons. Findings indicated that cover-cropping reduced nutrient loss, where cover cropping mixed with farmyard manure applications increased earthworm numbers. This increase in earthworm abundance improved infiltration and soil porosity through improved aggregate stability. Further investigation of cover cropping and organic matter additions effects on earthworm populations in UK agroecosystems following extreme rainfall events could determine whether sustainable applications could mitigate the loss of earthworm populations in frequently flooded arable areas (Kiss et al, 2021).

The ability of earthworms to improve soil properties and increase SOM within agroecosystems depends on the soil type, the organic matter application and the tillage management (Laossi et al, 2010; Blouin et al, 2013; Onrust and Piersma, 2019). In reduced tillage systems, the effect of earthworm decomposition of organic matter on improving the soil conditions for growing was higher under low quality soils (Bertrand et al, 2015a). In conventional tillage systems, the effect of earthworm populations on soil properties increased with increasing mass of organic matter application (Frazão et al, 2019), where applications of mixtures are more effective (Pelosi et al, 2014). This is possibly because mixing organic matter with soil is a better culture for endogeics (Lowe and Butt, 2005), which survive tillage management and fertilisation (Crittenden et al, 2014). The consistency of earthworm casts is significantly different between agroecosystems, which influences SOM storage within soil macroaggregates. Pulleman et al, (2005) determined that a higher amount of particulate organic matter (POM) consisted in earthworm casts under pastoral fields than organic and conventional fields. The total organic carbon within earthworm casts in pastoral and organic fields were higher than conventional. To utilise earthworms in the regulation or improvement of agroecosystem soils, the functioning of earthworms within the management systems must be assessed before decision making.

The assumption that converting from conventional tillage such as mouldboard ploughing to no-tillage will increase earthworm diversity is not always true. A field analysis of earthworms over an 8 year period under tillage and no tillage arable farming indicated that although there were general positive effects of no-till on taxonomic and functional indices, these were only in the short term and did not increase at experimental end (Pelosi et al, 2016). Longer-term effects (30 years) of no-till farming on cereal farming has highlighted that there are significantly higher number of *L. terrestris* populations and increased macro-aggregate density, possibly from casting (Singh et al, 2015). It has been calculated that converting from conventional ploughing to minimum tillage increases *L. terrestris* populations by 38%, which could have a significant impact on organic matter decomposition, soil aeration, runoff and compaction rates (Stroud et al, 2016c; Briones and Schmidt, 2017). Tillage may have a lesser effect on total earthworm abundance than the amount of organic matter applied, where a higher application of residues increased native earthworm abundances under no-tillage and conventional practices at a similar rate (Frazão et al, 2019).

Added to earthworm physical mediation of soil, there are significant implications of earthworm casting on waste organic matter decomposition and the assimilation of nutrients within agroecosystems. Earthworm casts produce granular aggregates high in soil porosity and moisture (Jongmans et al, 2001), which have high nutrient concentrations (van Groenigen et al, 2019) and microbial activity (Schrader and Seibel, 2001; Aira et al, 2009). Earthworm casts are macroaggregates combined of mainly the silt portion of soil and although initially very unstable in structure, casts can become more water-stable upon drying (Shipitalo and Protz, 1988). Earthworm species make different casts dependent on their feeding preference, size and soil type. Laboratory investigations of *A. caliginosa* casts have discovered that the water-soluble Mn, Zn and Fe increased under straw and clover additions (Bityutskii et. al, 2012). This resulted in a higher N biomass in cucumber plants over a 4 week period. In agroecosystems, casts are often disrupted by the raindrop impact, wet-dry cycles, cattle activity and disturbance by other soil organisms (Binet and Le Bayon, 1999; Decaëns, 2000; Blanchart et al, 2004), which are mainly aboveground impacts. Therefore, the lifespan of casts is predicted to be higher amongst belowground endogeic communities and soil porosity is regulated when the production and degradation of casts is balanced (Haynes et al, 2003; Bottinelli et al, 2010a). Further analysis into the impact of *A. caliginosa* cast age on aboveground (shoot biomass, N concentration) and belowground (root biomass, root length, ramification, soil N concentration) plant processes has been investigated by Agapit et al, (2018), where it was determined that plant roots forage N from casts. Nutrient rich casts could be key to crop growth in agroecosystems with no inorganic fertilization and could support other soil fauna in nutrient poor homogenous environments.

The type of organic waste applied in agroecosystems is now beginning to be considered in farm management and the implications for ecosystem services, where models (e.g. the Energy-Environment-Earthworm model) are being applied to extrapolate the impact of tillage, management and weather conditions on *L. terrestris*, to determine how no-tillage practices may impact their population proliferation (Johnston et al, 2017). Further development of this model and extending its reach to include other ecotypes may provide extensive information on the impact of tillage on earthworm species and soil properties.

2.5.4. Lumbricus terrestris populations in sustainable agroecosystems.

A significant response in the transition from conventional to sustainable agroecosystem practices is the increase in anecic populations, notably *L. terrestris* (Bertrand et al, 2015a; Stroud, 2020). This section will define the unique behavioural traits of *L. terrestris* populations and how an increased density of this species might influence the rate of waste organic matter decomposition and incorporation, earthworm species diversity and soil nutrient properties.

Lumbricus terrestris is the largest species of earthworm in the UK (Sims and Gerard, 1999), with a life cycle of between 4-8 years (Lakhani and Satchell, 1970). It is considered a keystone species by ecologists due to its unique species-specific feeding behaviours (Blouin et al, 2013), where it forms a permanent vertical burrow with a midden at the surface entrance of their burrows (Edwards and Bohlen, 1996). Middens are a collection of organic matter, casts and mineral soil and act as a food supply for *L. terrestris*, whilst also forming a microhabitat, regulating the temperature and air flow and providing protection from predators (Darwin, 1881). Middens are considered a hotspot for microbial activity, where increased concentrations of casting on the surface of organic matter can increase the rate of decomposition (Hamilton and Silman, 1989; Subler and Kirsch, 1999; Schrader and Seibel, 2001; Aira et al, 2009; Andriuzzi et al, 2015b; Stroud et al, 2016a). Field investigations into the interactions between other earthworm species and *L. terrestris* middens have discovered that juvenile earthworms and epigeic species are present at higher concentrations under and within middens than surrounding control soil (Butt and Lowe, 2007). The beneficial effects of *L. terrestris* middens on surrounding species could be due to increased food supply and shelter from predators (Maraun et al, 1999). A field study investigating the effect of *L. terrestris* midden behaviour in no-till systems found that earthworms combine surface wheat crops into midden structures and that middens enhance endogeic populations (Stroud et al, 2016a).

The action of *L. terrestris* populations enhance a number of soil physical and chemical properties. The presence of *L. terrestris* burrows within agroecosystems can significantly increase hydraulic activity, where there is an increased rate of water infiltration (Edwards and Bohlen, 1996). In no-till cropping systems, this may regulate soil moisture, enhance the drainage and improve nutrient transport to lower soil depths (Ehlers, 1975; Douglas et al, 1980; Edwards and Lofty, 1982; Edwards et al, 1990; Bicki and Guo, 1991; Carter et al, 1994; Willoughby et al, 1997; Armador et al, 2005; Andriuzzi et al, 2015a); all enhancing crop production (van Groenigen et al, 2014; Bertrand et al, 2015a). The effects of *L. terrestris* populations on infiltration rates in no-till agroecosystems was investigated by Willoboroughy and Kladiviko (2002), where the comparison of inoculated *L. terrestris* populations and controls (no additions) were compared in the field. There were significant differences between treatments where *L. terrestris* increased infiltration (*L. terrestris* = 0.80 mm min^{-1}; control = 0.71 mm min^{-1}), however the differences were low. The slow development of *L. terrestris* populations in no-till agroecosystems may inhibit their large-scale inoculation in agroecosystem settings by farmers. The development of populations could be improved by the inoculation of a higher density of *L. terrestris* and the introduction of waste organic matter (WOM) to stimulate initial growth (Lowe and Butt, 2002). An assessment of the earthworm ecology and soil conditions pre-inoculation could also determine whether *L. terrestris* will develop or disperse.

The constant regulation of the burrow wall creates a unique drilosphere, where casting on the burrow wall can enhance nutrient concentration (Schmidt et al, 1997; Jegou et al, 2000). Casting within the drilosphere can increase microbial nitrification, enhancing nitrogen transport to lower soil depths (Parkin and Berry, 1999; Schlatter et al, 2019). Improved soil aeration and moisture content within the burrow walls is suggested to be a good host for niche microbial communities and nutrient distribution (Devliegher and Verstraete, 1997). The distinct integration of fresh (< 8 years) plant derived C to depths of 15 cm can regulate distinct microbial and fungal populations compared with bulk soil (Stromberger et al, 2012). The elevated C and N concentrations caused by plant incorporation by *L. terrestris* can extend up to 4-8 mm around the burrow wall, where diameter could be larger closer to the surface below the midden complex (Andriuzzi et al, 2013). Even after death, *L. terrestis* burrows can be re used by other adults (Grigoropoulou and Butt, 2010; Nuutinen, 2011) and are utilised by plant roots (Cameron et al, 2014; Potvin et al, 2017). Therefore, an *L. terrestris* drilosphere can have long-term effects on the soil biodiversity and plant utilisation. However, the location of an *L. terrestris* drilosphere within a field can have different effects in on the soil bacterial diversity depending on the gradient (Schlatter et al, 2019). This can have significant effects on the nitrification of the soil, where downslope had higher bacterial diversity with a higher concentration of denitrifying bacteria.

Added to the drilosphere, the presence of a midden can significantly alter the chemical properties of soil beneath middens. Differences in ecosystem (forest or agricultural) and type of fertilisation (organic or inorganic) can influence the chemical properties of middens. Field investigations by Wilcox et al. (2002) recorded that the inorganic N concentration increased in soil below *L. terrestris* middens in no-till agroecosystems (organic fertilisation) and forests, yet in heavily fertilised agroecosystems, they decreased the inorganic N concentration (Wilcox et al, 2002). Analysis of the chemical composition of middens within no-till wheat agroecosystems indicated elevated concentrations of macronutrients P K and S (Stroud et al, 2016a). The effect of *L. terrestris* populations activity on the available micronutrients in forestry soil increased some elements (Ca, Mg, K) but decreased available P (Dobson et al, 2017). The effect of *L. terrestris* populations on the chemical composition and dispersal within soil may depend on the structure and chemical characteristics within its casts. *L. terrestris* casts on the surface of its midden and within its burrow wall. The positioning of *L. terrestris* casts nearer the burrow entrance and within the midden mean they are more prone to erosion following rainfall and extreme weather events (Binet and Le Bayon, 1998, 1999, 2001). They therefore have less of an effect on C and N storage than endogeics and an increased effect on nutrient dispersal.

L. terrestris is detritivorous and feeds on the soil surface, where it preferentially selects organic matter (Doube et al, 1997; Butt et al, 2005; Valckx et al, 2011; Rajapaksha et al, 2013; Ashwood et al, 2017b; Euteneuer et al, 2020) and can travel up to 0.7 m away from its burrow (Nuutinen and Butt, 2005). Recordings of the surface selection of barley straw indicated that under patchy distribution *L. terrestris* will reduce its activity around resource-poor areas, where it selected barley straw and mated more often around resource rich areas (Butt et al, 2003). This suggested how *L. terrestris* activity is triggered by lack of food resources, which may influence population development and dispersal in agroecosystems if organic matter is not applied homogenously. In forestry ecosystems, *L. terrestris* can modify its migration behaviour based on organic matter type, availability and conspecific population density (McTavish et al, 2013; Guy et al, 2018). In agroecosystems, the dispersal of *L. terrestris* populations following organic matter addition and changes to resource availability could influence nutrient mineralization (Menšík et al, 2018; Nuutinen and Butt, 2019). Therefore, waste organic matter type and the temporal scale of applications could have significant effects on the dispersal and availability of nutrients by *L. terrestris* in no-till agroecosystems.

The presence of *L. terrestris* populations in agroecosystems may reduce the spread of crop diseases. Laboratory and field investigations have determined that *L. terrestris* can feed on surface applied crop residues (Stroud et al, 2016a; Sizmur et al, 2017), where they have a preference of fungal types (Moody et al, 1995). The feeding and incorporation of straw residues into soil by *L. terrestris* populations has caused a reduction in the concentration of deoxynivalenol (DON) (a common mycotoxin developed in *Fusarium* species) (Wolfarth et al, 2016). The reduction of deoxynivalenol in fusarium infected soil has recently been calculated to be 20% higher under soil fauna activity, where the total mass reduction by earthworms was 24.3 mg kg^{-1} (Goncharov et al, 2020). *L. terrestris* can also significantly reduce the amount of *Fusarium culmorum*, a plant pathogen which can cause stalk and root rot in wheat cultivars (Meyer-Wolfarth et al, 2017), *Sclerotinia sclerotiorum*, a white mould that forms at the stems of crops such as oilseed rape and soybean (Euteneuer et al, 2019) and *Oculimacula yallundae*, a pathogenic fungus causing eyespot disease in wheat (Bertrand et al, 2015b). The utilisation of *L. terrestris* populations and manipulation of the detritivorous soil food web within cropping systems may supress fusarium and fungal based plant diseases (Bertrand et al, 2015a; Goncharov et al, 2020).

The positive effects of *L. terrestris* on soil properties have led to intensive production to ameliorate poor soil conditions (Butt et al, 1992). The Earthworm Inoculation Unit (EIA; Butt et al, 1997) is a technique used to increase *L. terrestris* population density within fields. Inoculation of *L. terrestris* may introduce populations to soil, however the development of populations once introduced can take a number of years. After a 13 year inoculation period, there were significant effects of tillage regime on *L. terrestris* population density, where 0.6 ind. m^{-2} was determined under conventional tillage and 4.3 ind. m^{-2} in no-tillage fields (Nuutinen et al, 2011). The positive effects of *L. terrestris* populations on soil porosity and increased erychtraid, nematode and earthworm density in the agroecosystem no-till was determined after 17 years (Nuutinen et al, 2017). Shorter term effects of *L. terrestris* inoculation within agroecosystems was investigated by Frazão et al (2019), where the effects of crop residue placement and tillage regime on earthworm populations was tested. The difference in *L. terrestris* population density between conventional tillage and no-tillage was lower than that of Nuutinen et al, (2011), however still significant. Findings highlighted the positive effects of surface residue applications on *L. terrestris* population density, however due to *L. terrestris* dispersal within the experimental field, it was not possible to determine density related effects on other earthworm populations. The time taken for *L. terrestris* populations to develop and the heterogeneous nature of their dispersal may disincline farmers from inoculation over a large scale.

There are some threats of enhanced *L. terrestris* populations on the performance of crops in agroecosystems. *L. terrestris* activity influences plant seedling recruitment, where the effect on plant development differs between plant seed size (Milcu et al, 2006). Large seed varieties are benefitted by *L. terrestris* burial whereas smaller species were repressed. Investigations into the herbivory of plants by *L. terrestris* observed foraging of legume leaves, but this behaviour was reduced under organic matter applications and was at a low rate of consumption, which did not influence overall plant growth (Kirchberger et al, 2015). *L. terrestris* activity can increase the emission of GHG through respiration and elevated N_2O emissions below their middens (Nieminen et al, 2015). Enhanced populations within no-till systems could increase the global warming potential (Evers et al, 2010; Lubbers et al, 2013). However, the emissions of earthworm populations could be outweighed by the increased carbon storage from their casting and incorporation of SOC (Bertrand et al, 2015a; Lubbers et al, 2015) and the overall emissions is significantly lower than the alternative tillage management methods (Soane et al, 2012). Residue incorporation depth in agroecosystems can significantly influence the rate of N_2O emissions (Paul et al, 2011), where surface applications increase emissions. *L. terrestris* activity may mitigate the effects of ploughing by incorporating organic matter, but also may increase N_2O release by emissions from their midden.

Determination of the total mass of organic matter removed by *L. terrestris* populations in no-till agroecosystems could indicate the rate of soil formation and nutrient addition to soil. The incorporation of organic matter by *L. terrestris* has been researched by Curry and Bolger (1983), who estimated that 100 g m^{-2} of *L. terrestris* biomass can incorporate 6-9 mg dry wt g^{-1} fresh wt day^{-1} and consume 1.34 kg soil m^{-2} yr^{-1}. However, the organic matter applied was *Salix aquatica cv. Gigantean*, which is not a common organic amendment in no-till systems. The effect of increased earthworm populations on the incorporation of organic matter in agroecosystems was investigated by Bohlen et al, (1997), where it was estimated that 840 kg ha^{-1} yr^{-1} of surface applied corn residues could be removed by increased earthworm population size. In this field experiment, the remaining 20% of surface litter was suggested to be *L. terrestris* burrows, therefore it was concluded that the rate of incorporation was mediated by *L. terrestris* density. The corn fields used within this experiment underwent spring tillage management, under no-tillage systems the mass incorporated could be higher. The incorporation of C and N from leaf litter with depth by *L. terrestris* and *L. rubellus* populations was investigated by Fahey et al, (2013), where increased litter removal was seen under *L. terrestris* populations, potentially due to their activity throughout the winter months. Over a period of two years the ^{15}C and ^{12}N concentration was significantly higher at lower depths around the *L. terrestris* burrow, highlighting how incorporation rate can correlate with enhanced nutrient concentration and carbon sequestration. This experiment was conducted in a forestry ecosystem with maple leaf litter; *L. terrestris* incorporation of organic matter in no-till agroecosystems could have different effects, dependant on the organic matter application and soil type.

3. GENERAL MATERIALS AND METHODS

3.1. Introduction

This thesis developed from a broad range of ecological interactions investigating the impacts of waste organic matter applications on earthworm species and soil properties to a narrower set of focussed conditions. The research direction was determined by reviewing pertinent literature and the availability of resources and study sites. This Chapter provides details of the methodological considerations taken for experiments within this thesis. It provides an outline of the earthworm species selected for laboratory experiments and indicates how they were collected and cultured. The waste organic matter types investigated during this research project and their treatment before application is defined in Section 3.4. Details of the chemical analyses conducted on soils is indicated in Section 3.5.

3.2. Earthworm species selection

Four earthworm species were selected for this research: *Lumbricus terrestris, Aporrectodea longa, Aporrectodea caliginosa* and *Allolobophora chlorotica.* Information on these species is provided in Table 3.1. Selection was based on abundance in UK agroecosystems (Sims and Gerard, 1999), ecological functioning and behaviour (Bouché, 1977), and the available literature on laboratory culture of these soil dwelling species (Lowe and Butt, 2005).

Table 3.1. An overview of the earthworm species used in laboratory experiments.

Earthworm Species	**Ecotype**	**General Information (adapted from Sims and Gerard, 1999)**	**References**
Allolobophora chlorotica (Savigny, 1826)	Endogeic (shallow-burrowing)	Geophagus; feeds on mineral soil; common in gardens, forests and pasture; co-dominant with *A. caliginosa*; pH range 4.5-8.2. Exists as two colour morphs: green and pink.	Butt, 1997; Lowe and Butt, 2008.
Aporrectodea caliginosa (Savigny, 1826)	Endogeic (shallow-burrowing)	Geophagus; feeds on mineral soil; common in agricultural land and grasslands; co-dominant with *A. chlorotica*; pH range 5.9-11.1.	Bart et al, 2018; Perrault and Whalen, 2006.
Aporrectodea longa (Ude, 1885)	Anecic (deep-burrowing	Feeds on surface organic matter; surface casting; common to cultivated soil and pasture; soil pH range 6.7-9.4.	Butt, 1993, 1998.
Lumbricus terrestris (Linnaeus, 1758)	Anecic (deep-burrowing)	Feeds on surface organic matter; surface casting; produces middens; common in grasslands and forests; pH range 6.2-10.	Butt et al, 1994; 2003; Butt and Nuutinen 2005; Nuutinen and Butt, 2003.

3.3. Earthworm sampling and laboratory techniques

3.3.1. Field sampling: earthworm assessment

Earthworm assessments were conducted at selected field sites to determine the effect of specific land management types on earthworm populations and to obtain an overview of the species richness within these environments. Field sampling of earthworms was used in Chapters 4 and 8 and followed methods described by Butt and Grigoropoulou (2010). For each sample point, a 0.1 m² quadrat was placed on the soil surface. The soil underneath the quadrat was removed with a spade to a depth of approximately 30 cm and hand-sorted for earthworms on a polyethylene sheet. Collected earthworms were contained in plastic bottles and preserved in 4% formalin. Following soil removal, a mustard vermifuge (5 g mustard powder L^{-1} water) was added to the soil pits to check for anecic individuals. A vermifuge is a liquid expellant that causes skin irritation for earthworms and can be either applied to the soil surface, where it will percolate into earthworm burrows, or via a targeted approach - injected directly into an earthworm burrow (Butt and Grigoropoulou, 2010). This causes the earthworms to leave their burrows. The ISO standard for earthworm extraction is a dilute formaldehyde solution (International Standards Organisation, 2006), however this chemical is carcinogenic at high levels of exposure and could negatively impact soil fauna (Eichinger et al, 2007). Therefore, the vermifuge used in this study was a mustard powder suspension at 5 g L^{-1} (Butt, 2000). Earthworms collected were then identified according to Sims and Gerrard (1999) in the laboratory and biomass was recorded. The data collected from these investigations were used to determine mean earthworm density (ind. 0.1 m^{-2}), biomass (g 0.1 m^{-2}) and species richness (n).

To provide a general overview of soil conditions, bulk soil was collected from each field assessed and stored in a plastic container (750 ml). On return to the lab, soil was sampled for pH, soil moisture and organic carbon content.

3.3.2. Earthworm Collection and Cultivation

The earthworms used in mesocosm and microcosm experiments (Chapters 5 and 6) were collected from agricultural pasture at Walton Hall Farm (SD 55050 28100) in Preston, UK. Earthworms were collected by digging and hand-sorting soil up to approximately 30 cm depth. Collected earthworms were placed in 750 ml plastic containers (obtained from Lakeland Ltd) filled with field soil. For the collection of anecics, a mustard vermifuge (Section 3.3.1) was used. Following extraction, anecic species were washed with water to remove the irritant from their skin, before placing in plastic containers filled with field soil (750 ml; Lakeland, UK).

Once transported back to the laboratory, earthworms were identified (Sims and Gerard, 1999) and separated into monocultures. Monocultures were transferred into 750 ml plastic containers (Lakeland, UK) previously filled with Kettering loam soil at 25% soil moisture content and left to burrow (Butt et al, 1994). To regulate airflow, small holes were made in the container lids (using a dissection needle) before earthworm addition. The number of earthworms stored per container differed between species (*L. terrestris* N = 3 ind. L^{-1}; *A. longa* N = 4 ind. L^{-1}; *A. chlorotica* N=10 ind. L^{-1}; *A. caliginosa* N= 6 ind L^{-1}; Lowe and Butt, 2005). Cultivated earthworms were fed with dried, rewetted horse manure (Section 3.4.1.). Horse manure was applied on the surface for anecic species and mixed within the soil for endogeics, at a homogenous particle size (< 1 cm for anecics, < 1 mm for endogeics) incubated at 15 °C (LMC, Kent) in 24 hr darkness (Lowe and Butt, 2005) for 28 days to allow equilibration from field conditions before use in experimental research (Fründ et al, 2010). To retain soil moisture, samples were sprayed weekly with water. Earthworms were refed biweekly at a rate of 20 g $adult^{-1}$ $month^{-1}$ for anecics and 10 g $adult^{-1}$ $month^{-1}$ for endogeics (Lowe and Butt, 2005).

For selected experiments, cultured earthworms were utilised. Cultured stocks of earthworms were developed by sampling adult stocks for cocoons. During biweekly refeeding, adult earthworms were removed and placed in containers with fresh soil and feed. Used soil was sampled for cocoons using sieves of decreasing mesh size (mesh size dependent on earthworm species; Lowe and Butt, 2005). Cocoons were stored on filter paper (Whatman no 1) in Petri dishes with sufficient water to cover them (Butt, 1991). Each week, water was refreshed to maintain oxygenation. Cocoons were stored in a fridge at 4 °C until they were required for use. To hatch the earthworms, they were transferred to a 15 °C incubator and monitored daily. Hatchlings were placed into 250 ml plastic containers (Lakeland, UK) filled with a 50:50 mixture of Kettering loam and milled horse manure (previously dried and rewetted) at 25% moisture content. Cultured juvenile earthworms were monitored weekly and earthworms were refed with horse manure as required (frequency was dependent upon species). Once developed from hatchling to sub-adult juvenile, they were transferred into 750 ml containers and cultured as for adults.

In laboratory experiments utilising *L. terrestris*, adults were purchased from Wigglywigglers.co.uk. It was decided that adult *L. terrestris* would be purchased due to the length of time required to cultivate them (mean cocoon incubation rate before hatching is 90 days at 15°C; Butt et al, 1992) and the experimental requirement to have them at approximately equal mass. Once received, earthworms were stored and prepared for experiments following the conditions stated by Fründ et al. (2010).

3.3.3. Microcosm Experimentation

Microcosm experiments were conducted to test the effects of waste organic matter type on earthworm health and development. In laboratory incubation experiments, earthworm health was defined by two factors. Firstly, growth rate, where juveniles under healthy conditions would gain mass and reach sexual maturity. Secondly, fecundity, where reproductive rate was determined by the mean number of cocoons produced over time (cocoons earthworm^{-1} year^{-1}) (Lowe and Butt, 2005). Cocoon collection was achieved by wet sieving soil through a set of sieves with varying mesh sizes, as appropriate to the earthworm species (Sims and Gerard, 1999; Butt 1991; 1997).

Specific details of each microcosm experiment are defined within the thesis (Chapters 5 and 6), however the conditions for incubation were the same throughout. For each sample, 500 g Kettering loam soil was prepared to 25% moisture content and added to 750 ml plastic containers with pierced lids. Juvenile earthworm monocultures of equal mass (N = 2) had their mass recorded and were placed into the labelled containers. Equal masses of waste organic matter were applied (surface for anecic; mixed for endogeic) and the samples were stored at 15 °C in darkness. Samples were removed from the incubator to refresh moisture content and refeed (as required). Experiments ended either when earthworms had reached sexual maturity and produced cocoons, or if there was 100% mortality. This differed between earthworm species based upon their life cycles (Sims and Gerard, 1999).

3.4. Soil and waste organic matter used in laboratory experiments

This section describes the organic matter types selected for use in experiments and where they were sourced. Table 3.2 indicates the waste organic matter types applied in microcosm analysis. Waste organic matter went through different pre-treatment processes before storage and subsequent application in experiments. Organic matter was heated to 105 °C before storage to prevent any degradation. Organic matter which was already treated or consisted of plant matter was air dried and liquid treatments were stored and applied during experiments at a weight/volume ratio.

The soil used for laboratory experiments throughout this PhD was Boughton Sterilised Kettering Loam (purchased from www.boughton.co.uk). This soil type was selected because loam-based soils support larger earthworm populations than other soils in temperate climates (Bouché, 1977) and is commonly used in earthworm laboratory culture (Butt et al, 1994; Lowe and Butt, 2005).

Table 3.2. Details of the waste organic matter used in laboratory experiments, its source and pre-treatment applied.

Type	**Pre-treatment**	**Location**
Horse manure	Dried 105 °C overnight.	Wigton, Cumbria, NY 37567 37732
Wheat (*Triticum aestivum*) straw	Air dried for 14 days.	Brook Lane Farm, Farington, Lancashire SD 52758 24517
Composted green waste (CGW)	Dried 105 °C overnight.	Global Renewables Ltd, Leyland, Lancashire, PR26 6TB.
Anaerobic digestate (solid fraction)	Air dried for 14 days.	Riverford Organics, Home Farm, Northallerton, Yorkshire SD 49870 18036
Anaerobic digestate (liquid fraction)	None: stored at 4 °C.	Global Renewables Ltd, Leyland, Lancashire, PR26 6TB.

Horse manure was utilised in this project as a control application due to its known positive impact on earthworm species (Lowe and Butt, 2005; Butt, 2011). It is also a common application in agriculture and horticulture (often as farmyard manure). To ensure the consistency of supply, horse manure used for laboratory earthworm culture was obtained from the stable of a single horse, which was under no horse medicaments detrimental to earthworm populations. The solid fraction of anaerobic digestate was stored outside under prevailing weather conditions (Figure 2.7), so a need for air drying before laboratory storage. Of the waste organic matter types described in Table 3.2, Chapters 4 and 5 explored the utilisation of all organic matter types listed, where later chapters focussed on the utilisation of straw residues.

3.5. Chemical Analysis

3.5.1. Macro- and micronutrient concentration

Chemical analysis was conducted on soils to determine whether there were any effects of waste organic matter application or earthworm behaviour on the abiotic conditions of soil. Soils were analysed for macro- and micronutrient concentration because the concentrations of these elements can influence crop growth. If there were any changes in nutrient conditions this could inform land managers of the potential effects on crops. Chemical analysis of soil was conducted in Chapters 4.4, 6 and 7.

3.5.1.1. Total nutrient concentration

Samples were prepared for total nutrient analysis by Microwave Digestion. Prior to digestion, soil samples were dried at 105 °C, milled and sieved to 1 mm. Using a balance accurate to 3 decimal places, 0.1 g of soil was weighed into a digestion vessel and 10 ml of nitric acid was added (70% analytical reagent grade, purchased from Fisher Scientific). To account for error, 'actual' soil mass was recorded. Samples were digested in a microwave digester (Milestone Ethos EZ) on a recommended temperature program (T1 20 °C to 200 °C in 10 minutes, then hold T1 at 200 °C for 15 minutes).

Following digestion, samples were diluted 10x using nanopure (18 um) water. A dilution factor was applied to the results (raw data result multiplied by 1,000) and a soil mass error (result multiplied by 0.1 g / recorded soil mass).

3.5.1.2. Available nutrient concentration

Samples were prepared for available nutrient analysis by ammonium acetate extraction. Prior to digestion, soil samples were dried at 105 °C, milled and sieved to 1 mm. Sieved soils were weighed (5 g) into 50 ml labelled centrifuge tubes and aliquots of 20 ml ammonium acetate (1 M) solution were added. Samples were vigorously shaken for 10 minutes then left overnight to react and settle. The following day, the supernatant was extracted, filtered (using Whatman no 1) and diluted 100x using nanopure (18 um) water prior to analysis. Following analysis, a soil mass error was applied (result multiplied by 5 g per recorded soil mass).

3.5.1.3. Inductively Coupled Plasma – Mass Spectrometry (ICP-OES) analysis

The ICP-OES used in this study was a ThermoScientific iCap 7000 series with radial detection. The analysis parameters of this study were derived from a standard method of samples used for agricultural soils analysis, published by ThermoScientific Ltd (Hannan, 2017).

The nutrients analysed in this study were Phosphorus, Calcium, Magnesium, Potassium, Manganese, Iron, Zinc and Boron. These elements were analysed because they are the main macro- and micronutrients found in agricultural soils that can be quantified on the ICP-OES.

Table 3.3. The wavelengths (nm) recorded for each element in ICP-OES analysis.

Element	P	Ca	Mg	K	Mn	Fe	Zn	B
Wavelength (nm)	213.618	183.801	202.582	213.618	293.910	240.488	206.200	208.959

Calibration standards were made by using a Fluka ICP Standard Mix 5 (purchased from Sigma Aldrich), where the following concentrations were made: 100 ųg kg^{-1}, 250 ųg kg^{-1}, 500 ųg kg^{-1} and 1000 ųg kg^{-1}. All calibrations had a correlation of 99.9% or higher.

3.5.2. Loss on Ignition (LOI)

Loss on Ignition was used to determine the soil organic matter content (%). The method used was adapted from Ball (1964). Soil samples collected from the field were dried at 105 °C to determine soil moisture content. Following this procedure, the soil was ground using a pestle and mortar and sieved to < 1 mm particle size. For each sample, 5 g soil was weighed (using a 3 decimal place balance) and placed into a pre-weighed crucible. 'Actual' masses for the crucible and soil were recorded. Crucibles were then placed in a furnace oven heated to 550 °C for 3 hours (Howard and Howard, 1990). Following this, samples were placed in a desiccator to cool down, before post-combustion mass determination.

3.5.3. Carbon and Nitrogen analysis

Samples were analysed for % CHN and C:N ratio using a ThermoScientific CHNS Organic Elemental Analyser. Prior to analysis, soil samples were dried (105 °C), milled and sieved (1 mm). Soil samples were mass determined (2-3 mg per sample; Mettler Toledo XP6 microbalance) and transferred into an aluminium tin foil capsule and sealed. Prior to analysis, the instrument was calibrated using 2,5-Bis(5-tert-butyl-2-benzo-oxazol-2-yl)thiophene (BBOT) standard (purchased from Fisher Scientific).

Samples were pyrolyzed at a temperature of 950 °C and directed through a pre-packed copper column using helium carrier gas (flow rate 140 ml/min) into a GC column, where C H N and S were separated and detected using a Thermal Conductivity Meter (TCD). Results will be presented in C-N ratios and concentration (%C and %N).

3.6. Statistical Analysis

Statistical tests in this thesis were completed using SPSS v.27. Each experimental Chapter was constructed to explore selected effects of organic matter applications on earthworm population dynamics. To determine how organic matter amendments applied in agroecosystems influence earthworm populations and soil functioning, the effects of selected waste organic matter applications on earthworm population development (biomass and reproduction) was sampled in field (Chapter 4) and laboratory (Chapter 5) conditions. To investigate how WOM application management in agroecosystems effects earthworm utilisation and soil functioning, controlled laboratory experiments were conducted to investigate the impact of WOM particle size on belowground (Chapter 6) and aboveground (Chapter 7) soil processes. The effects of earthworm density on organic matter utilisation was investigated in field conditions in Chapter 8. Statistical tests were conducted for each chapter based upon the experimental design. Therefore, statistical analysis will be outlined in each experimental Chapter in the respective methods sections.

4. EFFECTS OF ORGANIC MATTER APPLICATIONS ON EARTHWORM SPECIES IN THE FIELD.

4.1. Introduction

It has been widely demonstrated that the effects of organic matter applications have had positive impacts on earthworm abundance and species biodiversity, by providing a food source, protection from predation and through the regulation of soil properties such as soil moisture and erosion reduction (Blouin et al, 2013). Internationally, Sustainable Development Goals (UN, 2015) have been introduced to improve the management of soil, where a reduction in fertiliser usage and ploughing frequency has been encouraged to improve soil fertility. This has increased the utilisation of waste organic matter applications as fertilisers and the amount of no-till managed farms. The adoption of no-till farming with waste organic matter applications has increased earthworm abundances and changed the dynamics of earthworm species diversity, where more anecic species are present (Crittenden et al, 2014; Briones and Schmidt, 2017). The interactions between species may have different impacts on soil properties, which needs to be addressed to determine the impacts on soil conditions; information vital for crop development.

Although the effects of waste organic matter applications on earthworm populations are generally positive, there are many effects on earthworm population development (growth rate, reproduction, migration) under different waste organic matter types and application management (Lowe and Butt, 2005; Curry and Schmidt, 2007; Sizmur et al, 2017). This could cause differences in the soil organic matter transfer through the soil, which could have differing effects on decomposition and nutrient mineralisation, which indirectly influences soil ecosystem services (as discussed in Section 2.2.2.). This is particularly relevant when an increase in anecic populations occur, surface feeding earthworms that move organic matter vertically through the soil profile (Bouché, 1977). Some anecic species (such as *L. terrestris*) provide sources of waste organic matter to other populations through the formation of a midden (Section 2.2.3.). The positive effects of *L. terrestris* middens on juvenile populations of earthworms in forest ecosystems have been demonstrated (Butt and Lowe, 2007), where these middens are considered biological and chemical hotspots of activity (Hamilton and Silman, 1989; Schrader and Seibel, 2001; Butt and Lowe, 2007; Andriuzzi et al, 2015b; Stroud et al, 2016a).

This chapter compiles preliminary earthworm biodiversity assessments conducted to determine the effects of different waste organic matter applications on earthworm abundance, biomass and species richness. In addition, it examines the effects of *L. terrestris* middens in an agricultural setting. Specific objectives are provided associated with the relevant sections of this Chapter.

4.2. Biodiversity assessment of earthworm populations under applications of anaerobic digestate.

4.2.1. Introduction

The economic and greener incentive of anaerobic digestion has increased the utilisation by farmers (as described in Section 2.3.3.4). There have been numerous investigations on positive effects of anaerobic digestate applications on plant growth and its potential as a soil conditioner for arable land (Section 2.4.1.2; Möller and Müller, 2012; Nkoa, 2013; WRAP, 2016; Ren et al, 2020). However, effects of anaerobic digestate applications on earthworm populations is uncertain. If anaerobic digestate applications alter the species richness and density of earthworm populations, this may influence soil properties such as soil aeration, aggregation and moisture content, all of which could influence crop growth (Bertrand et al, 2015a). To determine the effects of anaerobic digestate applications on earthworm populations, a field survey of an experimental plot at Myerscough College was conducted in autumn 2015, following anaerobic digestate and standard practice fertiliser applications in spring.

4.2.1.1. Aim

To compare the effects of anaerobic digestate applications with inorganic fertiliser on earthworm populations.

4.2.1.2. Objectives

- To determine the difference in earthworm density and biomass under selected applications of anaerobic digestate and inorganic fertiliser.
- To examine whether the effects of anaerobic digestate caused a difference in the species richness of earthworm populations.

4.2.1.3. Hypothesis

There will be a higher earthworm population density and species richness under anaerobic digestate applications than applications of inorganic fertiliser and a control.

4.2.2. *Materials and Methods*

4.2.2.1. Site Location

Myerscough Lee Farm is an experimental arable field in Myerscough College, Preston, UK (SD 50423 39834). This field site had treatments of anaerobic digestate applied during the period of 2010-2013, in order to determine the impacts of organic waste on grass growth in comparison to a standard practice fertilizer.

4.2.2.2. Experimental Design

In this experiment, four treatments were applied:

NA: no treatment (control)
T1: Anaerobic digestate applied at 100 kg N ha^{-1} yr^{-1}
T2: Anaerobic digestate applied at 200 kg N ha^{-1} yr^{-1}
SP: Standard practice fertilizer application 100 kg N ha^{-1} yr^{-1}

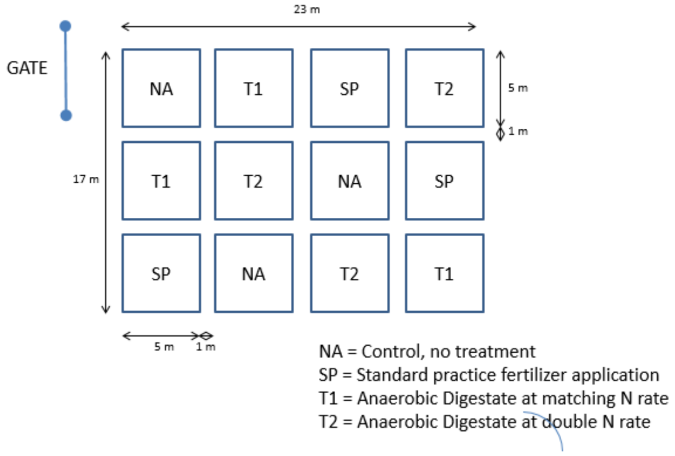


Figure 4.1. Diagram of the randomized block experimental design at Myerscough College (SD 50399 39823).

WOM treatments were applied evenly over 12 x 25 m² plots of turf, each separated by 1 m. There were three replicates of each treatment. A randomized block experimental design was applied (Figure 4.1). This design can identify any changes to earthworm populations influenced by field location.

4.2.2.3. Earthworm Sampling

Earthworms were sampled in October 2014. For each plot outlined in Figure 4.1, earthworms were sampled by digging and hand sorting an area of 0.1 m² soil, followed by an application of mustard vermifuge to remove anecics (method outlined in Section 3.3.1). Collected earthworms were stored in 4% formalin and transported to the lab, where they had their species identified (Sims and Gerard, 1999) and biomass determined.

4.2.2.4. Statistical Analysis

An increased earthworm density and biomass under anaerobic digestate treatments may confirm its benefits for earthworm health. An increased species richness under anaerobic digestate treatments may confirm how organic fertilisation may alter soil biological functioning. The variables earthworm biomass, earthworm density and earthworm species richness were tested for normality and equal variance. Using the randomized block experimental design, a General Linear Model (2-way ANOVA) was applied, where the effects of treatment and field row on earthworm density, biomass and species richness were analysed. Due to a low sample number (N=3) no statistical differences were determined between groups, therefore post-hoc tests were not applied.

4.2.3. Results

Anecic biomass increased under T1 applications compared with other treatments (Figure 4.2). There were no effects of anaerobic digestate or fertiliser on the biomass of other ecological groups. There were no significant differences in the mean total earthworm density between treatments and plot position (2-way ANOVA; df = 3; $p > 0.05$). This could be due to the low number of replicates, resulting in high bias in the statistical analysis. In future increased sample sizes will be used in future field analysis.

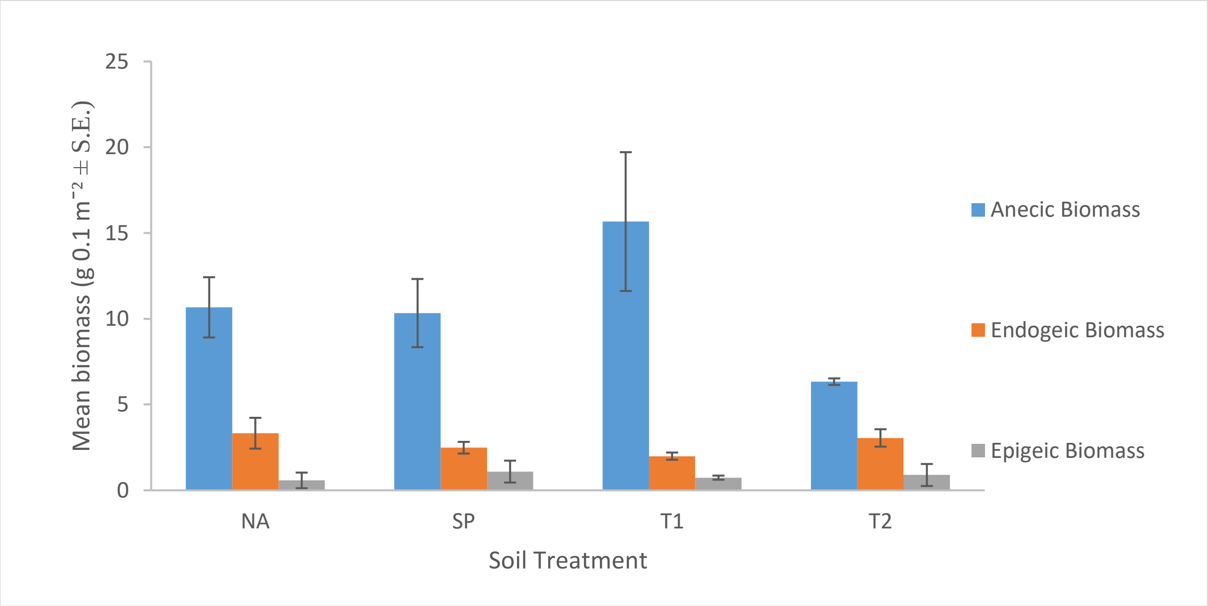


Figure 4.2. Differences in biomasses of earthworm ecological groups between organic waste treatments at Myerscough College. NA = No treatment; SP = Standard Practice Fertiliser (100 kg N ha^{-1} yr^{-1}); T1 = Anaerobic Digestate (100 kg N ha^{-1} yr^{-1}); T2 = Anaerobic Digestate (200 kg N ha^{-1} yr^{-1}).

A higher mean species richness was recorded under T1 anaerobic digestate treatment (Table 4.1).

Table 4.1. Mean species richness of earthworms (n ± S.E.) under applications of anaerobic digestate, standard practice fertiliser and a control. N=3.

NA	T1	T2	SP
4.67 ± 0.9	5.67 ± 0.3	4.67 ± 0.9	5.33 ± 0.7

The effects of organic matter treatment on earthworm density (separated by ecotype) is presented in figure 4.3. Higher mean anecic abundances were recorded under T1 treatments. The lowest abundance of anecic species occurred in T2 treatments. A 2-way ANOVA was applied to determine the effect of treatment type and plot position on earthworm abundance between species type. Findings indicated that there were no significant differences in the density of earthworms between treatments or plot positioning ($p < 0.05$).

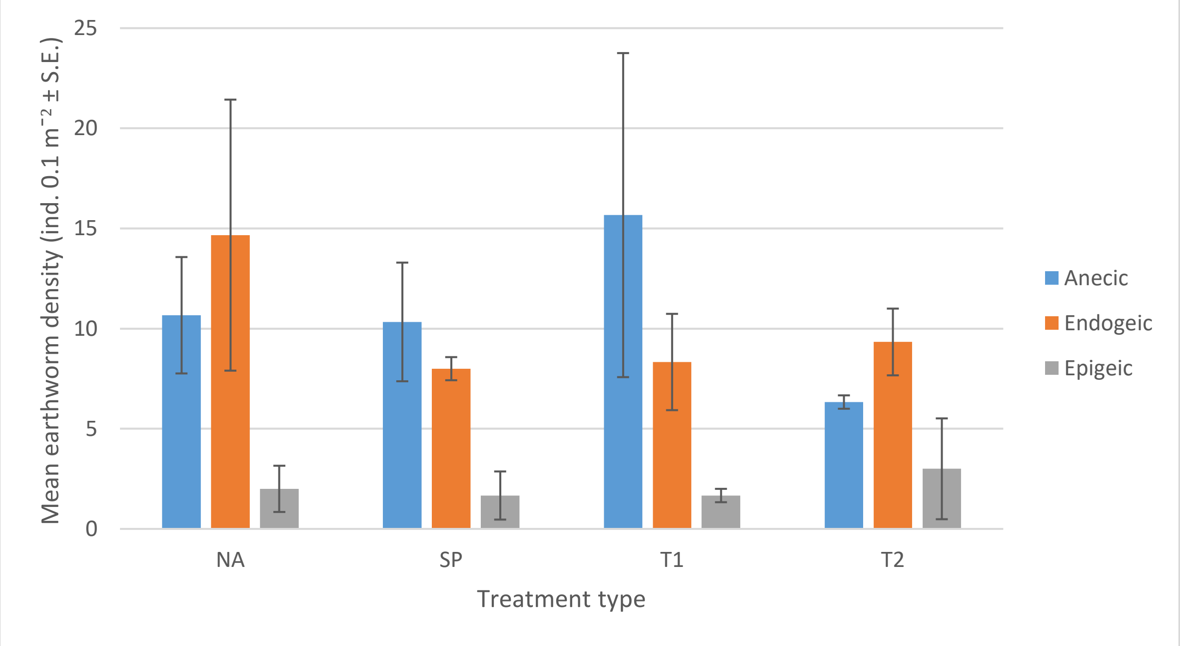


Figure 4.3. Differences in densities of earthworm ecological groups between organic waste treatments at Myerscough. NA = No treatment; SP = Standard Practice Fertiliser (100 kg N ha^{-1} yr^{-1}); T1 = Anaerobic Digestate (100 kg N ha^{-1} yr^{-1}); T2 = Anaerobic Digestate (200 kg N ha^{-1} yr^{-1}).

4.2.4. Discussion

This experiment provides preliminary evidence of the effects of anaerobic digestate applications on earthworm populations. A larger mean anecic biomass, density and richness was found under the T1 treatment. Anecic species feed on fresh surface organic matter; an increased density may increase the decomposition rate and nutrient mineralisation of anaerobic digestate (Blouin et al, 2013; Stroud et al, 2016a; Athmann et al, 2017). The bioturbation effects of anecic species could also increase the rate of SOM addition to the belowground ecosystem, increasing soil fertility. These effects could increase the nutrient availability to plants. Due to the experimental design, this field survey had a low number of replicates. This resulted in no statistically significant interactions between groups. It could be possible that the observed changes were small, which indicates that anaerobic digestate provides no earthworm response, or that more replicates are required to provide evidence that T1 is a positive earthworm application. The randomized blocking of the experimental design indicated that there were no significant differences in the earthworm density and biomass between the rows, therefore it is unlikely that the treatments were affected by plot position. Increased replication in future may increase the likelihood of determining significance within the dataset. Future experiments could explore the effects of anecic species on the decomposition and incorporation of waste organic matter.

Surprisingly, an increased anaerobic digestate application (T2) did not result in increased abundances or biomass of earthworm populations. This could indicate that the dosage of anaerobic digestate is considered before application. Applications at the same N rate at inorganic fertiliser could result in negative responses on earthworm population abundance and species biodiversity. The inputs of anaerobic digestate can differ dependent upon its inputs, where some reports have suggested that anaerobic digestate can consist of high metal concentration (Nkoa, 2013; Montusiewicz et al, 2020). Although earthworms can withstand soils under high metal concentration, the bioaccumulation effects of repeated applications could reduce earthworm populations. Added to this, a high concentration of heavy metals can cause phytotoxicity (Gell et al, 2011; Murtaza et al, 2017), which would also have negative effects on crop growth. There is a requirement for further investigations into anaerobic digestate and the effects of the inputs on the biofertilizer toxicity. Further field studies (Section 4.3) analyse the impacts of anaerobic digestate on earthworm populations and to determine the effect of anaerobic digestate on earthworm survival, growth rate and reproductive output, controlled laboratory experiments have been conducted (Chapter 5).

4.3. Organic Growth Media applications: the effects of plant type on earthworm species richness and density.

4.3.1. Introduction

The effects of waste organic matter on plant growth can differ due to the decomposition rate, nutrient concentration and the nutrient requirements of the plant (Möller and Müller, 2012; Nkoa, 2013). Earthworm abundance and biodiversity can have significant impacts on the decomposition rate and distribution of organic matter (Blouin et al, 2013; Frazão et al, 2019). Therefore, the interactions between earthworm abundance and organic matter characteristics could have a significant effect on plant growth. A preliminary field survey (Section 4.2) indicated that the effects of anaerobic digestate could be dosage dependant, where applications at half the N rate of standard practice fertiliser (100 kg N ha^{-1} yr^{-1}) is applied. Due to the positive effects of earthworm populations on soil health and organic matter decomposition, it is important that appropriate levels of organic matter are applied to maximise their decomposition effects.

Plant type can significantly impact earthworm density and species richness. Where grass species or trees predominate, there are different earthworm populations compared with agroecosystems supporting cereal crops and legumes. An increased biodiversity of plant species can increase earthworm species diversity and maintain populations, particularly in arable ecosystems (Roarty et al, 2017). The root structure of plants can impact the bulk density of soil, which can cause differing effects on earthworm populations. Therefore, the interactions between plant type and organic matter application could affect earthworm population dynamics. The effects of organic matter applications and plant type on earthworm population density and species richness are explored in the following section. This field survey investigated the effects of two waste organic matter applications, organic growth media (OGM) and anaerobic digestate (AD), on the earthworm abundance and species richness under different plant types.

4.3.1.1. Aim

To determine how waste organic matter applications influence earthworm population dynamics under different plant species.

4.3.1.2. Hypothesis

Earthworms will have a higher density and biomass under WOM treatments with increased organic matter N concentration and in plant species with a smaller root structure.

4.3.2. Materials and Methods

4.3.2.1. Background

Chisnall Hall, a restored colliery site, has been a research site for a Waste Resources Action Plan (WRAP) project since 2012 to investigate the effects of waste organic matter applications on short rotation forestry. Applications of organic matter (stated in Section 4.3.2.2.) were used at the field site from 2012-2015 with four plant species, where growth was recorded. The WRAP (2016) report concluded that there were positive impacts of anaerobic digestate applications when a higher percentage of dry matter was present in the anaerobic digestate. This experimental site was sampled in 2015 to determine the effects of organic matter type on earthworm populations under different plant types.

4.3.2.2. Experimental Design

This experiment followed a randomized block design (Figure 4.4). Six organic matter treatments were applied randomly over 3 blocks:

1. Anaerobic digestate low concentration (100 kg N ha yr^{-1})
2. Anaerobic digestate with organic growth media low concentration (100 kg N ha yr^{-1})
3. Organic growth media only (100 kg N ha yr^{-1})
4. Anaerobic digestate with organic growth media high concentration (200 kg N ha yr^{-1})
5. Anaerobic digestate high concentration (200 kg N ha yr^{-1})
6. Control plot with no organic matter application.

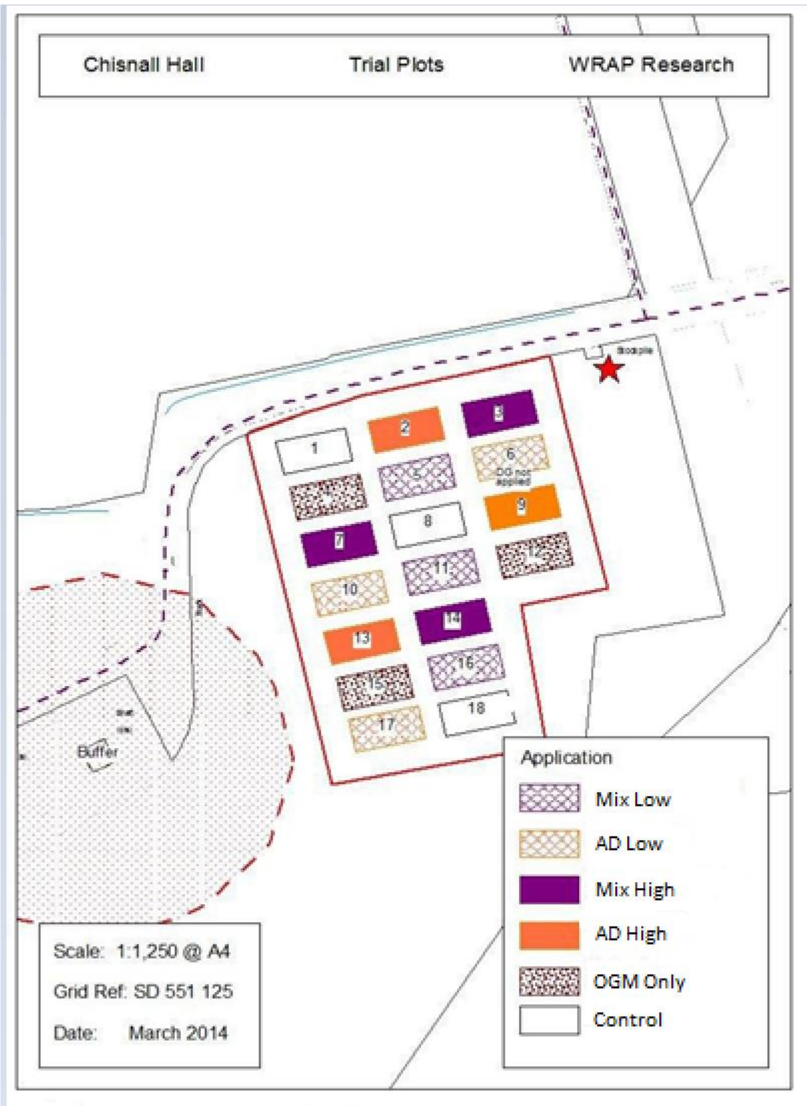


Figure 4.4. Diagram of the randomized block experimental design at Chisnall Hall (WRAP 2016).

Organic matter was applied annually over a period of 2 years (2013-2014). Each plot measured 10 m x 20 m. There were three replicates of each organic matter treatment. Plots were equally divided into subplots (10 m x 5 m) where 4 plant species were planted:

1. Cherry (*Prunus avium*)
2. Willow *(Salix* hybrids cv. Resolution and cv. Terra Nova*)*
3. Ash *(Fraxinus excelsior)*
4. Reed Canary Grass (*Phalaris arundinacea*)

4.3.2.3. Earthworm sampling

In 2015, this site was sampled for earthworms using a hand sorting method combined with a mustard vermifuge (details in Section 3.3.1). Collected earthworms were stored in 4% formalin and species identified (Sims and Gerard, 1999) in the laboratory. Recorded data was analysed to investigate any interactions in the effects of plant type and organic matter treatment on earthworm density (ind. 0.1 m^{-2}) and biomass (g 0.1 m^{-2}).

4.3.2.4. Statistical Analysis

The effects of interactions between organic matter type and plant type on earthworm density and biomass were analysed. Following tests for normality and equal variance, a two-way ANOVA was applied to the effects of organic matter treatment, plant type and block position on total earthworm density and biomass. Post-hoc Tukey multiple comparisons testing located any significantly different treatments.

To determine the effects of earthworm behaviour on soil functioning, earthworm data was separated into ecotype and analysed. The effects of organic matter treatment, plant species and block position on ecological group density and biomass were tested. Datasets for ecological groups were not normally distributed, therefore non-parametric Kruskal-Wallis tests were applied to each variable.

4.3.3. Results

The effects of organic matter type and plant species on total earthworm density and mass are presented in figures 4.5a and 4.6a. A higher mean total earthworm density and biomass was recorded under all organic matter treatments compared with the control. There were no interactions observed between the effects of treatment type and plant type on total earthworm density (2-way ANOVA: df = 15; F = 1.07; $p > 0.05$). Single main effects analysis indicated that there were significant differences in effect of treatment type on earthworm density (df = 5; F=8.22; $p < 0.001$). Post-hoc tests confirmed that the control and AD High were the significantly different treatments ($p < 0.005$). No effects of plant type on earthworm density and biomass was recorded ($p > 0.05$). There were no significant effects of block position on total earthworm density and biomass ($p > 0.05$).

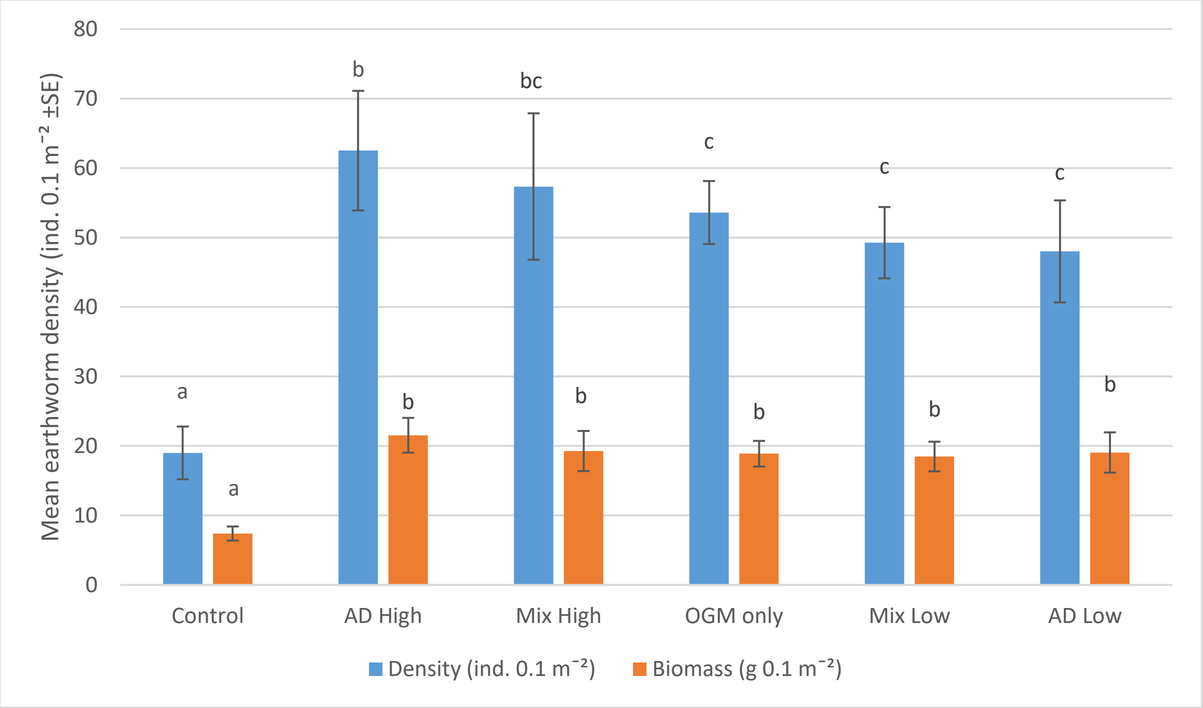


Figure 4.5a. Effects of organic matter type on the total mean earthworm density (ind. 0.1 m^{-2} ± S.E.) and biomass (g $0.1m^{-2}$) at Chisnall Hall. Significant differences between treatments are represented by different letters (one-way ANOVA; $p < 0.05$).

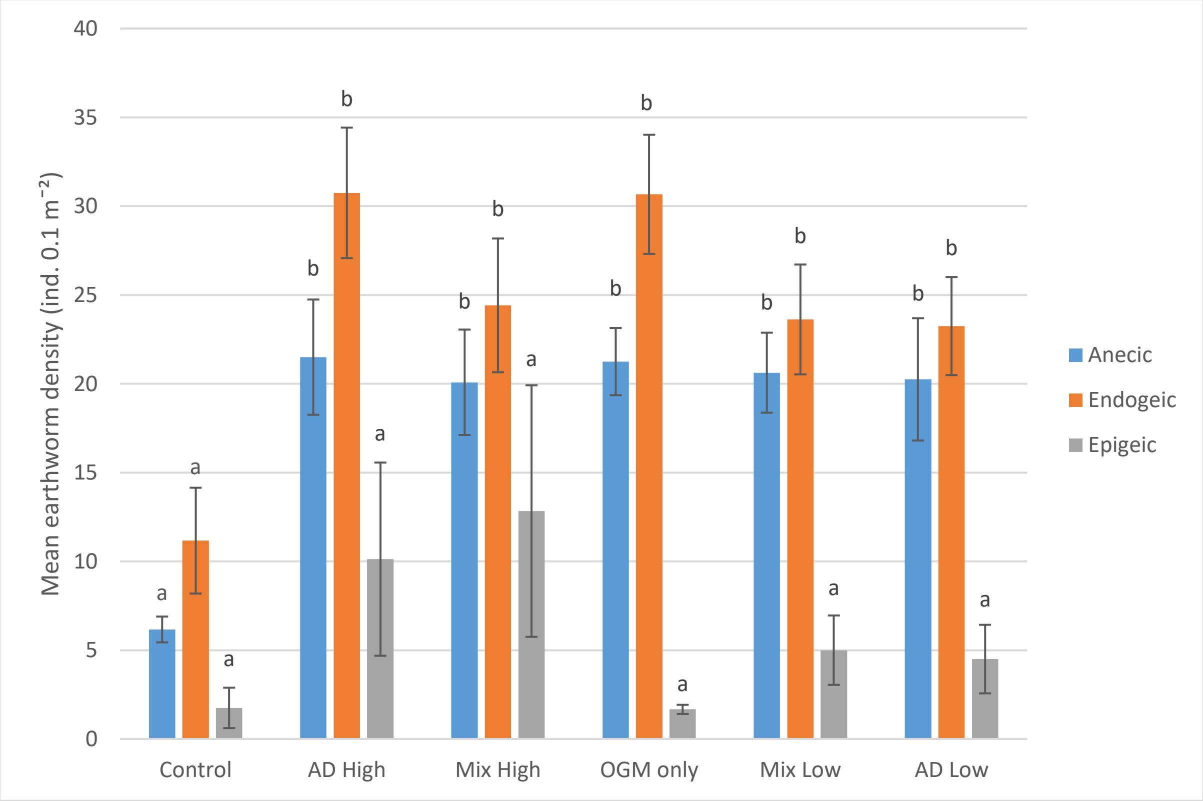


Figure 4.5b. Effects of organic matter type on mean earthworm density (ind. 0.1 m^{-2} ± S.E.), separated by ecotype. Significant differences between treatments are represented by different letters (one-way ANOVA; $p < 0.05$).

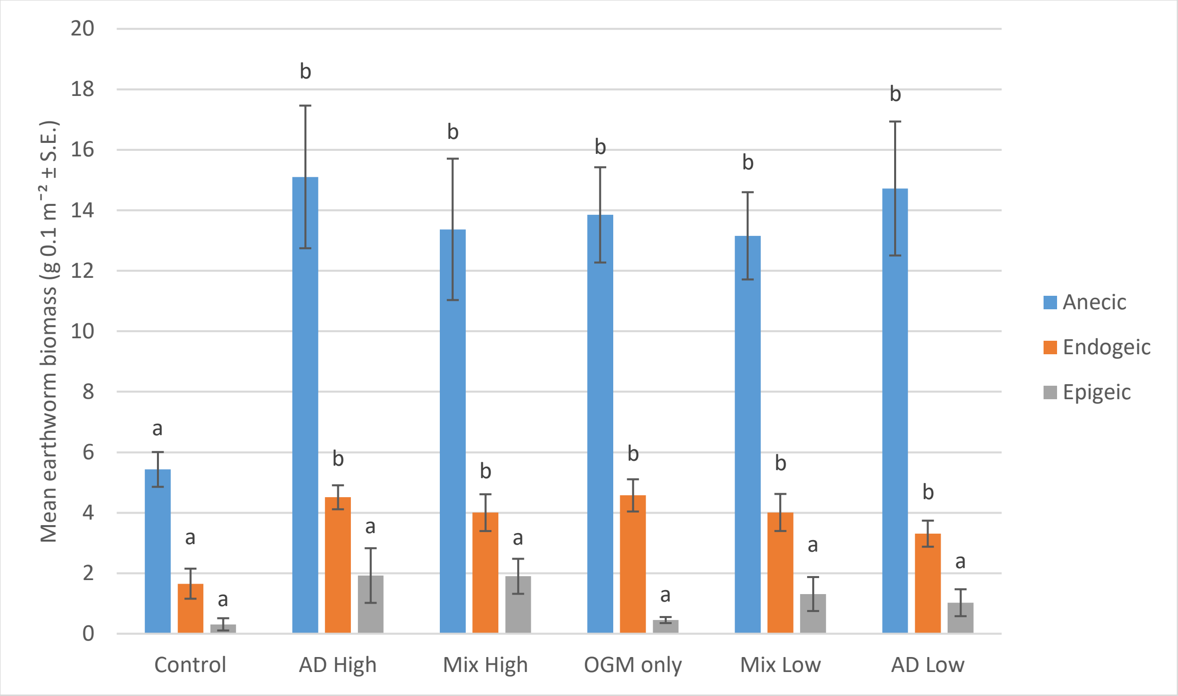


Figure 4.5c. Effects of organic matter type on mean earthworm biomass (g 0.1 m^{-2} ± S.E.), separated by ecotype. Significant differences between treatments are represented by different letters (one-way ANOVA; $p < 0.05$).

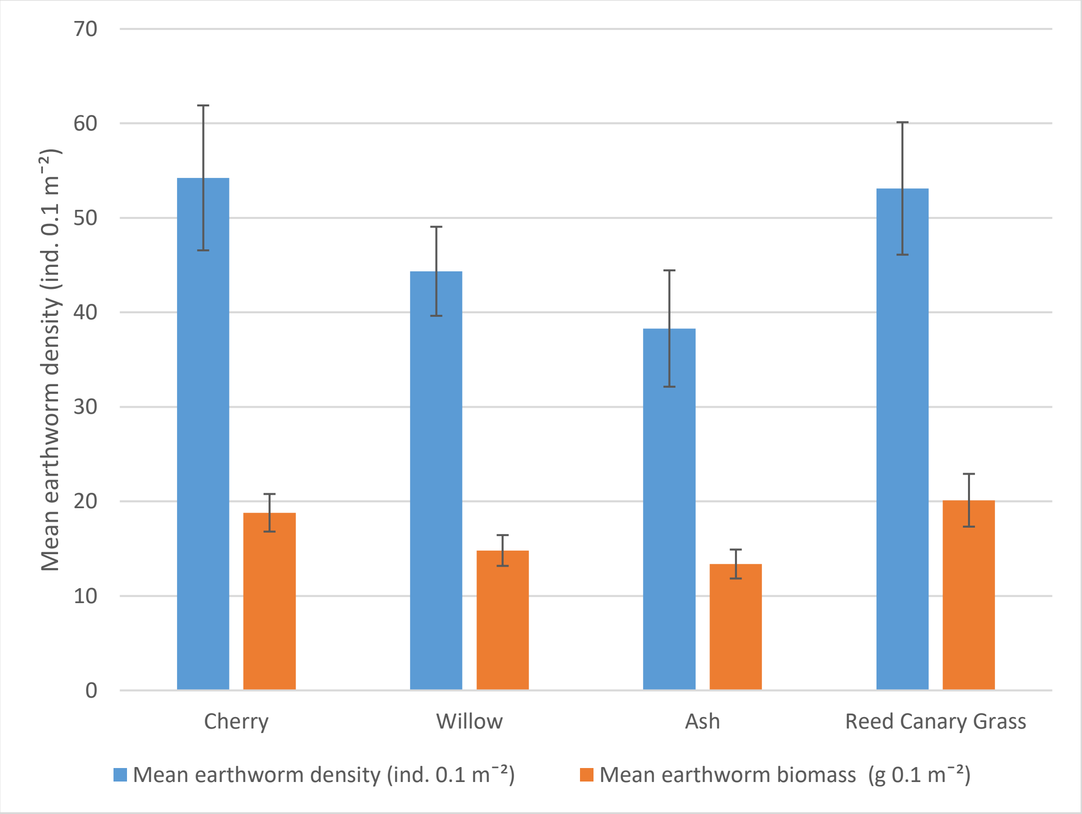


Figure 4.6a. Effects of plant type on mean earthworm density (ind. 0.1 m^{-2} ± S.E.) and mean biomass (g 0.1 m^{-2} ± S.E.) at Chisnall Hall. N=17.

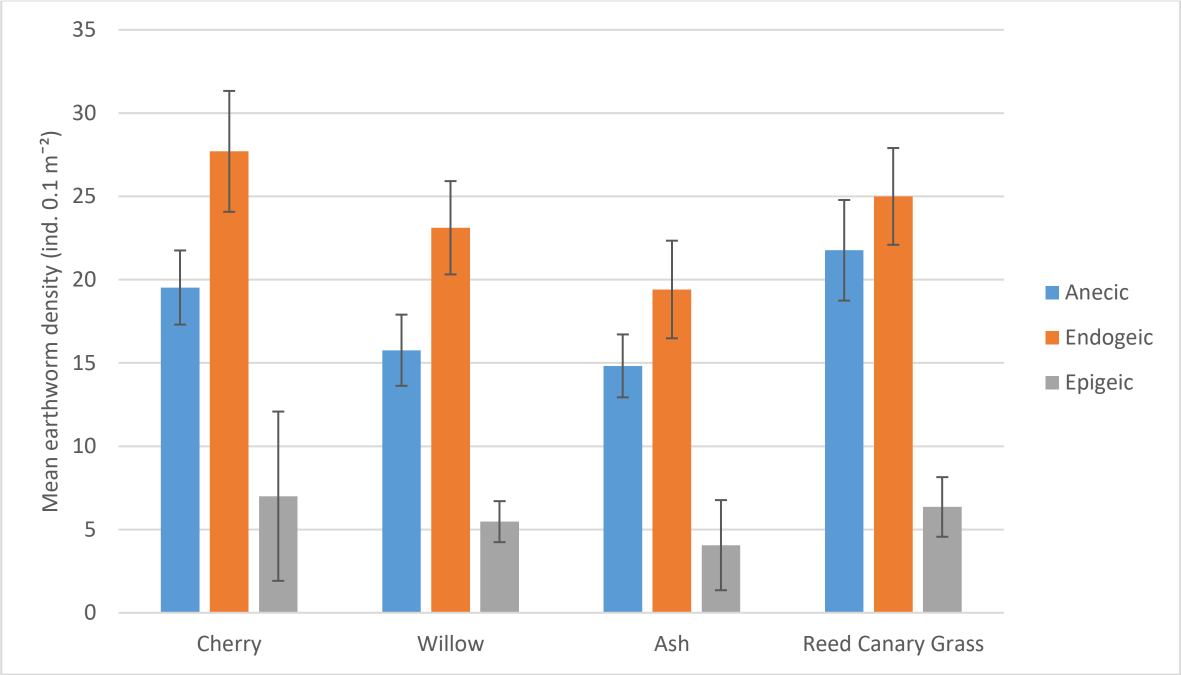


Figure 4.6b. Effects of plant type on mean earthworm density (ind. 0.1 m^{-2} ± S.E.), separated by ecotype.

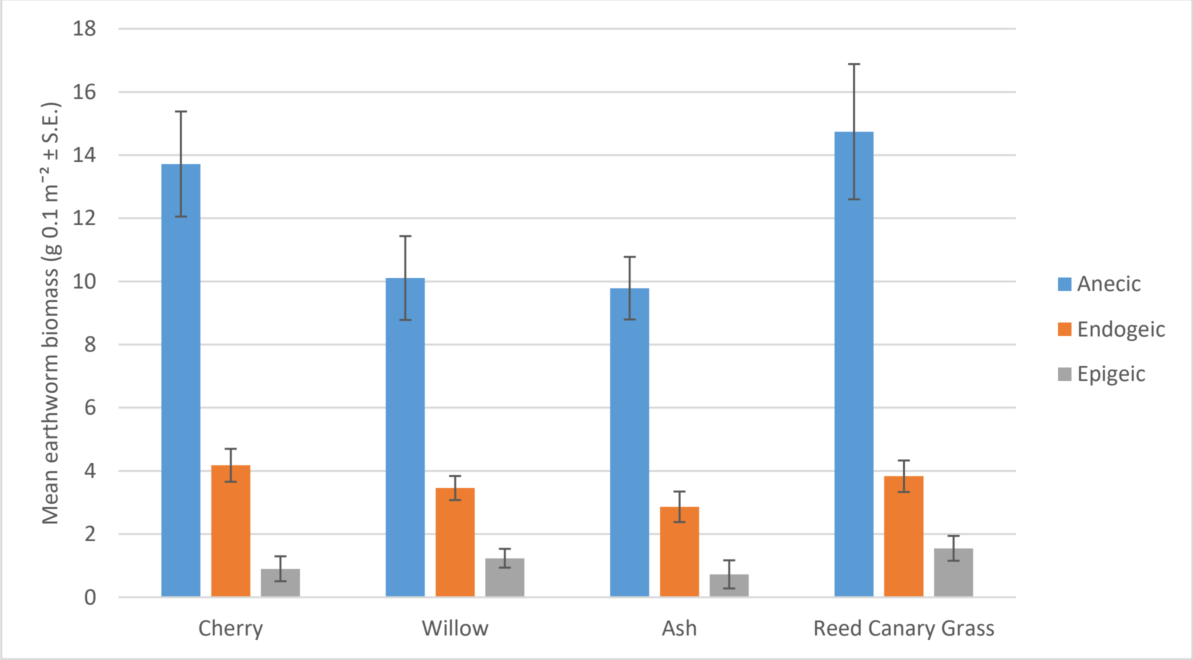


Figure 4.6c. Effects of plant type on mean earthworm density (ind. 0.1 m^{-2} ± S.E.), separated by ecotype.

To locate any differences within earthworm biodiversity, the earthworm density data was separated into ecotypes. The effects of organic matter application on the density and biomass of ecotypes are presented in figures 4.5b-c. The effects of plant type on the mean density and biomass of ecotypes are presented in figures 4.6b-c. Interactions between treatment type and plant type on the density of anecic and endogeic ecotypes were tested (Table 4.2).

Table 4.2. Statistical analysis results investigating the effect of WOM treatment type, plant species and block position on earthworm biomass (g 0.1 m^{-2}) and density (ind. 0.1 m^{-2}), when separated by ecological group.

Variable	Ecological Group	p value (Kruskall-Wallis)		
		Organic Matter	Plant Species	Block Number
Biomass (g 0.1 m^{-2})	Anecic	0.001	0.137	0.300
	Endogeic	0.003	0.293	0.551
	Epigeic	0.008	0.067	0.179
Density (ind. 0.1 m^{-2})	Anecic	< 0.001	0.173	0.994
	Endogeic	0.003	0.380	0.683
	Epigeic	0.015	0.072	0.307

There were no significant effects of block number on the density and biomass of all earthworm ecotypes ($p > 0.05$), which indicates that any potential environmental effects of field positioning on soil properties did not compromise any experimental findings. There were significant effects of WOM treatment on the biomass and density of all three ecological groups, where the control treatment was the significantly different group (Figure 4.5c). There were no significant effects of plant species on the biomass and density of all ecological groups.

4.3.4. Discussion

The positive effects of anaerobic digestate and organic growth media on earthworm populations were defined in this experiment, where total density and biomass of earthworms were higher under organic matter applications than a control (Figure 4.4a). The difference in N concentration (high and low) had no effect on abundance, therefore this implied that the optimum application of organic matter to sustain earthworm populations would be 100 kg N ha^{-1} yr^{-1}. This agreed with Section 4.2, where 100 kg N ha^{-1} yr^{-1} anaerobic digestate applications had the highest earthworm abundances. The decomposition characteristics and chemical differences between organic growth media and anaerobic digestate were not investigated in this experiment, however it is likely that the application of surface organic matter encouraged earthworm growth and reproduction regardless of amount applied (Curry and Schmidt, 2007). The metal concentration of the anaerobic digestate and organic growth media was not analysed in this experiment, however applications of waste organic matter at similar N rates to standard practice fertiliser (200 kg N ha^{-1} yr^{-1}) could increase metal contamination (Nkoa, 2014). Although this may not affect plant growth, the bioaccumulation of metals by earthworms can be toxic. Future investigations will assess the effects of waste organic matter on earthworm health.

There were no observed interactions between the effects of plant type and organic matter treatment on earthworm density and biomass. Qualitative comparisons of plant effects indicated that plant species with larger roots (willow and ash) had lower earthworm abundances than shallower root structured plants (Figure 4.6a). However, statistically these comparisons were not significant ($p < 0.05$) and cherry (which has a large root system) had similar earthworm abundances to canary grass. Earthworm burrows can attract plant roots due to the presence of nutrient-rich casts, however the effects of earthworm density on plant growth differs between plant species (Milleret et al, 2009; Cameron et al, 2014). Although this experimental design had randomized blocking, there were a low number of replicates (6 for each plant) and the plants grown were not well established (3 years at the time of sampling). Further sampling over time and increased replication of plant treatments may have exposed significance within the results. The effect of plant type on epigeic populations was almost significant (biomass $p = 0.067$; density $p = 0.072$; Kruskal-Wallis), further replications or adaptation of the experiment into controlled glasshouse conditions may have enhanced the likelihood of significance. As litter-dwelling species, epigeics maybe more influenced by plant foliage than other ecological groups (Bouché, 1977). The plants investigated in this survey were related to a reclaimed forestry ecosystem; there may be interactions between plants and organic matter applications in agroecosystems where stubble is maintained. The effects of stubble on earthworm population density and biomass, alongside potential utilisation of cereal residues will be explored in later experiments.

Although the plants investigated within this experiment were more replicable of a forestry ecosystem, the indication that waste organic matter application had more of an effect on earthworm density, biomass and species richness than plant type could suggest that within agroecosystems the organic matter applications are effective under most crop types. The effect of waste organic matter type on earthworms within an agroecosystem will be investigated throughout this thesis.

Separation of the earthworm population data into ecological groups identified some differences in the effects of organic matter type on density. Endogeic population size was significantly higher under high concentration applications of anaerobic digestate ($p < 0.05$). An increase in endogeic populations could increase the hydraulic activity of soil due to their semi-permanent burrowing and also increase nutrient concentration in the belowground ecosystem through increased casting, which may have positive impacts on plant growth (Massey et al, 2013; Le Couteulx et al, 2015; van Groenigen et al, 2019). An increase in earthworm population size may also increase the decomposition rate of organic matter, which would enhance soil organic matter content and soil fertility. An increase in decomposition based on earthworm activity could also increase the total amount of waste disposal, reducing waste to landfill. The decomposition of organic matter depends on a wide range of factors, where ecotype is one significant factor which can determine the transport of waste organic matter into soil. Future research will assess the effects of earthworm species on the decomposition of waste organic matter based on the waste type and its particle size.

This field survey highlighted the importance of organic matter type on improving soil conditions for earthworms in reclaimed systems, which could provide benefits to soil structure and nutrient cycling. This could be useful to explore in agroecosystems, particularly where soil may have been degraded or where organic matter is the main management source for enhancing soil (such as organic farming). Future investigations will assess the effects of organic matter applications in agroecosystem environments to determine whether these effects are similar and predict whether the changes in earthworm species diversity could affect soil functioning through the changes to SOM.

4.4. ASSESSMENT OF EARTHWORM POPULATIONS UNDER *L. TERRESTRIS* MIDDENS: IMPACT OF THE 'HOTSPOT'.

4.4.1. Introduction

Preliminary field investigations have suggested that waste organic matter applications can increase earthworm density and the abundance of anecic populations. Increased presence of epi-anecic populations such as *L. terrestris* in agroecosystems may significantly alter the biological community within the soil profile through its burrow-midden complex, which may enhance decomposition of organic matter and nutrient availability within soil (discussed in Section 2.5.4; Darwin, 1881; Hamilton and Sillman, 1989; Subler and Kirsch, 1998 Schrader and Seibel, 2001; Aira et al, 2009). Analysis of *L. terrestris* middens in forest ecosystems indicated that that soil beneath a midden had a higher earthworm abundance and species richness than surrounding soils (Lowe and Butt, 2005). Although extensive field experiments have explored the positive impact of reduced tillage systems on *L. terrestris* density (Kladivko et al, 1997; Ernst and Emmerling, 2009; Bogužas et al, 2010; Kuntz et al, 2013) and the effect of increased *L. terrestris* density on soil properties through increased bioturbation (Nuutinen et al, 2011; Capowiez et al, 2009, 2012; Frazão et al 2019), literature regarding middens in agroecosystems is less abundant than forestry ecosystems.

An investigation on the impact of *L. terrestris* middens on physical, biological and chemical soil properties in reduced tillage agroecosystems was conducted by Stroud et al (2016a). This field survey highlighted that *L. terrestris* abundance increased under no tillage, where midden density and size increased with addition of horse manure. Unlike in forestry ecosystems (Butt and Lowe, 2005), only endogeic earthworms were present within the midden structures. Added to the increased biological activity, there were higher concentrations of available micronutrients (P, K and S) in soil underneath middens. Within Stroud et al's (2016a) survey, juvenile earthworms were not identified to species level and were evaluated by number, therefore the impact of *L. terrestris* middens on earthworm biodiversity in agroecosystems was not confirmed. To help clarify this situation, a field survey was conducted to analyse the effects of *L. terrestris* middens on organic matter utilisation, earthworm biodiversity and chemical composition in a reduced tillage field.

4.4.1.1. Aim:

To investigate the effect of *Lumbricus terrestris* middens and their impact on biological and chemical soil properties.

4.4.1.2. Objectives:

- To investigate whether *L. terrestris* middens increase earthworm populations in no-till agroecosystems and if so, determine the earthworm species that utilise middens.
- To calculate midden size and structure and assess the particle size of the organic matter utilised in the field.
- To conduct chemical analyses on midden soil and determine whether middens influence soil macro- and micro-nutrient concentrations.

4.4.1.3. Hypotheses:

- Soil beneath *L. terrestris* middens will have a higher earthworm density and biomass than surrounding control soil.
- The chemical composition of soil surrounding the drilosphere will differ from surrounding control soil.

4.4.2. Materials and Methods

The experimental site for this field survey was Lilac field, Light Ash farm, Myerscough (National Grid Reference: SD 48735 39991; Figure 4.7), sampled during November 2019. To determine whether *L. terrestris* were present in the field, an initial investigation of this area was conducted in Autumn 2018, where 10 middens were found (results presented in Appendix I). In November 2019, Light Ash farm was fallow with spring barley stubble (*Hordeum vulgare*: *RGT Planet*) intact.

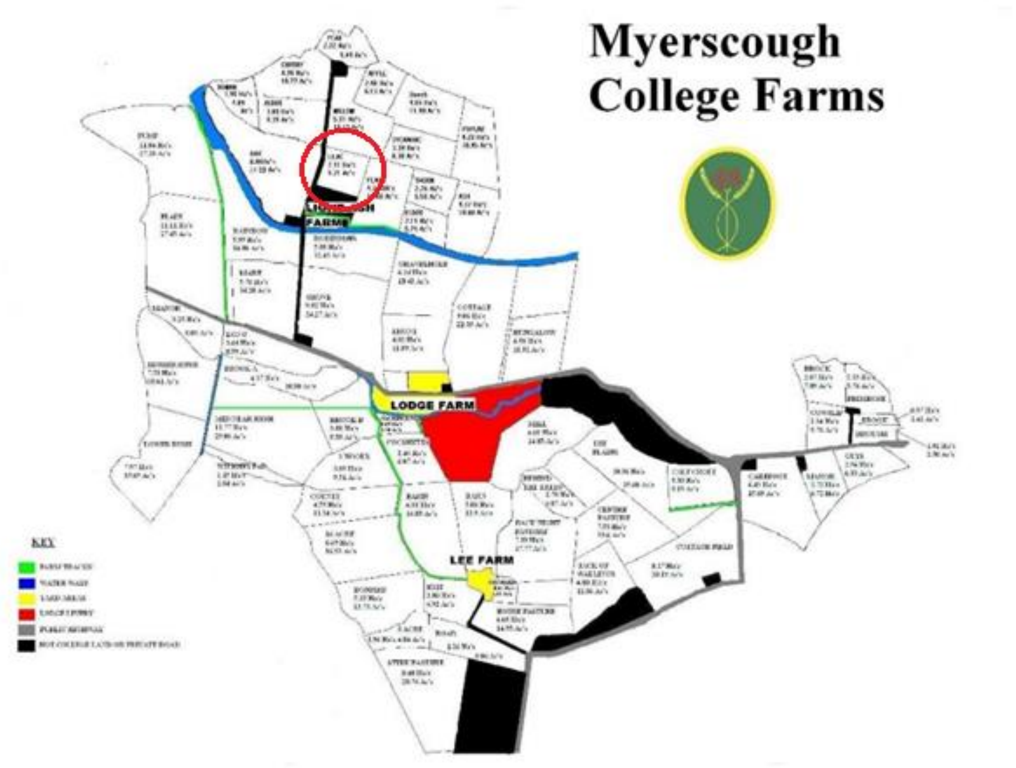


Figure 4.7. Myerscough College farms. Sampling was conducted in Lilac field (circled in red), Light Ash farm (National Grid Reference SD 48735 39991).

4.4.2.1. Midden Sampling

L. terrestris middens were determined using a method adapted from Butt and Lowe, (2007) and were identified as 5-10 cm diameter piles of organic matter containing barley straw and casts. Identified middens were carefully lifted to view the burrow beneath. Thirty samples were located. For each sample site, the midden and the soil directly below was collected using a 220 g auger (750 mm diameter x 600 mm depth). Soil cores with middens intact were placed upright into plastic sample bags to maintain structure. A control 'non-midden' soil core was taken 30 cm from the midden where no obvious signs of earthworm activity could be seen on the soil surface (Figure 4.11). Following midden sampling, 100 ml mustard vermifuge (5 g L^{-1}) was injected into the burrow in an attempt to extract the resident *L. terrestris* individual (Figure 4.9). Extracted individuals were washed with water and had mass determined in the laboratory, where juvenile specimens were grown to maturity. Midden and Non-midden cores were taken to the laboratory for organic matter content, earthworm and chemical analyses.

Figure 4.8. Sampling at Light Ash farm. Located midden sample (right) was cored using a 220 g auger. Soil with no organic matter cover 30 cm from midden was selected as a non-midden sample (left).

Figure 4.9. Mustard vermifuge addition to a *L. terrestris* burrow.

The location of *L. terrestris* middens were determined by their dispersal within the field, which can be influenced by environmental factors, soil conditions and earthworm behaviour. For this reason, *L. terrestris* middens were found in clusters. Alongside the sampling of *L. terrestris* middens and control soil nearby, an earthworm survey of the field was conducted by generally sampling for earthworms (methods detailed in Section 3.3.1.). Five soil pits (0.1 m^2) were located along a transect, then dug out and hand sorted for earthworms. Collected earthworms were stored in 4% formalin and species identified (Sims and Gerard, 1999) in the laboratory. The data collected was used to provide an overview of the earthworm community structure in Lilac Field, where the main organic matter source was barley stubble.

4.4.2.2. Midden Analysis

Middens were carefully removed from the soil surface using forceps and air-dried for one week. Once dry, middens were deconstructed using forceps. During this process, visible organic matter (> 0.5 mm) was removed from the midden and cleaned. Dried organic matter was mass-determined (g). Particle size of organic matter was determined by sieving the smaller material (2 mm mesh size) followed by measuring the individual particle size (mm) using a ruler. Visible surface organic matter was only present in midden samples, therefore particle size was not assessed for control - non-midden cores.

Midden soil cores were sub-sampled in two locations; the *L. terrestris* drilosphere (5 mm diameter of the *L. terrestris* burrow) and 20 mm away from the burrow (Figure 4.10). A 20 mm distance was selected because it was enough to avoid burrow influence, whilst being undisturbed during sampling. Two soil samples were analysed within midden samples to determine whether:

1. Chemical changes were associated with *L. terrestris* burrowing activity (A).
2. Chemical changes were associated with increased organic matter content (B).

Non-midden cores were sampled in one location, at the centre of the core (position A in Figure 4.10), using a spatula. Soil samples were dried at 105 °C, before organic and inorganic elemental analysis was conducted (methods detailed in Section 3.5).

Figure 4.10. Locations of the soil sampling in midden cores. Drilosphere soil is at point A, around the *L. terrestris* burrow. Midden soil was sampled 20 mm away from the *L. terrestris* burrow (B).

Following soil removal for chemical analysis, earthworms present within soil cores were removed. Juvenile earthworms present in each sample were placed into plastic containers (l. 150 mm x w. 150 mm x d. 75 mm), incubated in Kettering loam soil at 25% moisture content and fed an excess of horse manure, an effective earthworm culturing feed (Butt, 2011). These were incubated at 15 °C in 24 h darkness until the earthworms reached sexual maturity, at which point they were identified (Sims and Gerard, 1999).

4.4.2.3. Statistical Analysis

To determine whether there were any effects of *L. terrestris* midden presence on earthworm population dispersal and biomass, the total earthworm biomass and species richness within “midden” and “non-midden” samples were compared. Paired 2 sample t-tests were applied to determine whether the presence of an *L. terrestris* impacted the mean earthworm density and biomass within midden and non-midden samples. To determine any effects of earthworm population influences on soil functioning within soil middens, a one-way ANOVA was applied to determine whether there were any differences in ecotype distribution of earthworms between “midden” and “non-midden” samples.

To determine whether there were any effects of *L. terrestris* middens on the chemical distribution of soil, the soil macro and micronutrient concentration from 3 sample areas were compared: the drilosphere, soil beneath the midden, and non-midden (Figure 4.10). There were 13 elements analysed via ICP-OES. Where the data for an element was normally distributed, One-way analysis of variance (ANOVA) was used to determine whether there were any significant differences between the soil concentration at positions A (drilosphere), B (midden) and C (non-midden) in Figure 4.10. Post-hoc Tukey pairwise comparisons were conducted to locate the difference between groups. Where the dataset for an element was not normally distributed, non-parametric Kruskal-Wallis tests were applied. Correlation analysis was conducted to determine whether there was any relationship within the variables straw mass, earthworm density and earthworm mass with nutrient concentration.

4.4.3. Results

4.4.3.1. Lilac field survey

Lilac field had a mean pH of 6.8, a mean moisture content of 47.6% and a mean organic matter content of 18.2% (LOI). An earthworm survey identified that 6 species were present in the areas where middens were located (Table 4.3). A mean earthworm biomass of 10.48 ± 1.8 g 0.1 m^{-2} and a mean density of 68 ± 16 ind. 0.1 m^{-2} was recorded (N=5). The mean earthworm density by species is presented in Table 4.3. Although 6 earthworm species were identified, 82.4% were juveniles.

Table 4.3. Mean density (ind. 0.1 m^{-2} ± S.E.) of earthworm species at Lilac Field, Myerscough College, determined from earthworm sampling near midden areas (N=5).

Earthworm Species	**Mean density (ind. 0.1 m^{-2} ± S.E.)**
A. caliginosa	39.2 ± 9.5
L. rubellus	9.4 ± 2.4
A. chlorotica	3.8 ± 0.6
A. rosea	9.4 ± 2.1
L. terrestris	5.4 ± 2.6
A. longa	0.2 ± 0.2

4.4.3.2. Earthworm density and species richness in midden and non-midden soil

Earthworm density and species richness was compared between midden and non-midden samples. The mean earthworm density and biomass per sample was significantly higher within midden soils (2 sample t-test; $p < 0.001$; Figure 4.11). The mean earthworm density of midden samples was 3.26 ± 0.41 ind. treatment^{-1} compared with 1.12 ± 0.18 ind. treatment^{-1} within non-midden samples.

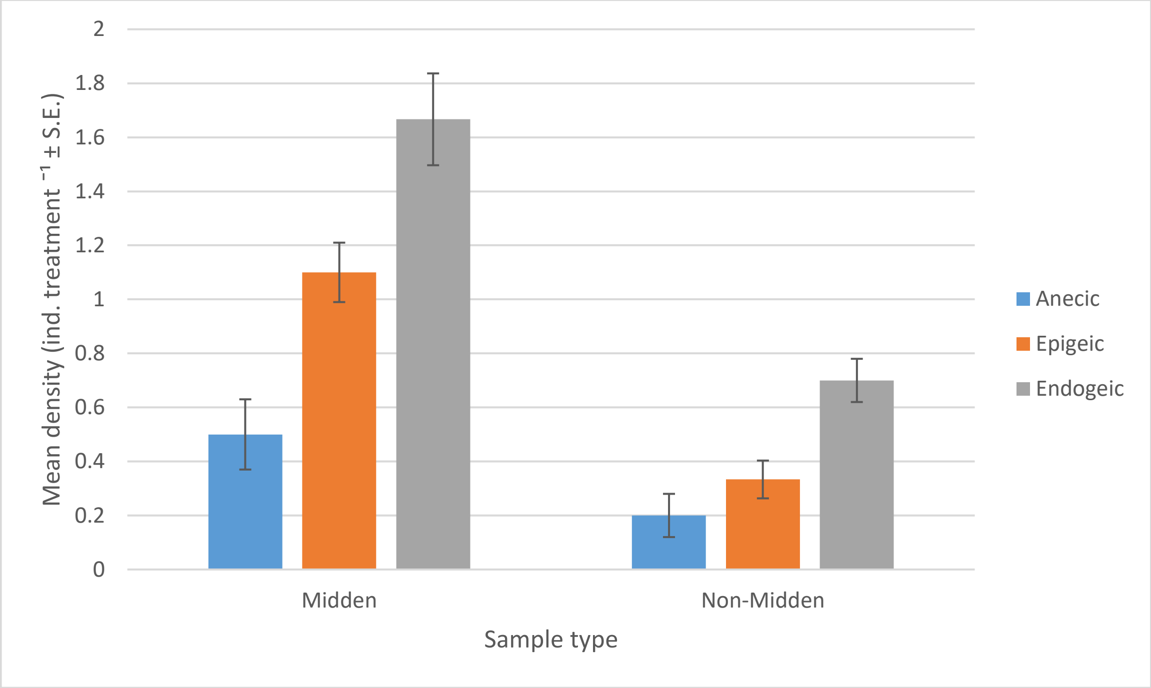


Figure 4.11. Mean earthworm density (ind. treatment^{-1} ± S.E.) between midden and non-midden cores, separated by ecological group.

There were significantly more earthworms within the midden samples compared with the non-midden controls ($p < 0.05$) and a higher species richness (midden N=7; non-midden N=4). Table 4.4 indicated the mean density of earthworm species recorded within midden and non-midden samples.

Table 4.4. Mean earthworm species density (ind treatment^{-1} ± S.E.) of juveniles grown to maturity collected from midden and non-midden samples (N=30). Statistically significant differences between midden and non-midden samples are presented by a different letter ($p < 0.05$; paired 2 sample t-test).

Earthworm Species	**Midden (ind. Treatment $^{-1}$ ± S.E.)**	**Non-midden (ind. treatment $^{-1}$ ± S.E.)**
L. terrestris	0.5 ± 0.22^{a}	0.2 ± 0.09^{a}
A. caliginosa	1.4 ± 0.14^{a}	0.63 ± 0.08^{b}
A. chlorotica	0.13 ± 0.08^{a}	0.07 ± 0.07^{a}
L. rubellus	0.76 ± 0.15^{a}	0.33 ± 0.08^{b}
L. castaneus	0.03 ± 0.03^{a}	0^{a}
O. tyrtaeum	0.1 ± 0.03^{a}	0^{a}
A. rosea	0.3 ± 0.08^{a}	0^{a}

There were significant differences between the density of earthworms by species within midden and non-midden soils ($p < 0.05$; Table 4.4) and a higher earthworm biodiversity within *L. terrestris* midden soils (midden N=7; non-midden N=4). However, Earthworm data from the midden and non-midden cores indicated that there was a trend between the biodiversity of earthworm ecotypes. Although the species richness and density were higher within midden samples, when considering ecotype, earthworm biodiversity between midden and non-midden soil was similar, where endogeic species were slightly more abundant in non-midden soils (Figure 4.12A-B).

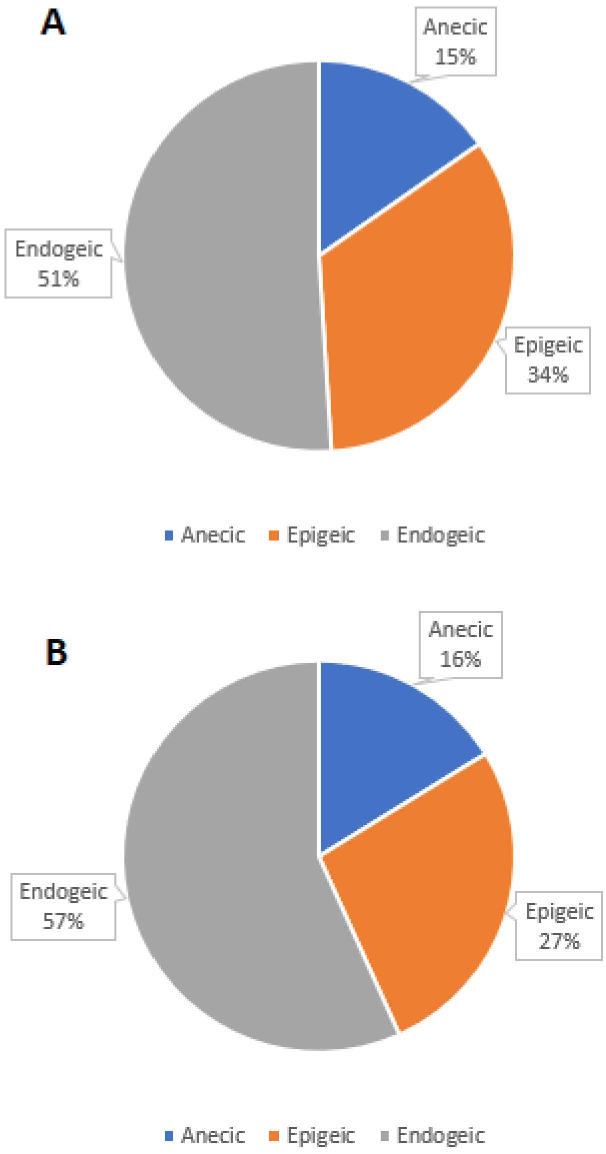


Figure 4.12. Difference in the ratio of earthworm species (%) by ecotype between midden (A) non-midden (B) soils.

Earthworm biomass was significantly higher in midden soils (total 9.2 g; sample mean 0.31 ± 0.06 g) than non-midden soils (total 3.05 g; sample mean 0.1 ± 0.01 g) ($p < 0.001$).

4.4.3.3. Midden analysis

The mean spring barley straw mass within the middens was 0.29 g ± 0.03. Soil moisture on average was higher in midden soils (39.2 ± 0.65%; Figure 4.13), however this was not significant ($p > 0.05$), due to a high variance within samples. Particle size measurements indicated that the mean straw particle length was 3.9 ± 0.1 cm^{-1}, with particle size distribution presented in Figure 4.14.

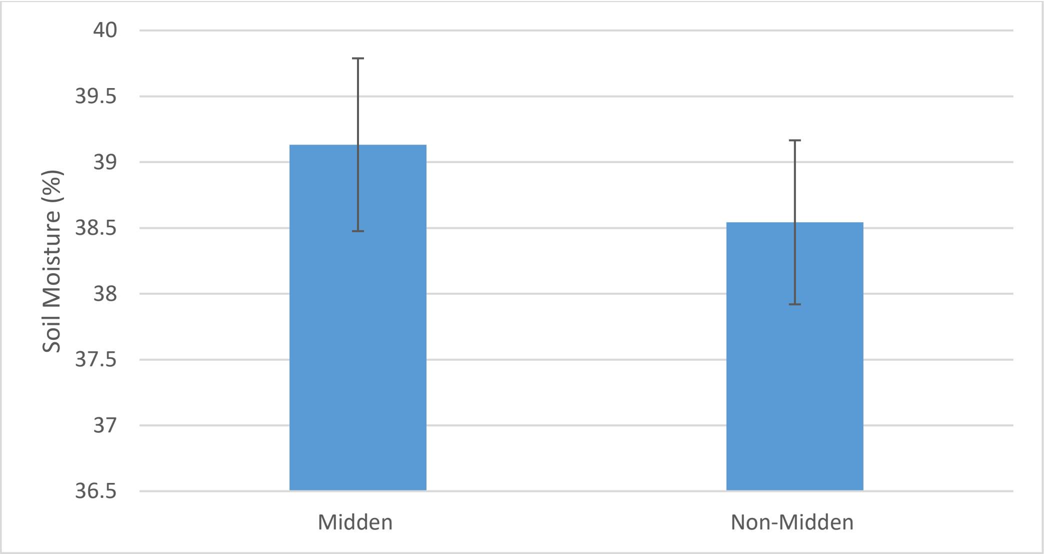


Figure 4.13. Difference in mean soil moisture (% ± S.E.) between midden and non-midden soil cores (N=30).

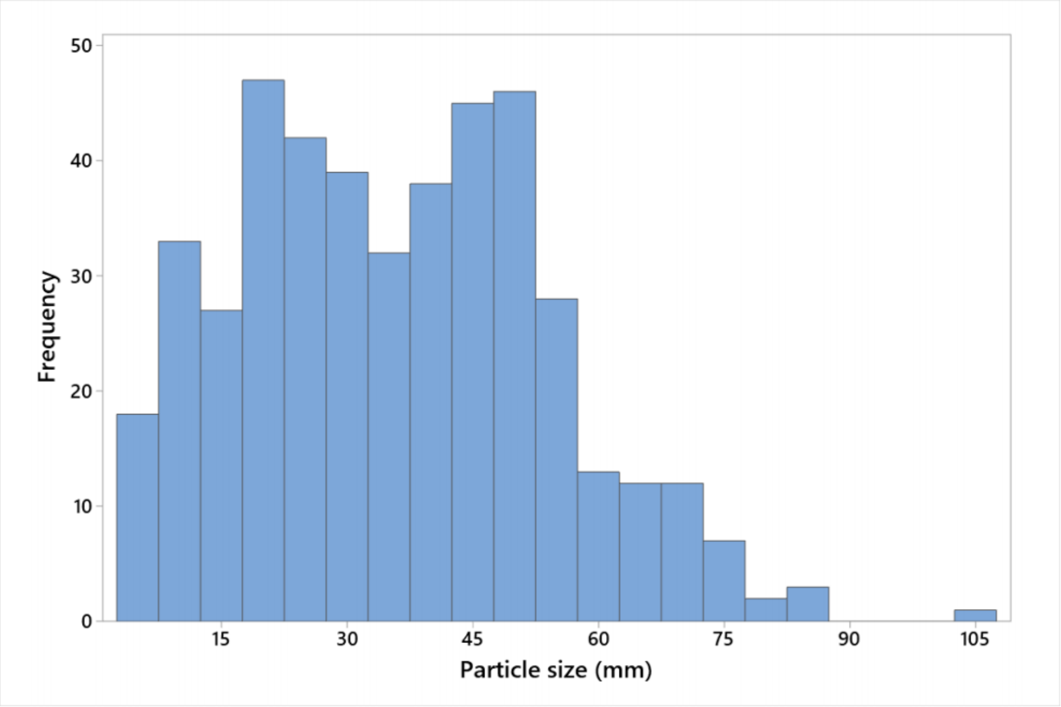


Figure 4.14. Spring barley particle size range within midden samples (N=30), collected at Lilac field, Myerscough College.

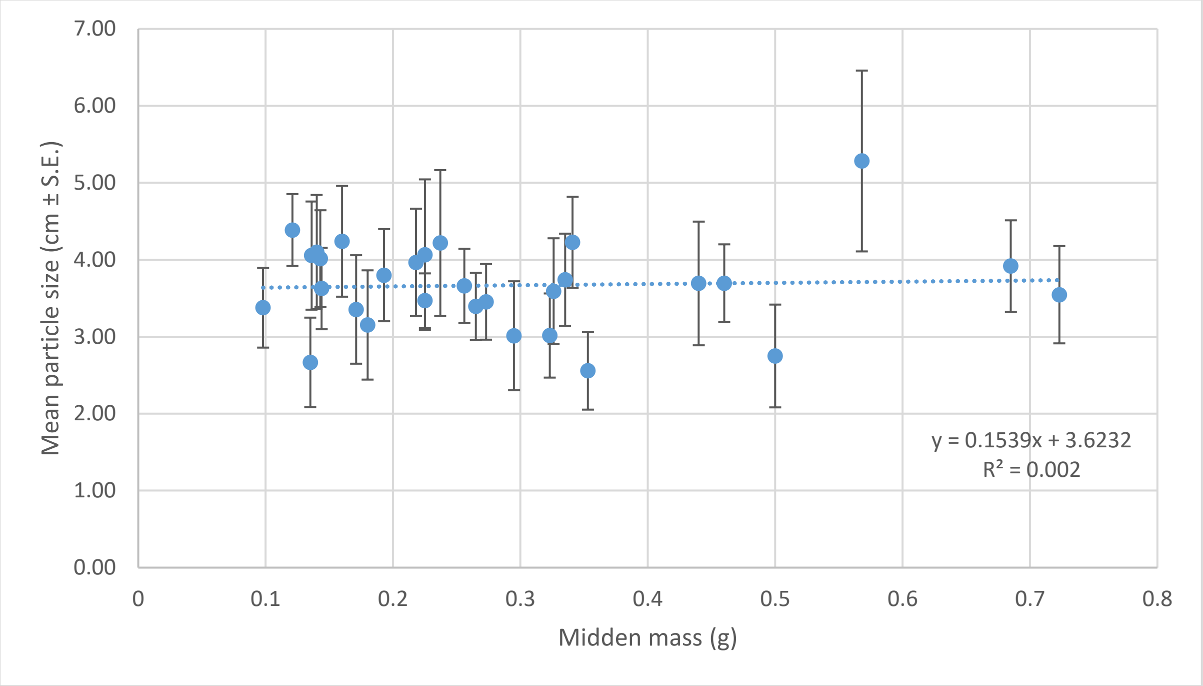


Figure 4.15. The relationship between straw mass (g) and mean particle size (cm ± S.E.) within midden samples collected at Lilac Field, Myerscough College.

There was no effect of midden mass on the mean particle size of the straw within midden samples (Figure 4.15), however this could be due to a low range of midden straw mass (0.1 - 0.7 g). There were no effects of earthworm density and biomass on the straw mass held within a midden ($r^2 = 0.0165$ and $r^2 = 0.0519$).

4.4.3.4. Chemical analysis of midden and non-midden soil

The results from organic chemical analysis are presented in Table 4.5. LOI and %C were lower in midden soils than non-midden soils, however due to the high range between samples, there were no statistical differences between the samples ($p > 0.05$).

Table 4.5. The mean (± S.E.) carbon and nitrogen concentration (%C, %N, C:N ratio, %LOI) of soil samples at A (drilosphere), B (midden) and C (non-midden) soil samples. Due to the mass of soil required, LOI was conducted for samples B and C only. N=30.

Analysis	A	B	C
C (%) (± S.E.)	7.66 ± 0.49	7.30 ± 0.44	7.88 ± 0.52
N (%) (± S.E.)	0.65 ± 0.03	0.62 ± 0.04	0.64 ± 0.04
C:N ratio (± S.E.)	9.12 ± 0.3	9.00 ± 0.4	9.52 ± 0.4
LOI (%)	N/A	18.01 ± 0.9	18.42 ± 1.3

The difference between the total nitrogen concentration of the drilosphere, midden and non-midden soil samples were negligible, where the mean %N was 0.65%, 0.62% and 0.64% respectively (Table 4.5.). A lower C concentration in midden soils with stable N concentration resulted in a lower mean C:N ratio in midden soils.

There was no linear correlation between spring barley mass and %C and %N concentration in midden soils (r^2= 0.0072 and 0.0132 respectively; Appendix II). There were also no effects of earthworm density and biomass on the C and N concentration in soil (Appendix II).

The mean total and available concentration of macro- and micronutrients are presented in Tables 4.6 and 4.7. There was a statistically significant difference within the total nutrient concentration between A (drilosphere), B (midden) and C (non-midden) soils (one-way ANOVA; $p < 0.05$) for all elements analysed. Post hoc (Tukey Kramer) tests determined that the significantly different group was A, which was lower in concentration than B and C for all elements recorded except Boron (Table 4.5).

Table 4.6. The mean total macro and micro-nutrient concentration (mg kg^{-1} ± S.E.) of soils at sample points A (drilosphere), B (midden) and C (non-midden) soils. Statistically significant differences within elemental groups are presented by a different letter (one-way ANOVA; $p < 0.05$).

Element	Mean Concentration (mg kg^{-1})		
	A	B	C
P	155 ± 5.5^{A}	232 ± 6.2^{B}	255 ± 6.1^{C}
Ca	5305 ± 417.6^{A}	6260 ± 374.7^{B}	7502 ± 1019.7^{B}
Mg	3389 ± 71.5^{A}	4124 ± 80.8^{B}	4011 ± 50.6^{B}
K	4260 ± 121.9^{A}	5593 ± 129.4^{B}	5418 ± 101.3^{B}
Mn	446 ± 17.7^{A}	627 ± 20.1^{B}	652 ± 15.0^{B}
Fe	24640 ± 814.8^{A}	29719 ± 924.0^{B}	29284 ± 889.3^{B}
Ni	28 ± 0.7^{A}	36 ± 0.6^{B}	35 ± 0.7^{B}
Zn	94 ± 2.3^{A}	113 ± 2.3^{B}	112 ± 2.3^{B}
B	12 ± 0.4^{A}	8 ± 0.2^{B}	8 ± 0.1^{B}
Na	142 ± 6.3^{A}	232 ± 6.1^{B}	217 ± 5.3^{C}
Co	12 ± 0.4^{A}	14 ± 0.4^{B}	14 ± 0.3^{B}
Cu	23 ± 0.9^{A}	30 ± 0.9^{B}	30 ± 0.9^{B}
Al	43477 ± 1659.6^{A}	95162 ± 2070.7^{B}	119004 ± 1580.7^{C}

Available nutrient concentration was analysed for midden and non-midden soil only (B) and (C). Due to the mass of soil required for the extraction (5 g), it was not possible to analyse the drilosphere. Unlike the total nutrient results, there were some macronutrients (Ca, Mg, K) that had higher concentrations within the midden soil. For elements that had a higher available concentration within non-midden soils, the difference was less than total nutrient concentration.

Table 4.7. The mean available macro and micro-nutrient concentration (mg kg^{-1} ± S.E.) at sample points B (midden) and C (non-midden) soils, conducted through ammonium acetate extraction. Statistically significant differences within elemental groups are represented by a different letter (paired 2-sample t-test; $p < 0.05$).

Element	Concentration (mg kg^{-1})	
	Midden	Non-midden
P	0.19 ± 0.005^a	0.28 ± 0.031^b
Ca	1107 ± 45^a	1080 ± 43^a
Mg	76.84 ± 2.6^a	63.98 ± 10.0^b
K	82.57 ± 5.9^a	67.62 ± 8.5^b
Mn	6.92 ± 0.3^a	8.69 ± 2.0^a
Fe	0.96 ± 0.1^a	1.61 ± 1.2^a
Ni	0.06 ± 0.002^a	0.07 ± 0.02^a
Zn	0.12 ± 0.01^a	0.13 ± 0.08^a
Na	7.11 ± 0.2^a	7.51 ± 1.1^a
Co	0.03 ± 0.001^a	0.03 ± 0.009^a
Cu	0.08 ± 0.003^a	0.11 ± 0.02^a
Al	1.21 ± 0.1^a	2.12 ± 1.1^a

Linear regression indicated the effects of midden straw biomass, earthworm density and earthworm biomass on the nutrient concentration. Of 126 tests, 5 significant relationships were determined. Findings indicated that an increase in straw biomass within a midden resulted in a decline in soil Ni and Co concentration (Ni $R^2 = 0.0396$; Co $R^2 = 0.0227$), however range in concentration between the samples was small (Ni = 10 mg kg^{-1}; Co = 40 ųg kg^{-1}). An increase in earthworm density resulted in an increase in Al concentration ($R^2 = 0.099$). An increase in total earthworm biomass below a midden caused an increase in Fe and Mg concentration (Fe $R^2 = 0.0666$; Mg $R^2 = 0.1248$), however this could have been due to extreme values within the dataset. Significant linear regression results are presented in Appendix II.

4.4.4. Discussion

4.4.4.1. Effects of midden presence on earthworm communities

As with previous results investigating *L. terrestris* and other organisms (Hamilton and Sillman, 1989; Maraun et al, 1999; Schrader and Schabel, 2001; Butt and Lowe, 2007; Stroud et al, 2016a) this survey suggested a positive effect of *L. terrestris* middens on other earthworm species, where there were 272% more earthworms present beneath *L. terrestris* middens than non-midden soil (Figure 4.11) and a higher species richness within midden samples (Table 4.3). This highlights the impact of middens as a biological hotspot in soil. Seven earthworm species were found in midden samples, which is lower than other studies (Butt and Lowe, 2007), but possibly more realistic for agroecosystem soils, which generally have a lower earthworm biodiversity than that of forest ecosystems (Blouin et al, 2013). Unlike Butt and Lowe (2007), no earthworm species were found within aboveground midden masses, only within midden soil. This could be due to the middens being smaller than that visualised in agroecosystems with applied organic matter (mean biomass from this field investigation was 0.29 ± 0.02 g; mean biomass for Stroud et al (2016a) was 6.65 ± 1.2 g), which provided a smaller habitat for epigeics. That said, this study highlighted that there were epigeics species present in soil underneath middens, of which juvenile *L. rubellus* was the most abundant (N = 23). The introduction of epigeic species not commonly found in arable ecosystem settings may accelerate organic matter decomposition and indirectly the nutrient mineralization of these residues into soil (Postma-Blaauw et al, 2006). Therefore, an increased density of *L. terrestris* populations in arable soils could enhance positive earthworm-mediated effects on soil properties, benefitting crop development. Previous (laboratory-based) research into the behaviour of *L. terrestris* and *L. rubellus* suggested that *L. rubellus* inhibits the growth of *L. terrestris* when surface organic matter is limited (Lowe and Butt, 2005). Further investigations into the effects of organic matter addition on the interspecific relationships between earthworms could determine how this affects the decomposition rate of organic matter and benefits soil properties.

Recent findings by Nuutinen et al, (2017) highlighted that although on a local scale *L. terrestris* middens enhance soil fauna populations (earthworms, nematodes and enchytraeids), at a field scale the populations were not significantly different, therefore *L. terrestris* development may lead to a patchiness of soil fauna. This survey supports the regional enhancement of *L. terrestris* middens on earthworm density and species diversity. The ecotype species richness was similar between midden and non-midden soils (Figure 4.12A-B), yet the density was higher (Figure 4.11). This indicates that physical soil functioning engineered by earthworm species may be the same between midden and non-midden soils, just heightened in *L. terrestris* midden areas. A regional localisation of soil functioning by earthworm populations and a minimal change in overall soil functioning over a field scale may not be beneficial within arable agroecosystems, which rely on homogenous soil conditions. However, other unique behaviours of *L. terrestris* (such as a high bioturbation rate) could have benefits on soil regulation within no-tillage areas. The field work reported by Nuutinen et al (2017) was a unique experiment where *L. terrestris* were inoculated (using the EIU technique, Butt et al, 1997), therefore it was possible to determine the impact of *L. terrestris* on a field scale compared to an area of low *L. terrestris* abundance. Further investigations explore the effects of *L. terrestris* population density on the rate of cereal residue bioturbation in arable soils (Chapter 8).

The conception of adult *L. terrestris* middens facilitating other earthworm species has been considered. Results from this survey suggest that midden samples had a higher earthworm biomass, an increased density and increased species richness. Of the earthworms found in midden samples, 86% of earthworms found within midden soils were juvenile. This implied that some facilitation occurred between *L. terrestris* and juvenile earthworms, which has also been observed in laboratory culture (Lowe and Butt, 2002), where juvenile *A. chlorotica* increased in mass compared with monocultures of *A. chlorotica*. It is likely that *L. terrestris* middens were the only source of nearby organic matter that endogeic species could access and provided shelter and moisture to encourage growth. This is supported as the two species that were significantly more abundant in *L. terrestris* middens were *L. rubellus* and *A. rosea*. Presence of a midden would have provided a habitat for epigeic *L. rubellus*, and protection from predation for *A. rosea*, an endogeic species found closer to the soil surface (Bouché, 1977). Although *L. rubellus* and *A. rosea* were more abundant in midden soils, it was not possible to determine whether middens improved the growth and development of these species as there was no control present or timed effects. The interactions between juvenile earthworm species and *L. terrestris* middens would have to be further investigated under controlled conditions in the laboratory to determine any positive effects on growth.

4.4.4.2. Particle size of barley straw held within middens.

This survey provided initial information on the structure and size of *L. terrestris* middens in an agricultural environment. Middens were smaller in average particle size and biomass in the field compared with other studies (Butt and Lowe, 2007; Stroud et al, 2016a), where the average particle size utilised by *L. terrestris* was 3.6 ± 0.1 cm. No crop residues were applied to the surface of Lilac field, therefore any straw utilised by *L. terrestis* was deposited during harvest and stripped from barley stubble, which was still left intact. The action of *L. terrestris* behaviour stripping straw from the epidermis of stubble was recorded in the field by Stroud et al (2016a). Although earthworms seemed to utilise the barley stubble for their burrows alongside weed species, there may not be enough organic matter present to forage in arable systems. This could inhibit *L. terrestris* populations and their benefits and has been seen in some arable systems (Stroud et al, 2016b). This supports findings by Stroud et al (2016a), where *L. terrestris* developed larger middens in areas with applied organic matter (horse manure) alongside stubble. The calorific value of straw is higher than that of horse manure or anaerobic digestate, which can increase the rate of *L. terrestris* growth (Sizmur et al, 2017). However, applications in field settings could be too large for *L. terrestris* to utilise as feed. The usage of straw in midden habitat development may be more useful to *L. terrestris* than the food availability, particularly when other digestible organic matter may be present. The addition of another organic matter source may also enable *L. terrestris* populations to retain mass and utilise barley straw as it further degrades with increased time. Future investigations in this thesis will determine how *L. terrestris* utilises cereal straw based on the modal particle sizes applied to the field.

The mean midden straw particle size was 3.9 cm which was uniform with increasing midden biomass (Figure 4.15). This suggests that *L. terrestris* may have a preference of particle size to utilise within its midden. However, due to the low amount of surface organic matter available, *L. terrestris* selection will have been limited, where observations indicated that burrows were situated near to stubble rows. Although *L. terrestris* is amongst the most surface-active earthworm species, it often does not forage further than the length of its body from the burrow (Edwards and Bohlen, 1996; Nuutinen and Butt, 2003). The ability of *L. terrestris* to return to its burrow was examined by Nuutinen and Butt (2005), where it was observed that in some circumstances *L. terrestris* can fully leave its burrow to source food, where its tail end can relocate the burrow wall, to a maximum of 0.7 m. In agricultural settings, organic matter resource deficiencies may encourage *L. terrestris* to populate nearer crop stubble, where *L. terrestris* stripping straw, leaves and stubble was recorded by Stroud et al (2016a). The effect of particle size may be a ‘luxury’ option, or the production of middens and food resource availability are two different entities. Further behavioural investigation into *L. terrestris* selection of straw particle and the impacts on midden structure are investigated in Chapter 7.

4.4.4.3. Effects of midden presence on the organic and inorganic nutrient concentration of soil.

4.4.4.3.1. Organic chemical analysis (C and N)

The mean C and N concentration between midden and non-midden samples was not statistically significant (Table 4.4). There may be other environmental factors which influence the C and N soil concentration alongside *L. terrestris* activity. This field study analysed a low number of replicates (N=30). Increasing replication may increase the likelihood of significant results in future. In this field survey, it was not possible to define midden age. A midden habitat is a constantly changing environment, where C and N dynamics may increase with increasing activity over time. The effects of earthworm activity on terrestrial C and N cycles is a growing area of research, where results are inconclusive. Previous field research determined that C and N levels in forest and agroecosystems increased under earthworm middens (Wilcox et al, 2002). Laboratory investigations have been designed to determine the impact of *L. terrestris* populations on C and N emissions (Niemen et al, 2015; Lubbers et al, 2017). Findings indicated that a higher earthworm density increased CO_2 emissions through respiration and N_2O emissions through high mineral content and increased soil moisture below the midden (Nieminen et al, 2015). The relationship between earthworm species (*L. rubellus* and *A. caliginosa*) activity and carbon distribution in soils was explored experimentally by Lubbers et al (2017). Findings indicated that increased earthworm activity enhanced CO_2 emissions and carbon incorporation into soils, there was a lower overall soil C concentration due to the enhanced organic matter decomposition. A more extensive study of the C and N dynamics of soil below *L. terrestris* middens in Finnish arable ecosystems was conducted by Sheehy et al (2019). This study analysed the C and N concentrations within macro- and microaggregates associated within middens. Although differences were observed between experimental fields and soil texture, it was evident that *L. terrestris* developed larger macroaggregates than surrounding bulk soil, which accumulated more microaggregates which were higher in SOC and N concentration. Future experiments could assess the flux in carbon and nitrogen within an *L. terrestris* midden environment to determine whether storage within casts outweighs the losses through respiration and surface degradation of organic matter.

Within midden samples, the effects of organic matter biomass, earthworm density and earthworm biomass on the C and N concentration was tested. It was hypothesized that middens with increased organic matter mass would increase the C and N concentration. It was also hypothesized that a higher earthworm density and biomass present within middens could increase soil nitrogen and carbon concentration, due to the evidence that earthworms mineralize nitrogen in their casts and produce SOC within macroaggregates (Agapit et al, 2018; Angst et al, 2017; van Groenigen et al, 2019). Results from this experiment indicated that there were no effects of midden straw mass, total earthworm density and total earthworm biomass on the C and N concentration in soil (Table 4.4; Appendix II). Research into the effects of earthworm density and biomass on carbon and nitrogen dynamics have mainly focussed around the impacts of changing tillage practices on earthworm density and concentration (Ernst and Emmerling, 2009, Umiker et al, 2009; Arai et al, 2013). The results from these experiments indicated how increased earthworm presence in no-till soils increased the macroaggregate fraction of soil, which stored more SOC and N. However, analysis of the effects of earthworms on C and N over a longer temporal scale indicated that the flux between organic matter decomposition and CO_2 and N_2O emissions (discussed by Lubbers et al, 2017) caused by increased earthworm density may result in there being no overall difference. Further research could investigate the effects of earthworms and organic matter applications on the C and N concentration under controlled conditions, where temporal changes can be quantified.

4.4.4.3.2. Macro- and Micronutrient concentration: comparisons between midden and non-midden soils.

Nutrient concentration analysis of midden and non-midden soils was conducted to determine whether *L. terrestris* increase the mobility (total nutrient concentration) and plant availability (available nutrient concentration) of macro and micronutrient concentrations. Research suggests that activity within an *L. terrestris* midden-burrow complex increases nutrient concentration in soils (Devliegher and Verstraete, 1997; Blouin et al, 2015; Stroud et al, 2016a). Of the total nutrient results, the drilosphere soil was significantly different in concentration to soil beneath a midden (B) and non-midden soil (C) (Table 4.5), where concentrations were lower. A number of factors could have caused this reduction. Higher levels of infiltration occur with the presence of *L. terrestris* burrow walls (Andriuzzi et al, 2015a), where in this experiment, the soil moisture content was higher underneath middens compared with non-midden soils (Figure 4.13). It could be possible that elements within burrow walls are leached down the earthworm burrow into a lower soil profile. This experiment analysed soil up to a maximum 60 mm depth, so it was not possible to determine whether there were any effects of leaching or burrow presence on the nutrient concentration. Future experiments could explore the total nutrient concentration of an *L. terrestris* burrow at increasing depth.

It is possible that *L. terrestris* reduced the total nutrient concentration through bioaccumulation, where *L. terrestris* consumes soil around the burrow edge to maintain its burrow wall. Research of earthworm bioaccumulation has been focussed around polluted soils and ecotoxicity, where the elemental focus was in potentially toxic elements (PTEs) and not macro- and micronutrients (Kennette et al, 2002; Ernst and Frey, 2007; Mombo et al, 2018). Bioaccumulation laboratory experiments have been conducted, but often not for *L. terrestris*. Investigations of the earthworm *Octadrilus* *complanatus* have indicated that this species can bioaccumulate aluminium, where bioaccumulation increased with larger applications (up to 3000 mg g^{-1}) (Billalis et al, 2013). *O. complanatus* a European and African earthworm species descendent from the Lumbricidae, with a similar life cycle and behaviour to *L. terrestris* (Monroy et al, 2007). It is possible that *L. terrestris* may bioaccumulate aluminium, which could explain the concentration difference (Non-midden Al > Midden Al > Drilosphere Al) presented in Table 4.5.

L. terrestris can bioaccumulate Zn from organic matter (Kizilkaya, 2005). When fed different organic matter types, the highest concentration of zinc bioaccumulated was from wheat residues. It was predicted the high bioaccumulation under this organic waste was due to its high C:N ratio. Although this paper related zinc bioaccumulation to nutrient uptake by birds and mammals of higher trophic levels, it highlighted that under straw applications (such as middens) the zinc may not be released from the organic matter as it is accumulated within *L. terrestris* biomass. This supports why total nutrient concentration may be lower around *L. terrestris* middens (Table 4.3). An earlier published experiment by Kizilkaya (2004) investigated the impact of sewage sludge applications on the bioaccumulation of Zn and Cu by *L. terrestris*. Findings indicated that applications of Zn and Cu increased the concentration within *L. terrestris* biomass, but the highest concentration was within *L. terrestris* casts. More research is required to determine the effects of *L. terrestris* bioaccumulation effects on the cycling of elements held within organic matter.

In contrast to the total nutrient concentration results, the available nutrient concentration within earthworm middens was higher than non-midden samples for selected elements (Table 4.6). The selected elements were Ca, Mg and K. This agrees with previous midden analysis by Stroud et al, (2016a) where K increased. However, although the differences in K concentration in this analysis were significant ($p < 0.05$), differences in K concentration between midden and non-midden samples were not as high within this experiment. The differences between concentrations could be due to a number of factors, such as crop type (wheat vs barley straw), the timing of the study in the harvest year (spring vs autumn) and the differences in soil types between areas (North West UK Vs Midland UK). The indication that the mean concentration of Mg and K were significantly different ($p > 0.05$), where the means were higher within midden soils supports the hypothesis that *L. terrestris* has a positive effect on nutrient availability to plants (Athman et al, 2017).

An increase in available nutrient concentration in soil beneath *L. terrestris* middens may be exclusive to certain nutrients, where there was a reduction in the concentration of some elements, such as phosphorus (Table 4.6). Phosphorus is not as mobile as other macronutrients such as N and K.

Therefore, in P-limiting environments, fertilization is the main method to increase concentrations. The concentration of some metals impacts the bioavailability of others, which may inhibit plant availability. For example, aluminium is a very abundant element in soil, but its presence at high concentrations, particularly in acidic soils, can limit plant growth (Kochian et al, 2005; Kochian et al, 2015). Aluminium can also bond to phosphorus making it less available to plant roots (Foy et al, 1978). In this analysis, it was found that aluminium concentration decreased around the midden soil samples in both available and total forms. High aluminium levels can cause phosphorus fixation, therefore low aluminium levels around midden soils may enhance P availability. Therefore, within a phosphorus-limiting environment, the presence of *L. terrestris* may assist phosphorus adsorption by crops.

It could be possible that the nutrient concentration was lower around an *L. terrestris* drilosphere because nutrients were concentrated within *L. terrestris* casts. The nutrient concentration of *L. terrestris* casts within middens was not analysed. This was due to the low mass of the middens and the field conditions influencing identification. Theoretically, casts deposited on the soil surface around the midden will have been analysed through the homogenisation of midden soil prior to soil extraction, however the ratio of cast to soil will be very low. Comparisons between the nutrient availability in casts compared to bulk soil have indicated that there are significantly higher concentrations held within casts (Clause et al, 2014; van Groenigen et al, 2019). Due to the initial fragility in cast structure and the high level of rainfall experienced within the UK, it is possible that cast-available nutrients are leached through the soil or eroded by surface runoff (the raindrop effect) (Le Bayon and Binet, 1999). Further laboratory research will identify whether *L. terrestris* casting mobilises elements in the soil.

4.4.4.3.3. Macro- and Micronutrient concentration: effects of physical and biological components within midden

To locate any observed differences in nutrient concentration between midden and non-midden soils (Table 4.5), correlation analysis was conducted to determine whether there was a relationship between the physical (straw mass) and biological (total earthworm density, total earthworm biomass) components of the midden area on soil nutrient concentration. Overall, no correlation was determined between 121 of 126 tests. Of the 5 observed relationships, 3 were caused by the presence of extreme values (Appendix II), which will not be discussed further. Findings indicated negative correlation between straw biomass with Ni and Co concentration. A reduction in Ni with increasing midden mass could be positive for crop growth and earthworm development, where Ni is phytotoxic at high concentrations, causing elongated roots (Wallace et al, 1977) and toxic for earthworm species (Gish and Christensen, 1973; Bigorgne et al, 2010; Hirano and Tamae, 2010). At low concentrations, Ni is important for crop growth due to its increase in urease, which encourages root and shoot development (Brown et al, 1987; Welsh and Shuman, 1995). Bioaccumulation of Ni by earthworms has previously been investigated, where findings suggested that *Eisenia fetida* increased the rate of bioaccumulation in degraded soils (Kilowasid et al, 2015). Schrader (1992), found that Ni was concentrated within the calciferous gland tissue towards the intestine, and not excreted. Cobalt is a micronutrient important for crops where it encourages stem growth and enhances nitrogen fixation in legumes (Welsh and Shuman, 1995). Co is considered an essential element for earthworms, as it is a component of the B12 vitamin (Neuhauser et al, 1984). To reduce plant iron deficiency, Co is regulated by keeping a neutral soil pH. The soil pH at Light Ash farm was 6.8, which is optimum to inhibit metal activity. As with Ni, investigations of bioaccumulation have focussed around *E. fetida* (Singh et al, 2014; Crossley Jr et al, 1995). Further investigations of the effects of the *L. terrestris* midden-burrow complex on soil chemistry is required to determine their impact on biogeochemical cycling in agroecosystems.

A number of factors could have influenced the lack of relationships between the physical and biological components of the midden with soil nutrient concentration. Firstly, *L. terrestris* midden sizes were small in comparison to other studies (Butt and Lowe, 2007; Stroud et al, 2016a), where the variance of organic matter between *L. terrestris* midden samples was low (0.098 g – 0.723 g). Larger and more developed middens may have encouraged further chemical changes in soil below. For correlation analysis, although the number of samples were acceptable (N=30), an increased number of replicates will increase the likelihood of a relationship. This field survey analysed middens over one field; enhanced sampling methods over increased geographical areas may provide more evidence on the effect of *L. terrestris* in agroecosystems. In this experiment, the most abundant organic matter available for midden development was barley stubble and weeds, which are lower in nutrient concentration than the harvested barley crop and less biodegradable than the leaf areas. There may be differences in nutrient concentration within midden samples if the crop selected is not homogenous. To improve this comparison, an increased sample size with repeated measures would be required and further laboratory experiments to control midden mass size and organic matter applications.

Overall, the findings from this midden survey highlighted a number of possible indirect benefits to agroecosystems by *L. terrestris*. Increased earthworm abundance supported evidence that middens are 'biological hotspots' within arable soils and that increased *L. terrestris* populations may enhance soil functioning by introducing more epigeic species. However, chemical interactions between *L.*

terrestris middens and surrounding soil was less clear. The results suggested that *L. terrestris* middens had some unique effects on the soil chemistry, where the total nutrient concentration reduced around the burrow and the available nutrient concentration increased for selected elements. The spatial patchiness of soil fauna created by *L. terrestris* middens may have a number of benefits to soil properties, notably an increased soil resilience through the spatial asymmetry of soil organic matter (van Apeldoorn et al, 2011) which may improve responses to environmental stresses (Brussaard et al, 2007; Harvey et al, 2019). *L. terrestris* dispersal has been monitored over long temporal periods (Nuutinen et al, 2017) and ecological models exist on their behavioural choices (e.g. Johnston et al, 2017), however the numerous environmental factors which influence their behaviour (Butt and Nuutinen, 2005) make spatial heterogeneity a challenging area of research.

Spatial patchiness of *L. terrestris* can be influenced significantly by many factors such as resource availability (Butt et al, 2003), particle size (Lowe and Butt, 2003), soil moisture (Butt and Nuutinen, 2005). Dispersal of *L. terrestris* usually occurs aboveground where they can migrate over long distances. Within agroecosystems, integrated management systems could enhance resilience (Szymczak et al, 2020), however frameworks to assess soil resilience within agroecosystems under environmental stresses are still to be determined (Peterson et al, 2019). Introduction of soil biological networks within these models may enhance knowledge on environmental resilience within agroecosystems. Further research on the effects of agroecosystem management on *L. terrestris* populations is required to determine how waste organic matter influences their spatial heterogeneity and build on the relationships determined by Sheehy et al (2019). This thesis will explore further the aboveground behaviour of *L. terrestris* when offered organic matter of different particle sizes and rates of degradation to determine how these factors influence midden development and earthworm growth and reproduction. Findings could indicate how spatial patchiness could be influenced by waste organic matter addition and recommend application methods for agroecosystem managers.

4.5. SUMMARY OF CHAPTER FINDINGS

- Waste organic matter can increase earthworm populations when compared with normal fertilisation procedures and a control plot.
- Although waste organic matter can increase earthworm abundance, this is impacted by plant type.
- The most common earthworm species found in arable systems were: *A. caliginosa*, *A. longa*, *A. chlorotica* and *L. terrestris*. These were subsequently used in laboratory experiments.
- *L. terrestris* can increase other earthworm population size and species diversity through the development of middens.

5. ASSESSMENT OF WASTE ORGANIC MATTER APPLICATIONS ON EARTHWORM SURVIVAL, GROWTH RATE AND REPRODUCTION THROUGH LABORATORY MICROCOSM EXPERIMENT

5.1. Introduction

Waste organic matter applications in agroecosystems can enhance earthworm populations and change the species diversity, which alters soil functioning. However, there are significant differences in the effects of applications on earthworm population size, growth and fecundity, where it has been suggested that the C:N ratio, lignin content and particle size may be limiting factors on earthworm assimilation of organic matter. To be applied in agriculture, waste organic matter has to reach a UK PAS 110 standard, to ensure that it is not toxic to the environment and human health.

This chapter will assess the effects of common agricultural waste organic matter applications on earthworm health, by assessing the effect on growth rate and reproduction. Laboratory studies were conducted at UCLan, Preston, to investigate the effect of waste organic matter content on growth and reproduction rates of four earthworm species: *A. longa*, *A. caliginosa*, *A. chlorotica* and *L. terrestris*. These earthworms have different burrowing and feeding activities and are common in agricultural settings, where they were the most abundant in Section 4 experiments.

Five waste organic matter types were selected: horse manure, anaerobic digestate (dry fraction), anaerobic digestate (wet fraction), composted green waste and wheat straw residues. These were selected to reflect different agricultural practices. Horse manure could be used as a waste organic matter treatment, however for this experiment was used as a control treatment, due to it being a known effective substrate for earthworm cultivation (Butt, 2011).

5.1.1. Aim

To determine the effects of selected waste organic matter applications on earthworm health and fecundity.

5.1.2. Objectives

To examine the effects of selected waste organic matter treatments on the growth rate of juvenile earthworm species.

To investigate the effects of selected waste organic matter treatments on the reproductive rate of adult earthworm species.

5.1.3. Hypothesis

There will be differences in earthworm rate to maturity based on organic matter type.

5.2. Materials and methods

Earthworm growth and reproduction microcosm experiments were conducted under controlled laboratory conditions in the earthworm research group lab at UCLan in Preston, UK from the period of 2015-2016. For these experiments juvenile earthworms were sampled from Walton Hall farm, Preston, UK, following the procedure described in Section 3.3.2.

5.2.1. Organic matter treatments

In this experiment, five organic matter treatments were selected. The organic matter type and its source is defined in Table 5.1. Prior to experimental start, the %N was measured for each waste treatment using CHN analysis (method described in Section 3.5.3).

Table 5.1. Information on waste organic matter applications used.

Experimental Abbreviation	**Waste organic matter type**	**Source**	**N (%)**
AD	Anaerobic Digestate (Dry Fraction)	Riverford Organics, Home Farm.	0.91
HM	Horse Manure	Wigton, Cumbria	1.21
ST	Wheat straw residues	Farington, Lancashire	0.45
LAD	Liquid Anaerobic Digestate	Global Renewables, Chorley, UK	0.86
CGW	Composted Green Waste	Global Renewables, Chorley, UK	0.31

Prior to the experimental start, waste organic matter types AD, HM, ST and CGW were dried at 105 ^{o}C for 24 hours and then blended using a Magimix blender (Compact 3100) to reduce the particle size and stored in a dry plastic container in the dark. LAD was stored in the fridge at 4 ^{o}C until experimental start.

5.2.2. Earthworm species selection

The earthworm species selected for this experiment were *A. longa*, *A. caliginosa*, *A. chlorotica* and *L. terrestris*. These species were selected because they are commonly found in UK soils (Sims and Gerard, 1999) and were the most abundant species in field survey trials (Section 4). Each earthworm species has differing burrowing and behavioural characteristics (Section 2.2; Bouché, 1977), which may influence their response to the feeds selected (Curry and Schmidt, 2007) and soil functioning (Blouin et al, 2013).

Following sample collection, earthworms were prepared for the experiment using the procedure defined in Section 3.3.3 and contained in incubators under full darkness at 15 °c. Juvenile earthworms of equal masses were used for the monocultures (Table 5.2).

Table 5.2. Information on the earthworm species used in this experiment.

Earthworm Species	Number of juvenile earthworms used per treatment (n).	Average individual earthworm biomass (mean ± S.E.)	Average earthworm biomass per treatment (mean ± S.E.)
Apporectodea longa	2	0.47 ± 0.02 g	0.93 ± 0.04 g
Apporectodea caliginosa	3	0.28 ± 0.01 g	0.83 ± 0.05 g
Allolobophora chlorotica	3	0.088 ± 0.0007 g	0.26 ± 0.002 g
Lumbricus terrestris	2	1.28 ± 0.03 g	2.55 ± 0.08 g

5.2.3. Growth experimental design

Preparation of the samples differed between earthworm size and ecological grouping. For smaller earthworm species (*A. longa*, *A. caliginosa* and *A. chlorotica*), plastic containers of 500 ml volume (Lakeland, UK) were used. To account for the deeper burrowing behaviour and larger size of *L. terrestris*, larger 1 L plastic containers were used, as recommended in Butt and Lowe (2005). Organic matter application method differed between ecological grouping. For anecic earthworms (*L. terrestris* and *A. longa*), Kettering loam soil (500 g for *A. longa* samples, 1 Kg for *L. terrestris*) was pre-wetted to 25% moisture contrent and added to the plastic containers. Juvenile earthworms were placed on the surface and left to burrow. Dried organic matter was homogenised to an equal particle size (< 1 cm) using a Magimix 4150 W blender and 5 g was re-wetted and added to the soil surface. Liquid anaerobic digestate was surface applied at 10 ml volume. Although a higher mass:volume ratio to other treatments, this enabled the liquid digestate to be more evenly surface applied. All organic matter treatments were applied in excess; therefore this was not expected to compromise the experiment.

For endogeic earthworms (*A. caliginosa* and *A. chlorotica*), 5 g dried organic matter (< 1 cm particle size) was mixed with 500 g dry Kettering loam soil before pre-wetting to 25% moisture content. Soil with organic matter was added to 500 ml plastic containers and juvenile earthworms were placed on the surface and left to burrow. For liquid anaerobic digestate samples, Kettering loam was made to 25% moisture content before addition to ensure the organic matter was evenly mixed. This also ensured the overall moisture content under this treatment was equal for anecic and endogeic earthworm treatments. A diagram of the experimental design of the 4 earthworm samples is demonstrated in Figure 5.1.

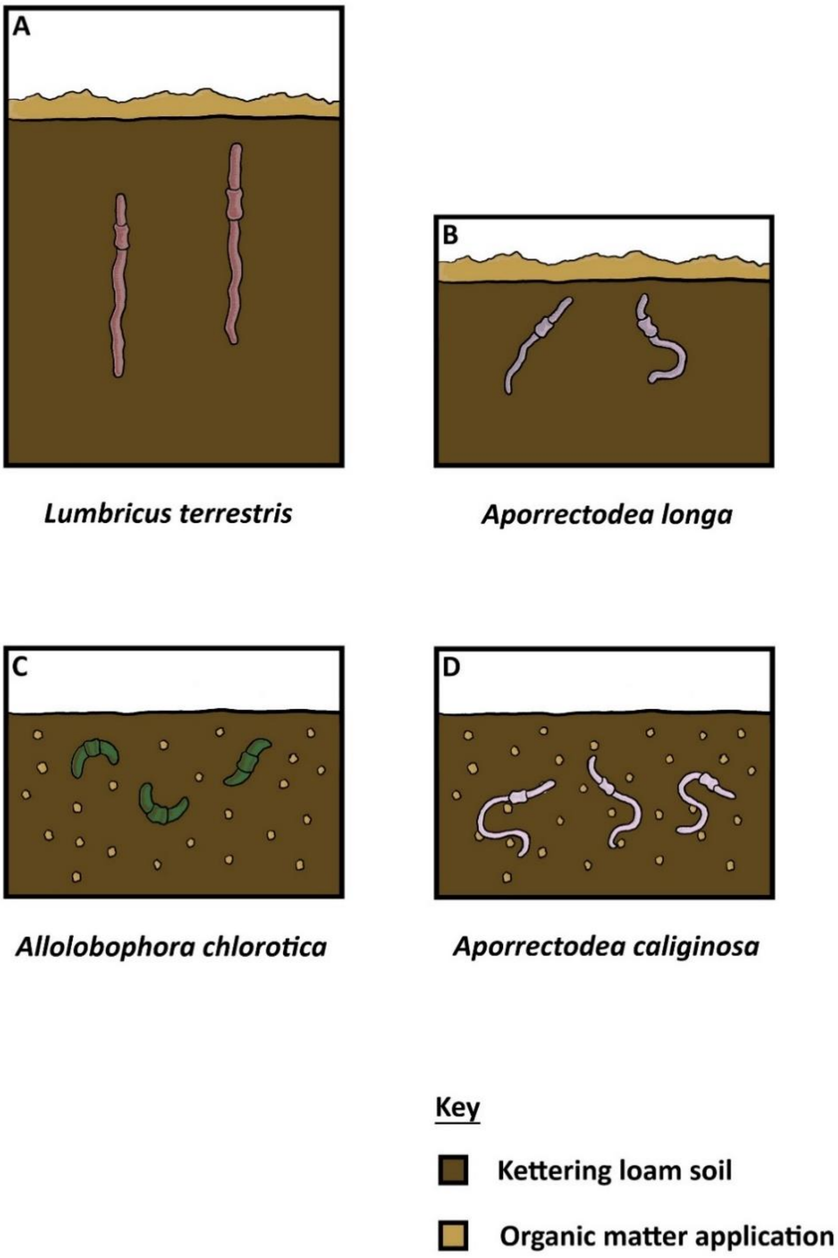


Figure 5.1. A diagram of the growth and reproduction experiment sample design for each earthworm species (*A. caliginosa*, *A. chlorotica*, *A. longa* and *L. terrestris*). For anecic *L. terrestris* and *A. longa*, equal masses of organic matter were surface applied to each sample. For endogeic *A. chlorotica* and *A. caliginosa*, equal masses of organic matter were mixed with Kettering loam, as recommended in Lowe and Butt (2005).

There were 5 replicates per organic matter type alongside 5 replicates of a control treatment with no organic matter application. Treatments are for the four growth experiments are defined in Table 5.3.

Table 5.3. Matrix of laboratory growth experiments undertaken. Type of earthworm species vs organic waste type.

	Horse Manure	**Anaerobic Digestate**	**Straw**	**Liquid Anaerobic Digestate**	**Composted Green Waste**	**Control**
A. longa	X	X	X	X	X	X
A. caliginosa	X	X				X
A. chlorotica	X	X	X			X
L. terrestris	X		X			X

The 4 earthworm growth and reproduction experiments were not conducted during the same time period. Available waste treatments were first tested on *A. longa*. Treatments giving poor results were not examined further. Organic waste treatments in subsequent growth and reproduction experiments tested effects on selected earthworm species.

Throughout the duration of experiment, samples were incubated at 15 °C under full darkness. Earthworms had their masses determined every two weeks. To determine earthworm mass, samples were destructed using the following method. Any remaining surface applied organic matter was firstly removed using forceps and placed onto a clean aluminium tray. Below soil was destructed in a plastic tray and earthworms were located. To remove attached soil, earthworms were washed with clean water and dried before determining mass. Growth rate was recorded as the mean change in sample biomass (g ± S.E.).

Following mass determination, an additional 5 g fresh organic matter was applied. For anecic earthworms, soil contained in the plastic tray was deposited back into the sample container and earthworms were placed onto the soil surface and left to burrow. Any remaining organic matter was placed back on the surface from the aluminium tray. The additional 5 g fresh organic matter was re-wetted and applied to the surface. For endogeic species, 5 g organic matter was added to the plastic tray and mixed with the soil, before depositing back into the sample container and re-introducing the earthworms. This maintained the sample design in Figure 5.1 throughout the experiment.

This bi-weekly mass determination and re-feeding of earthworm samples continued until earthworms were clitellate. Once the earthworms reached sexual maturity, reproduction (cocoon production) (n ± S.E.) was recorded alongside earthworm mass change. Samples were placed in a plastic tray and earthworms were located and removed for mass determination. Following the procedure outlined in Section 3.3.3., soil and organic matter was sampled for cocoons using appropriate mesh sizes for earthworm species. Collected cocoons had their mass determined then placed in petri dishes with wetted filter paper (Whatman no 1) inserted, where they were stored at 4 °C in the fridge.

Experiments were ended once the earthworms for successful treatments had reached sexual maturity (clitellate) and after there were over 8 successive weeks of reproductive activity. The timing was different for each earthworm species (*A. longa* = 32 weeks; *A. caliginosa* = 32 weeks; *A. chlorotica* = 24 weeks; *L. terrestris* = 40 weeks) due to temporal differences in life cycles (Edwards, 2004).

5.2.4. Statistical analysis

To determine whether there were any effects of organic matter type on earthworm growth rate, the change in earthworm biomass over time between treatments was statistically analysed (SPSS v. 27). Due to the difference in the number of WOM treatments between earthworm species (Table 5.3), statistical tests were performed for each earthworm species separately. Following testing for normality and equal variances, nonparametric Friedman’s tests were applied on the biomass data to determine whether there were any significant differences between treatments. If a significant difference occurred, post-hoc Wilcoxon tests were used to define the significant group.

To determine whether there were any effects of organic matter type on reproduction, the difference in total cocoon production (n) between treatments was analysed. Following normality testing, nonparametric Kruskal-Wallis tests were applied to determine the difference in mean cocoon production between organic matter treatments. Post-hoc Dunn-Bonferroni tests located which treatments were significantly different.

5.3. Results

5.3.1. A. longa

Of the organic matter types applied, 3 treatments had successful earthworm growth rates (Figure 5.2), where earthworms reached sexual maturity over the experimental period. Two treatments, LAD and CGW were unsuccessful, where the mortality rate was higher and faster than the control.

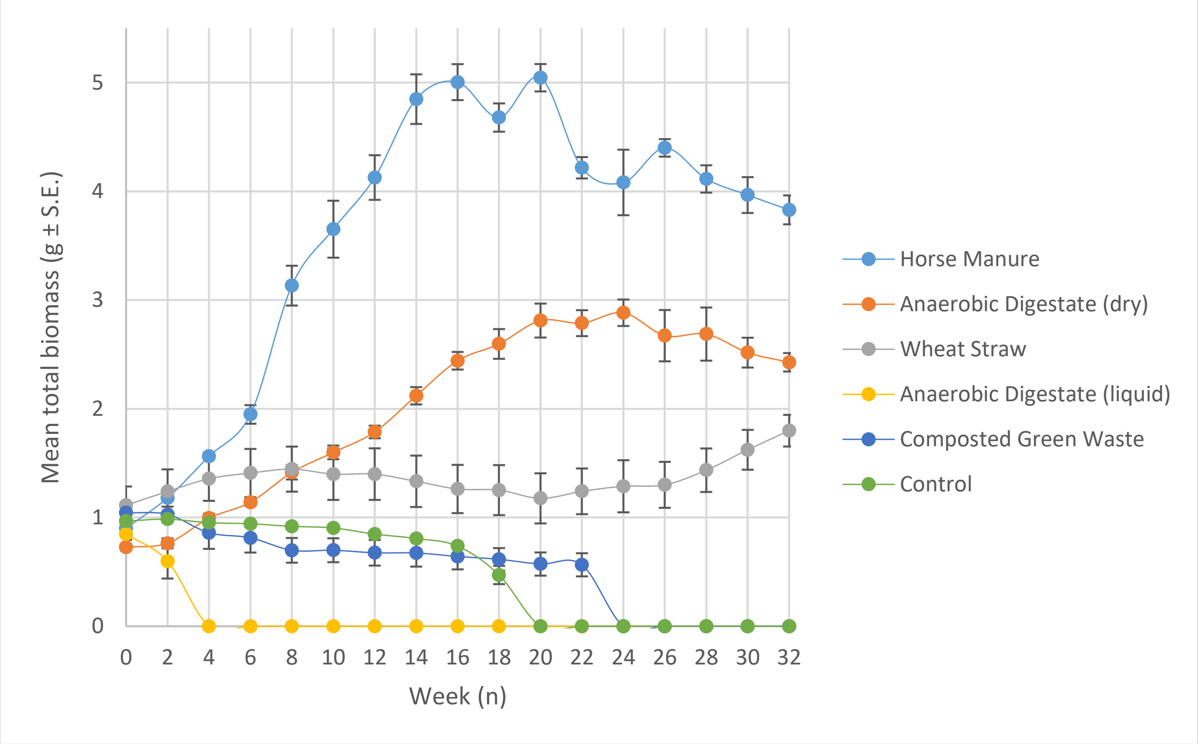


Figure 5.2. Changes in mean biomass (g ± S.E.) of *A. longa* (N=2) (32 weeks) under horse manure, anaerobic digestate (dry), wheat straw, liquid anaerobic digestate and composted green waste mono-applications with a control soil treatment. N=5.

Applications of horse manure resulted in a faster growth rate, where *A. longa* reached sexual maturity at 10 weeks. Anaerobic digestate had 70% adults at 20 weeks, where under wheat straw applications 60% of *A. longa* were adult at experimental end (Figure 5.3). Following sexual maturity, *A. longa* biomass reduced as they began to copulate. Mean adult biomass was lower under anaerobic digestate and wheat straw applications (HM = 2.5 g; AD = 1.4 g; ST = 0.9 g). The effect of organic matter application on biomass change over time was significantly different between all treatments (Friedmans test; x^2 (2) = 623.355; $p < 0.001$). Post-hoc Wilcoxon signed rank tests confirmed that all treatments were significantly different ($p < 0.001$).

Under LAD and CGW applications the mortality rate was 100%, where *A. longa* did not gain mass throughout the experiment. The mortality rate was faster under LAD applications than the control, which suggests that it is toxic. These unsuccessful treatments were not applied in future experiments.

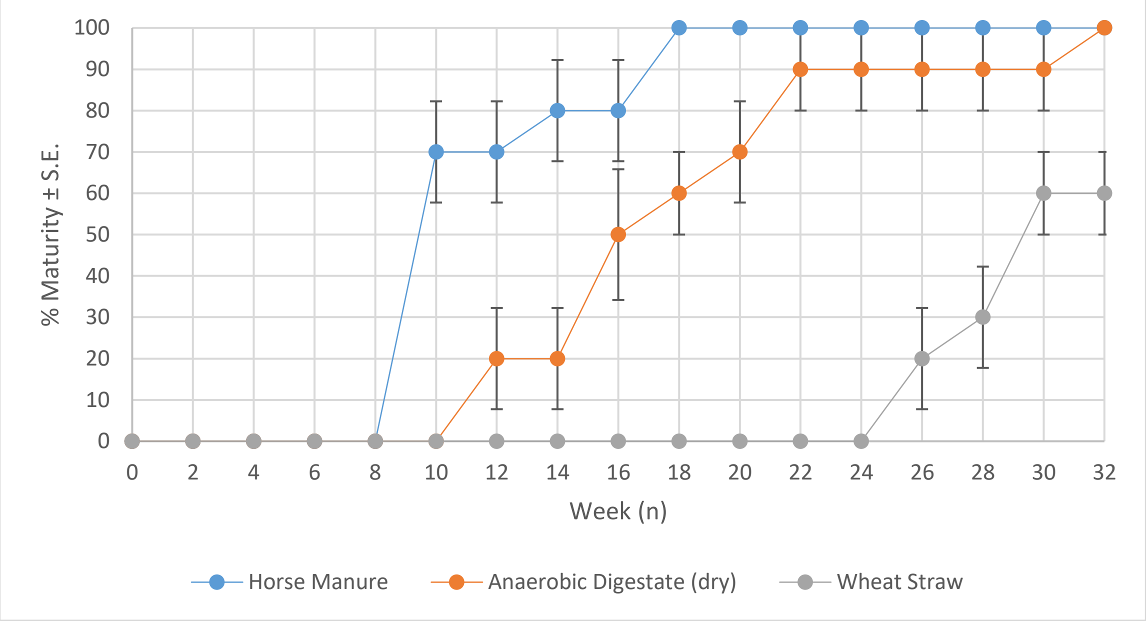


Figure 5.3. Rate of *A. longa* attainment of sexual maturity (% ± S.E.) under dry anaerobic digestate (AD), wheat straw (ST) and horse manure (HM) applications.

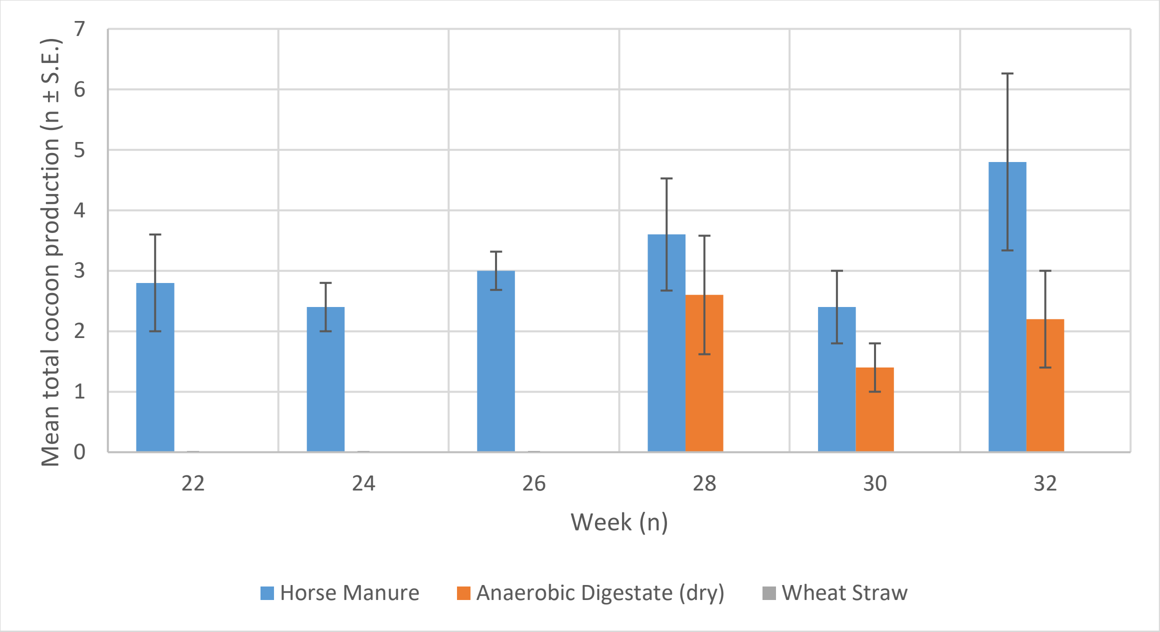


Figure 5.4. Mean number of cocoon production (n ± S.E.) by *A. longa* (32 weeks) under horse manure (HM), wheat straw (ST) and dry anaerobic digestate (AD).

A. longa reached sexual maturity under all positive organic matter treatments, but the time taken to reach maturity was different between treatments (Figure 5.3), where 60% of *A. longa* were mature at experimental end under ST treatments.

Cocoons were produced under HM and AD applications (Figure 5.4). Cocoons were produced earlier under HM applications (week 22). When both applications were producing cocoons, there was no statistical difference in the rate of cocoon production between treatments for the weeks 28-32 (Mann Whitney-U; $p > 0.05$).

5.3.2. A. caliginosa

The growth rate of *A. caliginosa* under AD and HM treatments was similar (Figure 5.5). Initially, the mean growth rate of *A. caliginosa* was higher under HM treatments, however both treatments reached 100% sexual maturity at 20-22 weeks (Figure 5.6). The effect of organic matter application on biomass change over time was significantly different between treatments (Friedman's test; x^2 (2) = 807.248; $p < 0.001$). This could have been caused by the differences in biomass between the two treatments earlier on in the experiment, or the high variance in adult biomass (SE was approximately 0.1 g from week 6).

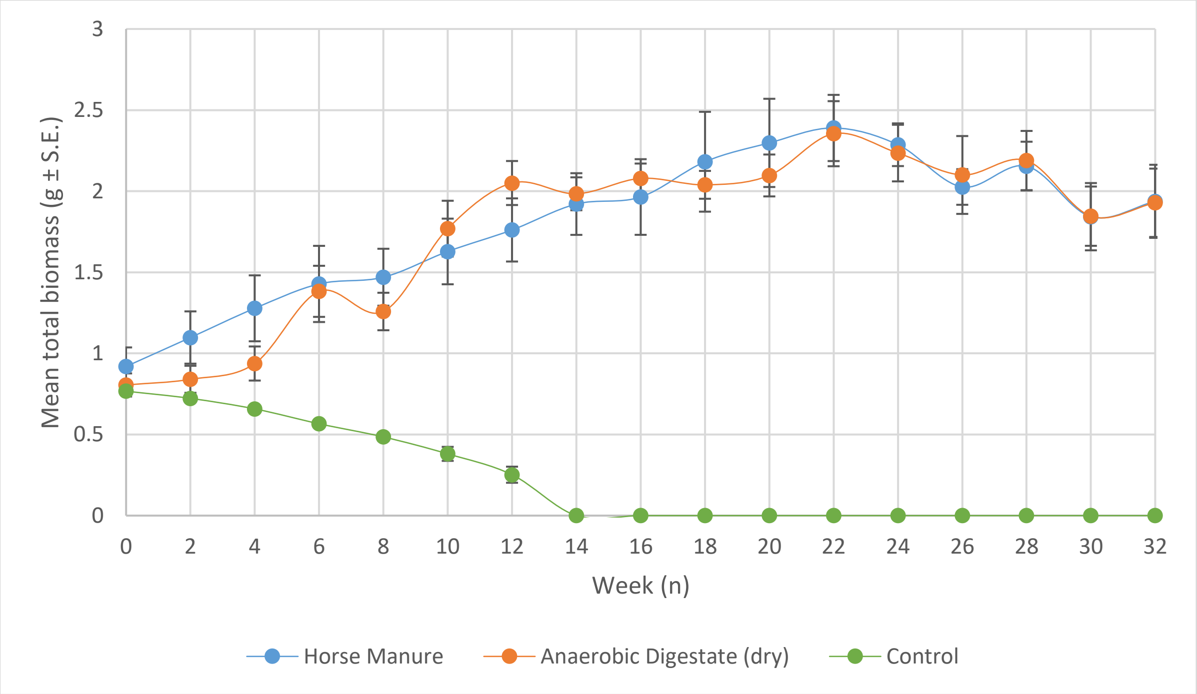


Figure 5.5. Changes in mean biomass (g ± S.E.) of *A. caliginosa* (N=15) over time (32 weeks) under horse manure (HM), anaerobic digestate (AD) and a control (C) soil application.

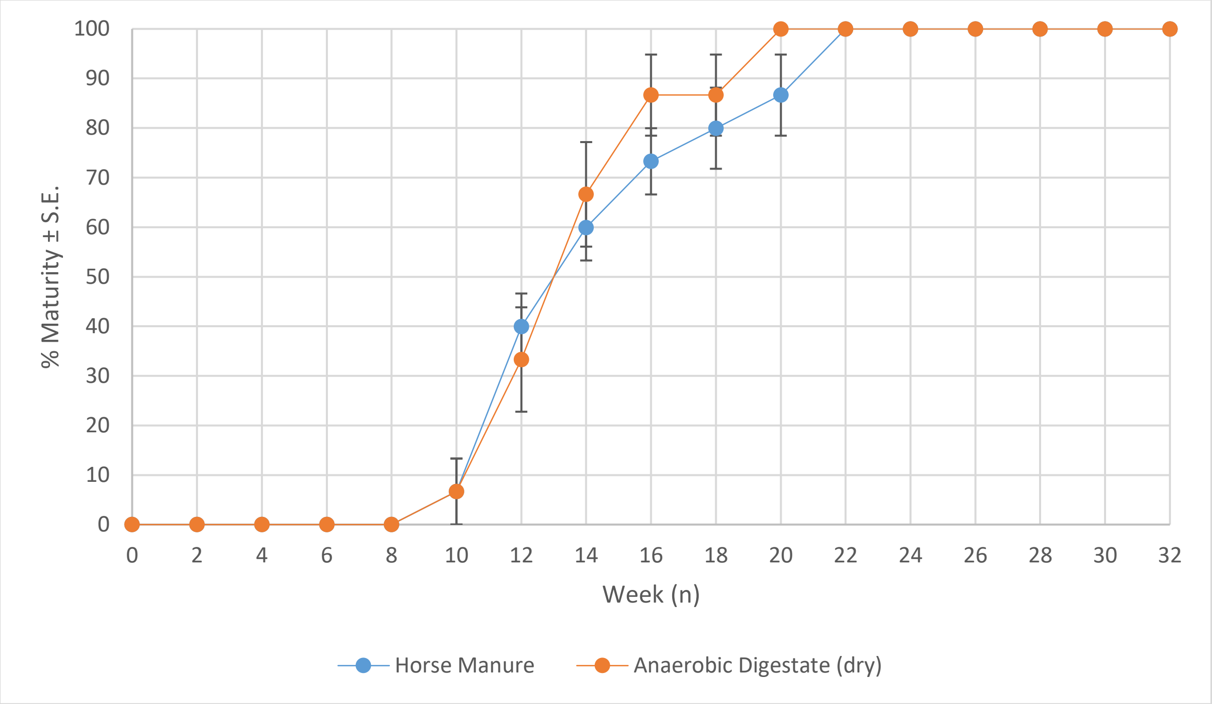


Figure 5.6. Rate of *A. caliginosa* attainment of sexual maturity (% ± S.E.) under dry anaerobic digestate (AD) and horse manure (HM) applications.

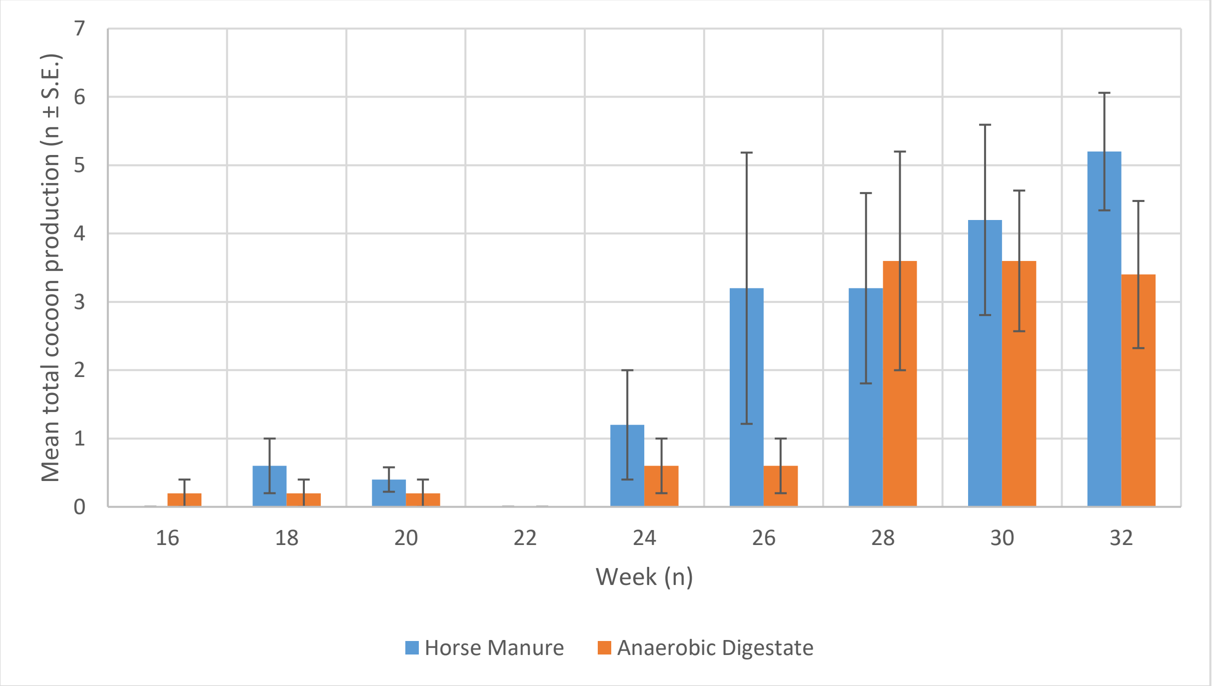


Figure 5.7. Mean number of cocoon production (n ± S.E.) by *A. caliginosa* (32 weeks) under horse manure (HM) and dry anaerobic digestate (AD).

Both AD and HM treatments produced cocoons (Figure 5.7.). At weeks where both treatments resulted in copulation (18-20; 24-32) there were no significant differences in the effect of organic matter treatment on the rate of reproduction (Mann Whitney-U; $p < 0.001$).

5.3.3. A. chlorotica

The effect of organic matter application on biomass change over time was significantly different between all treatments (Friedmans test; x^2 (2) = 893.613; $p < 0.001$; Figure 5.7). Post-hoc Wilcoxon signed rank tests confirmed that all treatments were significantly different ($p < 0.001$). Adult biomass was higher under HM and AD treatments (Figure 5.8), and the rate to sexual maturity was faster under HM applications (Figure 5.9).

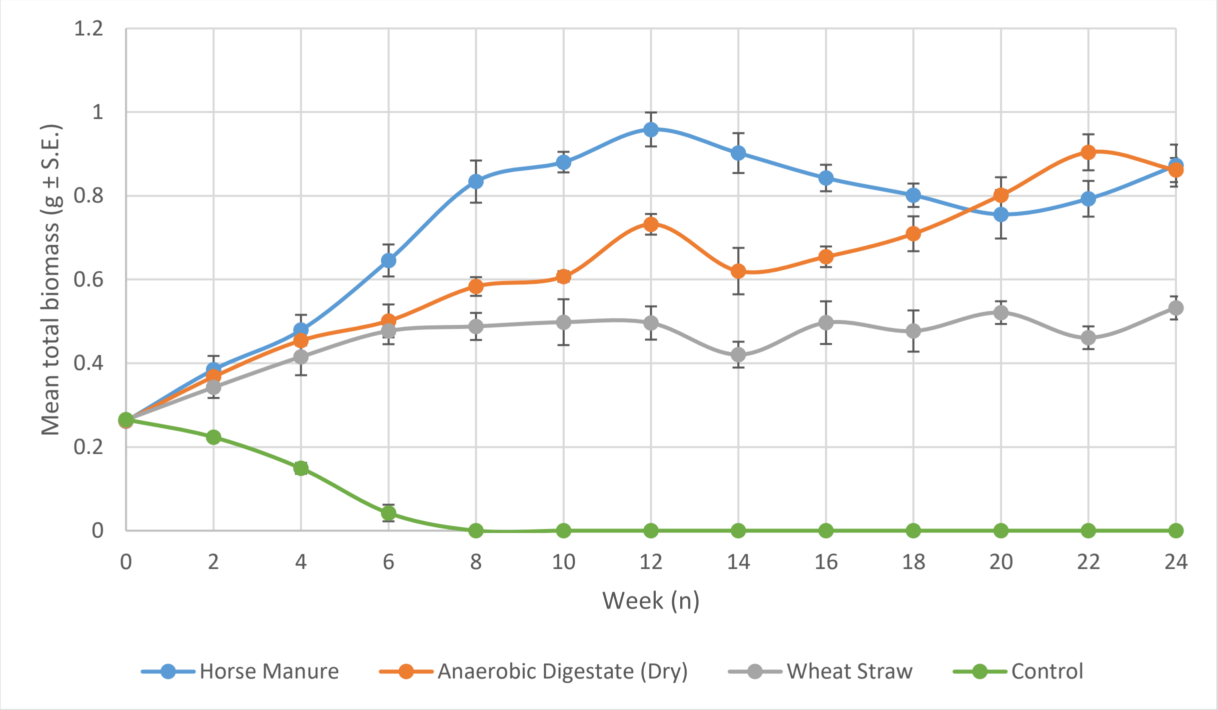


Figure 5.8. Changes in mean biomass (g ± S.E.) of *A. chlorotica* (24 weeks) under horse manure (HM), dry anaerobic digestate (AD), wheat straw (ST) and a control (C) soil application. (N=5).

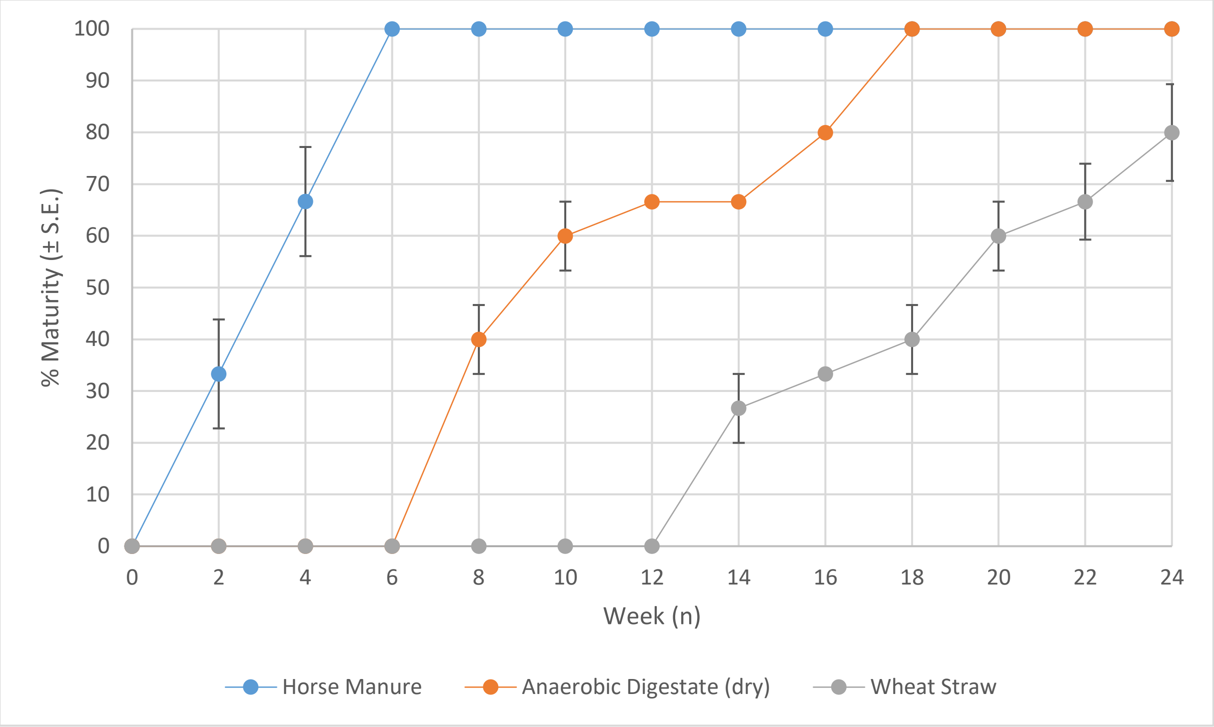


Figure 5.9. Rate of *A. chlorotica* attainment of sexual maturity (% ± S.E.) over 24 weeks under dry anaerobic digestate (AD), wheat straw (ST) and horse manure (HM) (N=5).

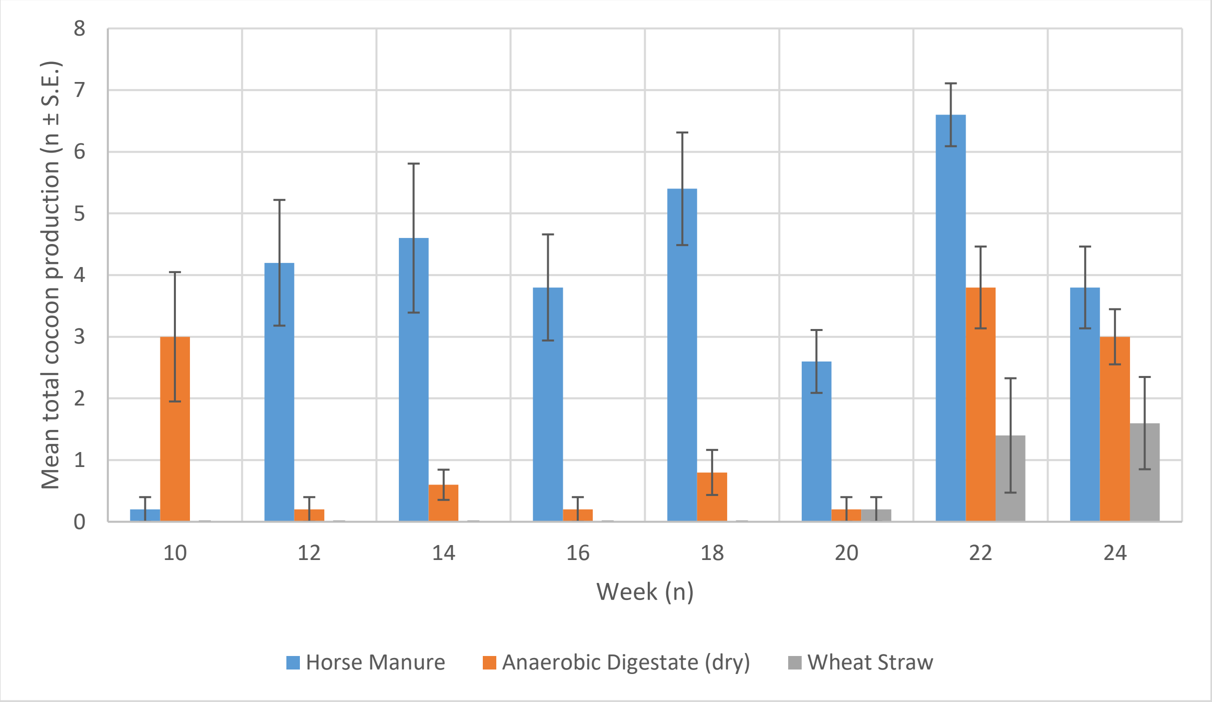


Figure 5.10. Mean cocoon production (n ± S.E.) by *A. chlorotica* (24 weeks) under horse manure (HM), wheat straw (ST) and dry anaerobic digestate (AD).

The number of cocoons produced over the experimental period was higher under HM applications, where copulation began at 10 weeks (Figure 5.10). This resulted in the statistical difference between waste organic matter applications being significant in weeks 12-20 where there was a higher proportion of adult *A. chlorotica* under HM treatments (Figure 5.9). At week 24, when there was a high proportion of adults under all treatments, there was no significant difference in the rate of cocoon production ($p > 0.05$).

5.3.4. L. terrestris

The effect of organic matter application on biomass change over time was significantly different between treatments (Friedman's test; x^2 (2) = 529.495; $p < 0.001$). Horse manure had a significantly higher mean *L. terrestris* adult biomass and reached sexual maturity at a faster rate than ST applications (Figure 5.11). Both treatments produced cocoons, however the abundance and frequency of cocoons was higher under horse manure (Figure 5.12).

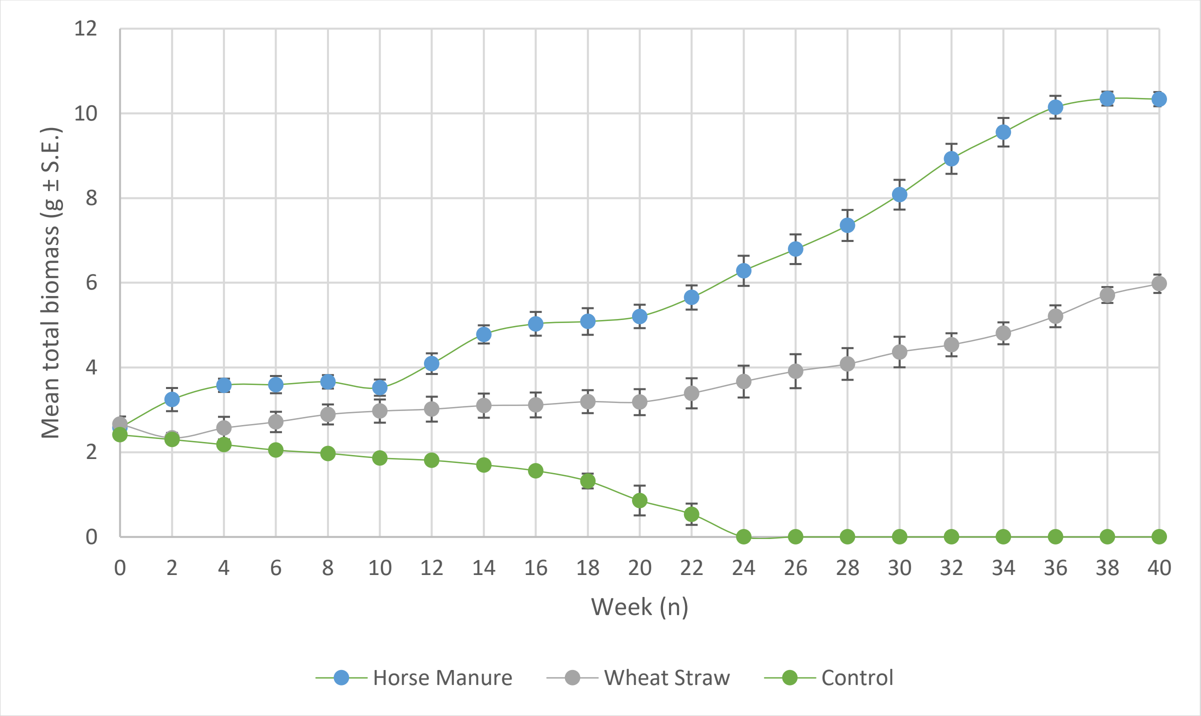


Figure 5.11. Changes in mean total biomass (g ± S.E.) of *L. terrestris* (40 weeks) under horse manure (HM), wheat straw (ST) and a control (C) soil application. N=5.

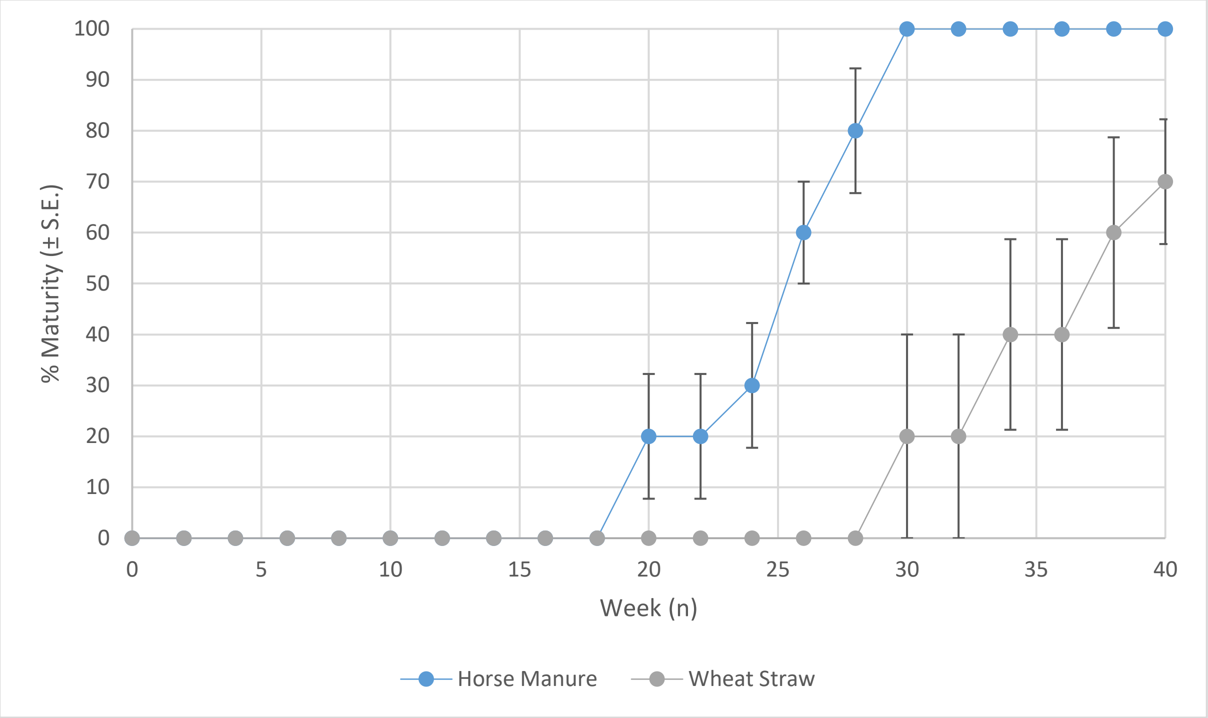


Figure 5.12. Rate of *L. terrestris* attainment of sexual maturity (% ± S.E.) over 24 weeks under wheat straw (ST) and horse manure (HM) applications (N=5).

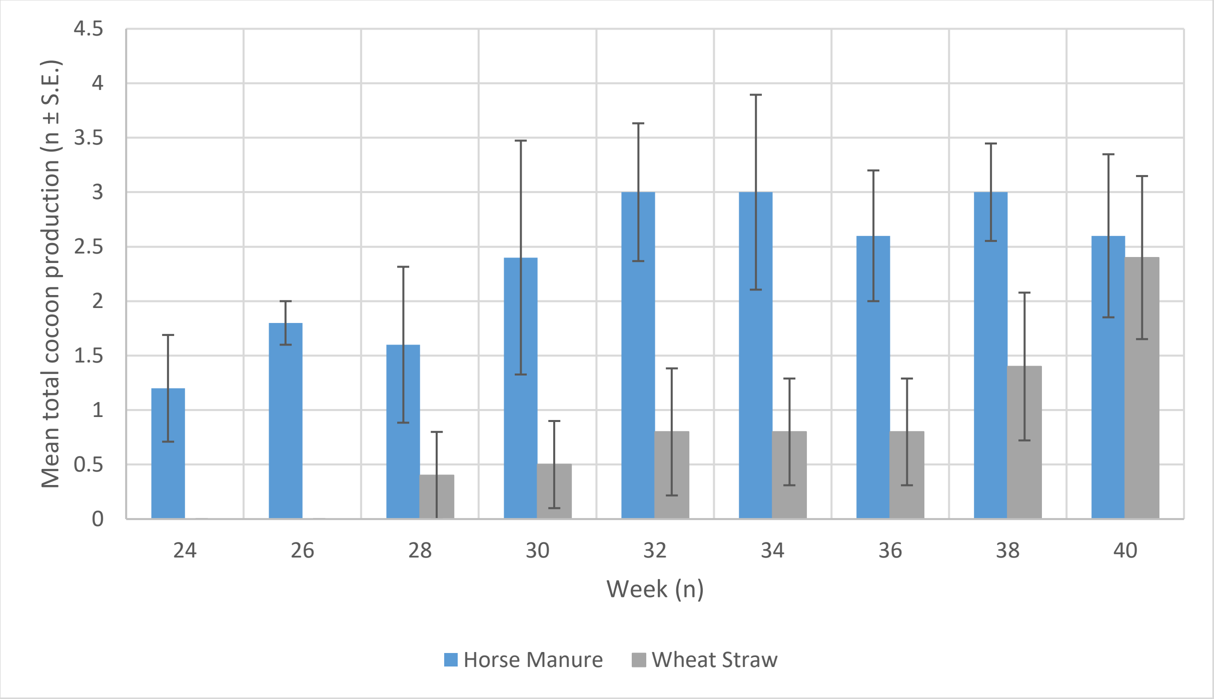


Figure 5.13. Mean cocoon production (n ± S.E.) by *L. terrestris* (24 weeks) under horse manure (HM) and wheat straw (ST) (N=5).

As with *A. chlorotica* treatments, the cocoon production was significantly higher under HM treatments when there was a higher percentage of adult individuals (Figure 5.13). When the % mature adults increased under ST treatments (weeks 38 and 40), the difference in reproductive rate was not significant ($p > 0.05$).

5.4. Discussion

5.4.1. Effects of waste organic matter type on earthworm growth rate

This experiment indicated that waste organic matter type had significant effects on earthworm growth rate, which differed between earthworm species. For all earthworm species, horse manure was the most effective feed, where the growth rate was significantly higher under all populations. Previous studies on the effect of organic matter type on *L. terrestris* monocultures indicated that horse manure increased the growth rate compared with birch leaves (Butt, 2011), however the rate of cocoon production was lower and the effect of food quality on growth changed with *L. terrestris* life stage. There were significant differences in the growth rate of *L. terrestris* between horse manure and wheat straw applications (Figure 5.11), but due to the experimental time period it was not possible to determine whether the application of straw influenced *L. terrestris* growth rate at later life stages. The effects of selected food types on *L. terrestris* species was investigated in the field and laboratory by Sizmur et al, (2017), where it was indicated that under controlled laboratory conditions, a higher calorific value of milled cereal straw increased the growth rate. However, in the field, horse manure applications had a higher effect on *L. terrestris* growth rate. Although the particle size used in this study was uniform between treatments, the particle size was larger (5 mm), which could have had some influences on the growth rate of *L. terrestris*. The positive effects of a particle size reduction on the growth rate and development of *L. terrestris* and *A. chlorotica* species have been indicated (Lowe and Butt, 2003), however the practical implications of applying milled straw onto agroecosystems could limit the utilisation on farms. Future experiments will explore the interactions between earthworms and organic matter at different particle sizes.

The effects of waste organic matter applications on the growth rate endogeic earthworms were different to anecic. Applications of AD and HM resulted in similar growth rates of *A. caliginosa* populations (Figure 5.5) and time to sexual maturity (Figure 5.6). *A. caliginosa* populations are upon the most abundant in temperate agroecosystem settings (Pelosi et al, 2018) and can withstand tillage management (Crittenden et al, 2014). The positive effects displayed by anaerobic digestate applications on the growth rate of *A. caliginosa* suggests that it would increase earthworm populations in agroecosystem settings. This could cause an increase in soil organic matter concentration in soil and nutrient availability to crops. There were differences in the growth rates of *A. chlorotica* under AD and

HM treatments (Figure 5.8), however at experimental end (week 24) there were no significant differences in *A. chlorotica* biomass ($p < 0.05$). There could be a number of reasons why there were growth differences between anecic and endogeic monocultures. First, geophagus earthworms feed on larger quantities of soil, therefore the process of organic matter decomposition in the gut is different (Curry and Schmidt, 2007). Second, the effects of organic matter nitrogen concentration could have a larger effect on the growth rate of earthworms with a higher biomass, which have a different metabolic rate (Shipitalo et al, 1998). The effects of litter quality can have significant effects on the growth rate of earthworms, particularly in soils low in C and N (Cesarz et al, 2016). Although the amount of nitrogen provided by AD and HM was different (Table 5.1), the amount provided by AD (%N = 0.91) could be sufficient for the growth of earthworms with a lower biomass. ST had the lowest nitrogen concentration of the three successful treatments and had the lowest effect on growth when compared under *A. chlorotica*, *A. longa* and *L. terrestris* populations. Alongside the relationship between earthworm biomass and nutrient requirements for growth, a reduction in C:N ratio is suggested to increase earthworm assimilation of organic matter (Curry and Schmidt, 2007). The relationship between C:N ratio and growth rate of earthworms was supported in this experiment. Further investigations on the chemical components of organic wastes may provide information on the impacts on earthworm growth and fecundity.

Liquid anaerobic digestate and composted green waste applications were not successful treatments and did not increase the growth of *A. longa* populations (Figure 5.2). It is suggested that the liquid fraction of anaerobic digestate could be toxic to earthworms, due to the mortality rate of *A. longa* being faster than the control. Recent findings from Moinard et al, (2021) indicated a short-term (2 week) negative effect of digestate on earthworm population density, where negative effects subsided over the long-term (2 years) and earthworm density was 150% higher. Due to the organic matter being a liquid, it is possible that it provided no nutrition to earthworms, however the faster rate of mortality suggests a toxic effect. To confirm whether liquid anaerobic digestate is toxic to earthworms, an ecotoxicological assessment could be conducted (Elliston and Oliver, 2019). The metal concentration of this waste was not analysed, however previous studies have suggested that liquid anaerobic digestate from waste treatment plants can be high in PTE Pb, Cu and Zn (Coelho et al, 2018), where dilution is recommended to reduce phytotoxicity. A high metal concentration can be toxic to earthworm species (Domínguez-Crespo et al, 2012; Leveque et al, 2013). At non-toxic levels, earthworm populations such as *L. terrestris* can increase the mobility of PTE in soil (Sizmur et al, 2011), which could be phytotoxic. Anaerobic digestate can also be high in ammonia emissions, which could be toxic to earthworms in laboratory settings (Edwards and Bohlen, 1996). Under CGW applications, *A. longa* decreased in biomass at a similar rate to the control, which suggests that there is no nutrition provided to earthworms by this treatment. Although CGW can provide organic matter to soil and improve plant growth (Milinković et al, 2019), previous surveys of fields where CGW has been applied have suggested an earthworm collapse (Stroud et al, 2016c). This experiment correlated with these findings. Future experiments could analyse the chemical, physical and biological characteristics of organic wastes from different suppliers, to determine which waste inputs could create toxic environments for soil fauna communities.

5.4.2. Effects of waste organic matter type on reproduction

The reproductive output of earthworms was significantly higher under HM applications, where the total number of cocoons produced was 95 under *A. longa* species. Reproduction was limited by time to maturity, where under ST and AD applications, this was slower than HM. When all earthworms were mature, the rate of cocoon production was not significantly different between applications. This could indicate that the waste organic matter types HM, ST and AD had no effects on fecundity, just that copulation started earlier under treatments where adulthood was reached sooner. A change in temporal dynamics of cocoon production of earthworms could cause a reduction in the growth rate of an earthworm population. In the field, the temporal changes of seasons could have significant effects on the hatchling time of cocoons, which are temperature and moisture sensitive (Holmstrup et al, 1990, 1991, 1992, 1994, 1996; Holmstrup and Westh, 1994; Nuutinen and Butt, 2009). In the laboratory, the hatchling rate of *L. terrestris* cocoons when cultured under different organic matter feeds was explored by Butt (2011). Findings indicated that there were no significant differences in the hatchling rate between horse manure and birch leaves. The hatchling rate of cocoons produced was not recorded in this experiment. The health of cocoons and rate of hatchlings would determine whether there are any impacts of the waste organic matter treatment on earthworm population development. Therefore, the effect of waste organic matter type on the long-term population development of earthworm species is still to be explored. To determine the effects of waste organic matter applications on the long-term population development of earthworm species, this investigation could be extended to investigate whether the higher cocoon production over time caused by HM applications resulted in an increased number of hatchlings compared to other treatments.

5.4.3. Implications of application type on earthworm population development

The difference in the growth rate and fecundity of *A. longa* under HM, ST and AD may cause implications for population growth and development in the field. Although *A. longa* and *A. chlorotica* species grew to maturity under all treatments and reproduced, the rate of growth and reproduction over the experimental period was significantly longer under treatments with less N (Table 5.1; HM >

AD > ST). Under a temperate climate, there are temporal windows where the optimum conditions for growth and reproduction occur (autumn and spring) (Blouin et al, 2013). Changes in the rate of growth from juvenile to sexual maturity may affect population redevelopment, particularly for endogeic species with shorter life cycles. For example, under straw it took 30 weeks for *A. longa* to reach sexual maturity; in the field, there is less than 30 weeks of optimum growth conditions in autumn or spring before diapause, therefore the population would not develop as fast as under horse manure, where sexual maturity was reached within 8-16 weeks. It has been reported that straw residues and composted green waste applications have impacted the development of *L. terrestris* populations, where a population collapse occurred after 3 years (Stroud et al, 2016c). The low N concentration in ST and CGW could have caused this reduction. Applications of mixtures of AD or HM with ST could have reduced the population collapse. It has been recorded in *Eisenia fetida* monocultures that the type and ratio of organic matter mixtures can have an impact on earthworm cocoon production, where the cocoon production was significantly higher under a mixture of cattle manure and sewage sludge than 100% concentration treatments (Li et al, 2016). Although it is noted that *Eisenia fetida* are epigeic earthworms often used in vermiculture, the observed effects of waste organic matter mixtures may have significant effects on the cocoon production of soil dwelling earthworms, particularly epi-anecic earthworms such as *L. terrestris*, where the selection of organic matter is often determined by litter quality (Hoeffner et al, 2018). Future experiments could explore the effects of mixed organic matter applications on the population growth of earthworms and the temporal effects of growth rate on population development in the field.

An increased earthworm biomass is related to increased rate of organic matter bioturbation, which could enhance the rate of SOM addition into the soil and nutrient mineralization (Lévêque et al, 2013, 2019). Increased bioturbation through the optimum waste organic matter application could increase soil fertility and in no-till systems, mitigate the effects of ploughing. However, in the field, there are other biotic and abiotic soil properties which influence earthworm health and abundance, such as soil moisture, pH, bulk density, predation (Blouin et al, 2013). Waste organic matter applications in agroecosystems also provide other benefits to earthworm population health and development, such as midden development, burrow moisture regulation, camouflage from predators (Butt and Lowe, 2007; Andriuzzi et al, 2015a; Bertrand et al, 2015a). The utilisation of organic matter is also limited by particle size and the decomposition stage (Lowe and Butt, 2003; Sizmur et al, 2017), which was uniform in this experiment. Although intra-specific interactions can occur between earthworms in culture (Butt, 1998; Lowe and Butt, 1999; Lowe and Butt, 2002), the negative effects of inter-specific interactions between conspecifics (Edwards and Lofty, 1976; Uvarov, 2009) could influence earthworm population development and the dispersal and utilisation of organic matter in the field. To determine the fate of organic matter in soil mediated by earthworms, future investigations will explore the impacts of the positive treatments determined in this experiment (ST, AD and HM) under different application settings and earthworm species combinations.

5.5. Summary of Chapter findings

- Applications of horse manure, wheat straw and anaerobic digestate (dry fraction) increased the growth rate of earthworm populations.
- Applications of anaerobic digestate (liquid fraction) and composted green waste did not increase the growth rate of *A. longa*, where anaerobic digestate (liquid fraction) showed toxic effects.
- The recorded differences in earthworm growth rate under horse manure, wheat straw and anaerobic digestate (dry fraction) applications could have been caused by differences in N concentration between treatments.
- A faster growth rate resulted in a higher cocoon production within the experimental period under horse manure applications.
- Once sexually mature, there was no observed effects of waste organic matter type on the rate of cocoon production between species.

6: ORGANIC MATTER PARTICLE SIZE: EARTHWORM PREFERENCE AND INCORPORATION RATE

6.1. INTRODUCTION

Investigations of the decomposition and incorporation rate of organic matter by earthworm species have suggested that numerous factors influence selection and utilisation (discussed in Section 2.5.1.). This chapter investigates the impact of organic matter particle size on selection, utilisation and incorporation by specific earthworms into the belowground ecosystem. Effects of particle size on earthworm feeding capabilities have been suggested by researchers (Boström and Löfs-Holmin, 1986; Boyle, 1990; Lowe and Butt, 2003; Clause et al, 2017; Sizmur et al, 2017), such that a smaller particle size may accelerate organic matter decomposition. The size of e.g. agricultural residues may therefore be important. It has been noted that *L. terrestris* may utilise organic matter for different purposes, with larger particle sizes used for midden development, which may enhance other earthworm species development (Chapter 5; Lowe and Butt, 2007). The effects of crop residues on soil moisture regulation may also pose benefits to earthworms under drought conditions, regulate soil temperatures during winter and therefore enhancing survival.

Growth experiments with commonly found UK earthworm species (Chapter 5) indicated that horse manure and anaerobic digestate were the most effective organic matter feeds of those tested. Wheat straw was also an effective feed, which maintained earthworm mass, but entailed a slower growth and reproductive rate. Research by Sizmur et al (2017) indicated that milled straw feed can increase earthworm mass at a faster rate than horse manure. It is possible that the particle size used for the growth experiments (Chapter 5) was too large for most earthworm species. Applications of milled organic material may enhance feeding of soil fauna and improve growth conditions under culture, however milled organic matter is rarely applied to the field. This chapter seeks to investigate the effects of particle size of organic residues on earthworm use and associated effects on the soil.

6.1.1. Aims

1. To determine the effects of wheat straw residue on the growth and utilisation of earthworm populations under selected particle sizes.
2. To measure the effects of bioturbation on the distribution of nutrients and organic matter through a soil profile.

These objectives will be investigated through 3 inter-linked controlled laboratory experiments, with specific objectives, defined throughout. Sections 6.2 to 6.4 introduce the experiments, describe the methodology and present the results. Results from sections 6.2 to 6.4 are then discussed in Section 6.5.

6.2. EXPERIMENT 1: A LABORATORY INVESTIGATION OF WASTE ORGANIC MATTER INCORPORTATION BY SELECTED EARTHWORM SPECIES.

6.2.1. Introduction

Growth trials indicated that horse manure and anaerobic digestate were the most effective feeds, crop residues (such as wheat straw) are common organic matter applications in sustainable agroecosystems post-harvest and have been shown as a proven feed for earthworms in the laboratory (Sizmur et al, 2017) and field (Stroud et al, 2016a). Numerous factors may have reduced the growth rate of earthworms with straw applications in growth trials (Chapter 5). First, the particle size was greater than 1 mm (the particle size used by Sizmur et al, 2017) and could have been too large, particularly for *A. caliginosa* (Boyle, 1990; Lowe and Butt, 2005). Second, the C:N ratio of wheat straw was significantly higher than horse manure and anaerobic digestate, which may have resulted in a slower growth rate. Third, the mineralization rate of applied straw is slower than other organic matter types (Abbasi and Khaliq, 2016; Gao et al, 2016), so as a feed it might be more sustainable for earthworm populations, but over a longer time period.

A microcosm experiment was designed to investigate the effects of surface applied wheat straw and of horse manure on *L. terrestris*, *A. longa* and *A. caliginosa* under controlled laboratory conditions. Milled applications (1 mm particle size) were used to ensure that earthworms could easily feed on both organic matter types. Organic matter was surface applied to replicate a no-till agroecosystem. To determine potential benefits of both applications, a 50:50 mixed application was also included as a treatment. In addition to earthworm growth rate, the incorporation of organic matter over the experimental period was recorded.

It is hypothesised that the consumption and incorporation of organic matter residues will be higher under *L. terrestris* populations than *A. longa* and *A. caliginosa*, due to a larger mean body mass and size (Sims and Gerard, 1999). It is also predicted that anecic species (*L. terrestris* and *A. longa*) will incorporate at a faster rate than endogeic *A. caliginosa* due to their feeding behaviour, where they are more inclined to feed on the soil surface (Bouché, 1977). This experiment had equal numbers of earthworms per sample to assess the reproductive rate under selected feeds. To regulate earthworm biomass, this would have required a high number of *A. caliginosa* per vessel (N=15), which would have caused intraspecific competition (Eriksen-Hamel and Whalen, 2007a).

6.2.1.1 Objectives

- To determine the effects of wheat straw residues and horse manure applications on the growth rate of earthworm species *A. caliginosa*, *A. longa* and *L. terrestris* at milled (1 mm) particle size.
- To measure the incorporation of surface applied organic matter by *A. caliginosa*, *A. longa* and *L. terrestris* at milled (1 mm) particle size.

6.2.1.2. Hypotheses

- Incorporation of organic matter will be higher in anecic (*L. terrestris* and *A. longa*) than endogeic species (*A. caliginosa*).

- Milled organic matter will increase the growth rate of all earthworm species.

6.2.2. Materials and methods

6.2.2.1. Experimental design

An experiment was designed to determine the effects of 3 waste organic matter (WOM) treatments (horse manure, wheat straw and a 50:50 mixture) on the growth rate and reproduction of 3 earthworm monocultures (*Lumbricus terrestris*, *Aporrectodea longa* and *Aporrectodea caliginosa*) when surface applied, at milled particle size. The effect of earthworm species on the removal of WOM from the surface was also analysed. The full experimental design for this laboratory experiment is presented in Figure 6.1.

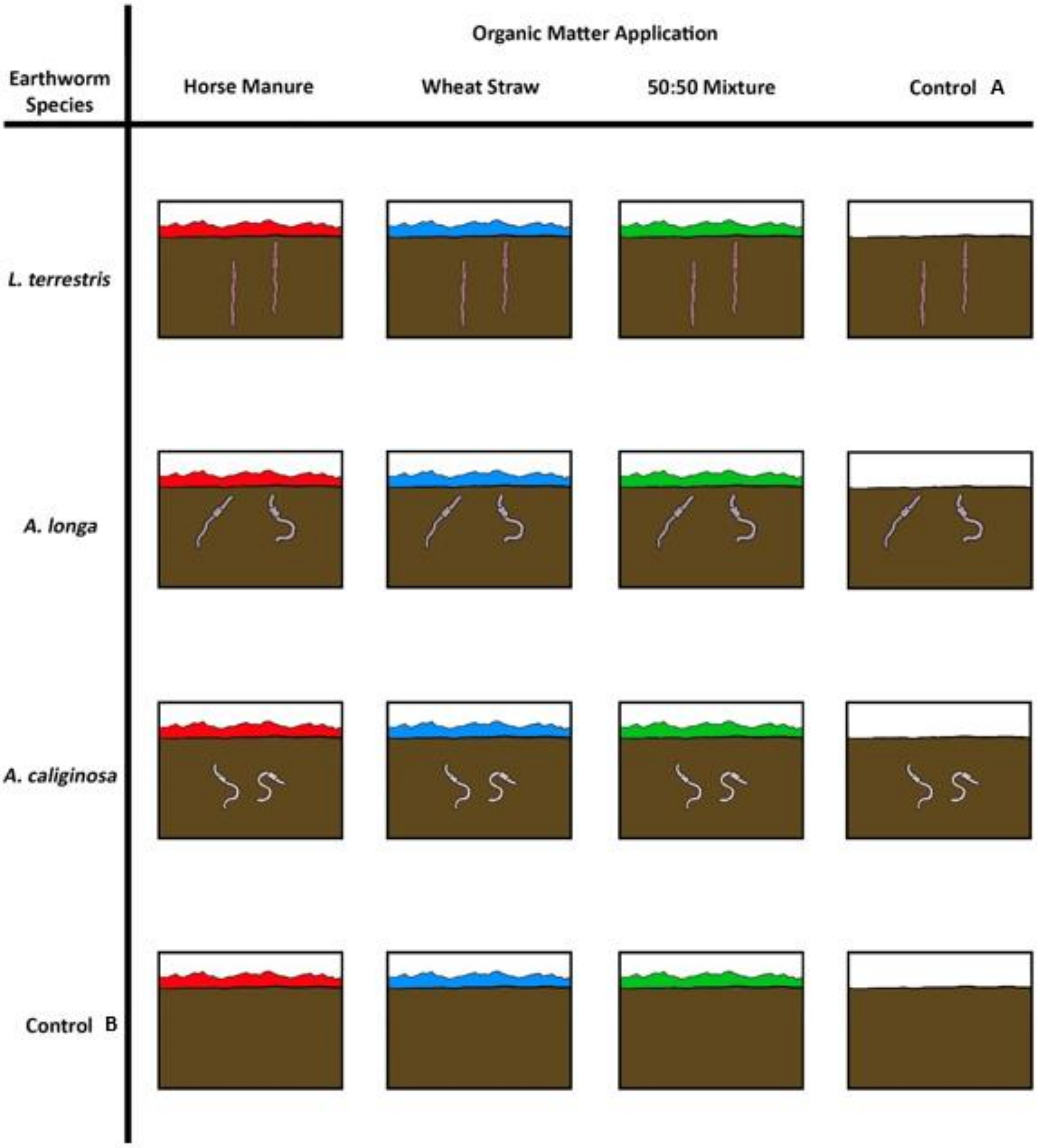


Figure 6.1. Diagram of the experimental design. Three treatments of organic matter applications (horse manure (red), wheat straw (blue) and a 50:50 mixture (green)) were added to three sets of earthworm monocultures (*L. terrestris*; *A. longa* and *A. caliginosa*) with controls of no organic matter addition (A) and no earthworm addition (B). N=5.

6.2.2.2. Sample preparation

To ensure that earthworm species for each treatment were of equal masses and age, they were cultured from cocoons under controlled conditions (25% moisture content; 15 °C; 24 hr darkness) at the University of Central Lancashire. To avoid experimental bias, earthworms were cultured with a mixed feed of horse manure and wheat straw residues (Rajapaksha et al, 2013).

Monocultures of earthworms (N=2) were mass determined and placed in 750 ml plastic containers (Lakeland, UK) containing 500 g of sterilised Kettering loam (Boughton, UK) at 25% moisture content. Samples were left over night in an incubator (15 °C; constant darkness) to equilibrate (Fründ et al, 2010). Thereafter, milled organic matter (IKA A11 analytical mill) to 1 mm particle size was provided. Dried organic matter (5 g) was rewetted before being surface applied. In addition, two sets of controls were applied:

1. Control A: Earthworm monocultures with no organic matter application
2. Control B: Organic matter application with no earthworms added.

Control A determined whether earthworms increased in mass under the organic matter applications and Control B determined whether the earthworm monocultures increased removal of organic matter from the soil surface.

Samples were maintained in temperature-controlled incubators (LMS, Kent) for 8 weeks. A further 5 g of organic matter was applied after every second week (total 20 g organic matter application) and samples were sprayed with water at the same time to maintain soil moisture content. At experimental end, remaining surface organic matter was carefully removed using forceps, dried at 105 °C and mass was determined. Earthworms were also removed, had masses determined and soil was destructively sampled and surveyed for cocoons (method in Section 3.3.3).

6.2.2.3. Statistical Analysis

Total earthworm biomass for each sample was recorded at experimental beginning and end. The change in biomass throughout the experiment was calculated by subtracting the starting vessel biomass from the biomass recorded at experimental end. Statistical analysis was conducted on the mean change in total earthworm biomass (g) to determine whether there were any effects of organic matter type on the growth rate of earthworm monocultures. Following normality and equal variance testing, nonparametric Welch F tests were conducted to determine whether there were any significant differences in the mean biomass change within earthworm monoculture groups. Post-hoc Games-Howell tests located the significant differences between the 3 treatments.

The mass of organic matter incorporated was determined by subtracting any remaining organic matter left on the surface at experimental end from the initial mass applied. Kruskal-Wallis tests were applied to determine whether there were any effects of earthworm species or organic matter type on the mean mass of waste incorporated. Post-hoc Dunn's tests were applied to locate any significant treatment effects.

6.2.3. Results

6.2.3.1. Effects of treatment on earthworm growth rate

Mean biomass change over the 8 week period is presented in figure 6.2, during which there was 100% survival for all treatments including the control with no organic matter addition. All adult earthworm species increased in mass over the experimental period under WOM application, where there was a mean reduction in mass under all control treatments (*L. terrestris* = 52.4% ± 1.5 ; *A. caliginosa* = 64.6% ± 2.5; *A. longa* = 48.5% ± 2.6). The total biomass increase was highest with *L. terrestris* under all organic matter applications (max mean increase 2.25 ± 0.2 g under horse manure), where there was a statistical difference in the biomass increase over the experiment between earthworm species ($p < 0.05$). There were no significant differences in reproductive rate between organic matter treatments, where all earthworm treatments produced cocoons by experimental end (mean reproductive rate of all samples: *L. terrestris* = 1.8 coc. ind.$^{-1}$ 8 weeks^{-1}; *A. caliginosa* = 2.2 coc. ind.$^{-1}$ 8 weeks^{-1}; *A. longa* = 2.4 coc. ind.$^{-1}$ 8 weeks^{-1}).

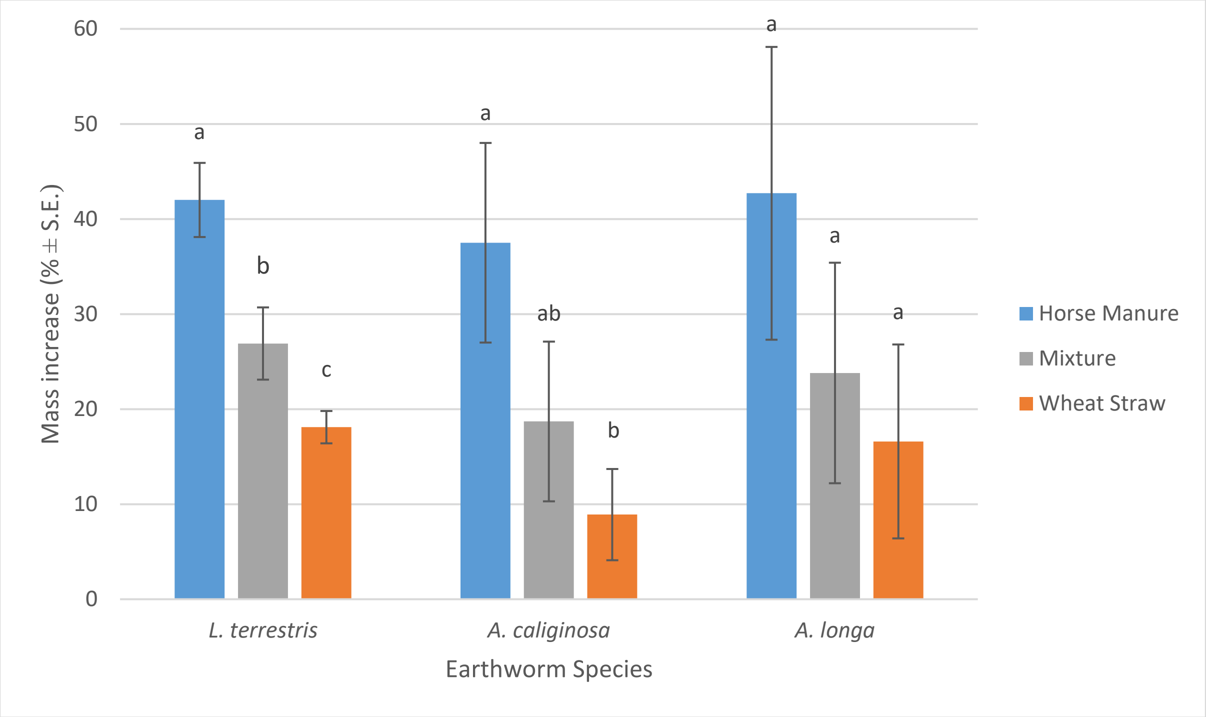


Figure 6.2. Mean biomass change (% ± S.E.) of *L. terrestris*, *A. caliginosa* and *A. longa* monocultures (n=2) under milled horse manure, wheat straw and a 50:50 mixture after 8 weeks incubation at 15 °C. For biomass of each earthworm species, different letter between OM treatments denote a significant difference at $p < 0.05$.

In terms of g per initial g of mass, the relationship between organic matter application and earthworm growth rate was similar, where the mass increase was highest under horse manure applications. The effect of organic matter type on earthworm growth rate was only significant for *L. terrestris* (Welch F test, $p < 0.05$). Multiple comparisons testing (Games-Howell) indicated that all three treatments were significantly different to one another, where horse manure > 50:50 mixture > wheat straw. Although not significantly different to the other treatments, horse manure also showed greatest biomass increase for *A. caliginosa* and for *A. longa*.

6.2.3.2. Effects of earthworm species on organic matter removal

Organic matter was removed from the soil surface under all earthworm species and WOM treatments. At experimental end, no WOM was removed from the surface under control (B) treatments, where no earthworms were added. For organic matter treatments, the highest mass incorporated in all earthworm species was with horse manure applications (Figure 6.3). Straw removal was different within a) earthworm species and b) organic matter type. A significant difference was observed for straw removal within earthworm species (df = 2; F = 43.64; $p < 0.001$), where a post-hoc Dunn's test confirmed that the significance lay with the *L. terrestris* treatment. Although a higher mass of horse manure was removed by *L. terrestris* (Figure 6.3), the effect of organic matter type on removal between treatments was not significantly different (df = 2; F = 2.07; p = 0.147).

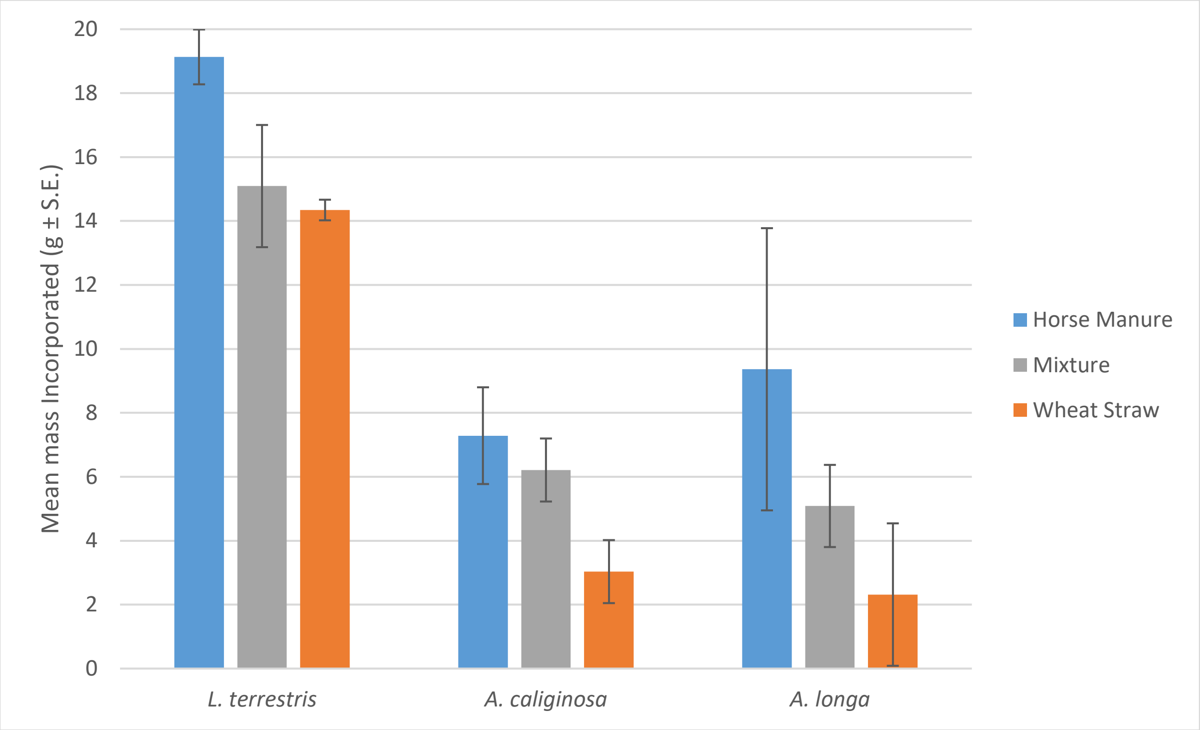


Figure 6.3. Mean mass (g ± S.E.) of organic matter removed by *L. terrestris*, *A. caliginosa* and *A. longa* monocultures over 8 weeks. N=5.

Unlike *L. terrestris*, observations suggested that *A. longa* did not incorporate organic matter into the soil profile; feeding and casting was conducted simultaneously on the soil surface. At experimental end, the determination of 'incorporated organic matter' and 'surface organic matter' was difficult because organic matter and casts were combined. For this experiment, organic matter on the surface combined with casts was determined as surface organic matter.

6.3. EXPERIMENT 2: STRAW PARTICLE SIZE PREFERENCE: CHOICE CHAMBER INVESTIGATIONS.

6.3.1. Introduction

This was conducted to determine whether the amount of straw incorporated into soil with time was limited by particle size and whether earthworm species had a preference over organic matter particle size. Similar earthworm choice chamber experiments had previously been designed by Rajapaksha et al (2013) and used by Ashwood et al (2017b) and Butt et al (2020) to determine whether earthworm species have a preference for different types of organic matter. This experiment replicated this method to determine whether *L. terrestris* and *A. caliginosa* had a preference for wheat straw at either 1 cm or 1 mm particle size.

6.3.1.1. Objective:

- To determine wheat straw residue preference of adult *A. caliginosa* and *L. terrestris* monocultures with 1 cm and 1 mm particle sizes through controlled choice chamber experiments.

6.3.1.2. Hypothesis:

- A smaller particle size will be preferred by both earthworm species, as suggested by Lowe and Butt (2003).

6.3.2. Materials and methods

6.3.2.1. Choice chamber design

To assess straw particle size preference of *L. terrestris* and *A. caliginosa*, a modified choice chamber, as designed by Rajapaksha et al (2013), was set up under controlled environmental conditions. This soil-mediated system provided more natural conditions for soil-dwelling earthworms and allowed the quantification of organic matter selected over time, without disturbing their habitat. Circular aluminium foil trays (diameter 0.16 m and depth 0.03 m) and Eppendorf tubes (diameter 0.01 m and depth 0.04 m) were used as the basis of the soil chambers. Eppendorf tubes and caps were separated, and a 0.01 m hole was drilled into the Eppendorf tube caps, which permitted the passage of earthworms. Equally spaced holes (approx. 0.01 m diameter) were made in the foil tray wall, enabling drilled caps to be affixed to the inner side and Eppendorf tubes to be attached from the outside (Figure 6.4). Trays were filled with Kettering loam (25% moisture), a proven substrate for earthworms (Butt et al, 1994).

Figure 6.4. Choice chamber mesocosm (with aluminium foil lid removed), viewed from above (tray diameter = 0.16 m; tray depth = 0.03 m; cap diameter = 0.01; cap depth = 0.04 m). Wheat straw removal from tubes by *L. terrestris* monocultures (N=2) when supplied with 2 particle sizes (1 cm and 1 mm) over 24 days.

Earthworms were introduced into each tray as designated and sprayed with water. To prevent moisture loss and earthworms from escaping, trays were covered with a sheet of aluminium foil, held in place with a rubber band. To ensure air circulation, two holes were made in each sheet using a mounted needle. Trays were then incubated in full darkness for 24 hours at 15 °C, for earthworms to equilibrate.

Air dried wheat straw (previously collected from Farington, UK) was separately cut into two particle sizes, 1 cm and 1 mm. For 1 cm particle size treatment, wheat straw was manually measured and cut using scissors. For 1 mm particle size, wheat straw was milled using an IKA 200 analytical mill and sieved to ensure uniformity. Individual masses of each cap-less Eppendorf tube were recorded. Tubes were filled with dry straw particles and soaked with water for two hours, then excess was drained through inversion onto absorbent tissue paper. Masses of straw-filled tubes were recorded (2 g tube^{-1}) and then attached to the choice chambers, which were then replaced in the incubator for experimental start. To monitor moisture variation throughout the experiment, control samples containing no earthworms were prepared and measured alongside samples.

Wheat straw removal by earthworms was measured by recording the mass loss of individually labelled food tubes over time. Choice chambers were examined every 3 days throughout the experiment, where tubes were removed, weighed and reaffixed in the same position. Following mass determination, each tray was sprayed with equal amounts of water to maintain soil moisture content.

This process was repeated for 24 days. At experimental end, the number of surviving earthworms and their masses were recorded.

Adult *L. terrestris* used in this experiment were purchased from wigglywigglers.co.uk and upon arrival were acclimated to laboratory conditions for 8 weeks prior to the experimental start (Fründ et al, 2010). Adult *A. caliginosa* were cultured in the laboratory following the method described in Section 3.2.2.

6.3.2.2. Experimental Design

This experiment tested the food preference of wheat straw particle size by monocultures of adult earthworms: either *L. terrestris* or *A. caliginosa*. The particle sizes tested were 1 cm and 1 mm. The number of individuals used per sample (*L. terrestris* N= 2; *A. caliginosa* N=10) was based on mean biomass per tray, which overall was 10 ± 0.2 g for *L. terrestris* and 10 ± 0.05 g for *A. caliginosa*. For each choice chamber, 4 tubes were filled with 1 cm particle size, and 4 tubes with 1 mm particle size wheat straw (*Triticum aestivicum*). Tubes were randomly arranged around each tray. There were 10 replicated trays for each earthworm species. Particle size preference was assessed by calculating the mean mass loss of litter in individual food tubes, measured every 3 days over a period of 24 days.

6.3.2.3. Statistical Analysis

Particle size selection behaviour was assessed by calculating the mass (%) of straw remaining in each tube over time. The remaining amount of straw was associated with earthworm preference, where at experimental end the highest remaining (%) for non-preferred and the lowest remaining (%) for preferred. For each sample, the total mass removed per sample was calculated for each particle size by subtracting the remaining mass from the original mass, having removed the tube mass. The point of 50% total waste organic matter removal was taken as the criterion for statistical analysis, (Doube et al, 1997; Rajapaksha et al, 2013). Following normality and homogeneity of variance testing, a one-way ANOVA with repeated measures was applied to determine whether there were any significant differences between the means of 1 cm and milled (1 mm) straw removal by each earthworm treatment. Post-hoc Tukey Kramer multiple comparisons tests determined the timing where the differences occurred.

6.3.3. Results

A similar preference for milled straw was observed in each species investigated (Figure 6.5 and 6.6.). At experimental end, there was a 100% survival of both earthworm species, but with a mean mass loss over the experiment of 4.8% for *L. terrestris* and 3.6% for *A. caliginosa*.

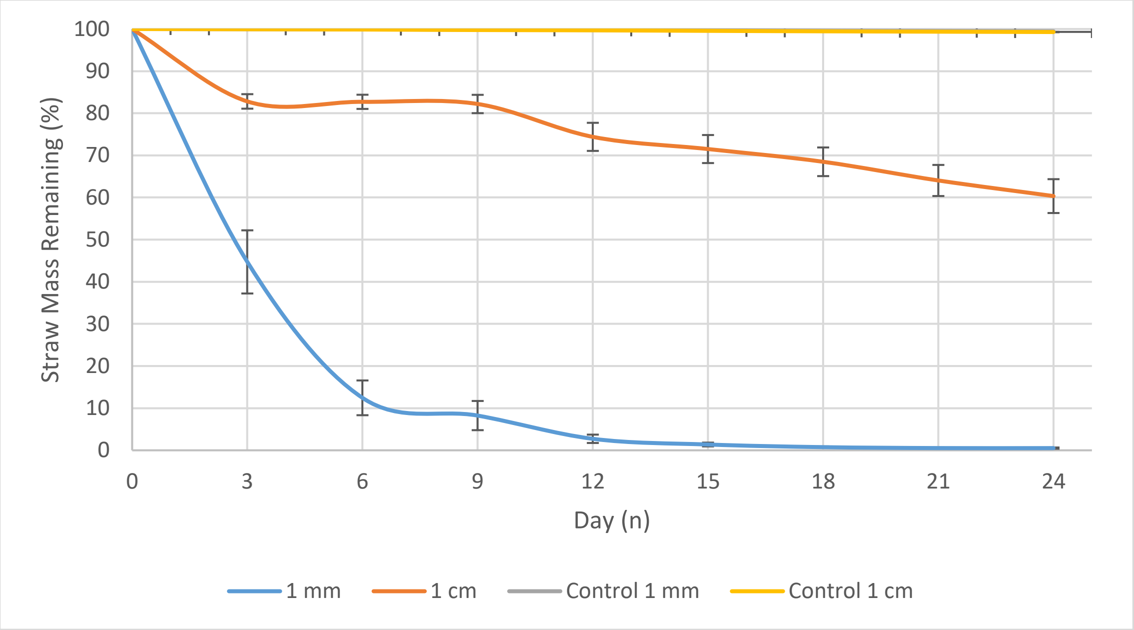


Figure 6.5. Mean straw residue selected (% ± S.E.) by adult *L. terrestris* (N=2) over a period of 24 days with 1 cm and 1 mm particle size treatments. N=10.

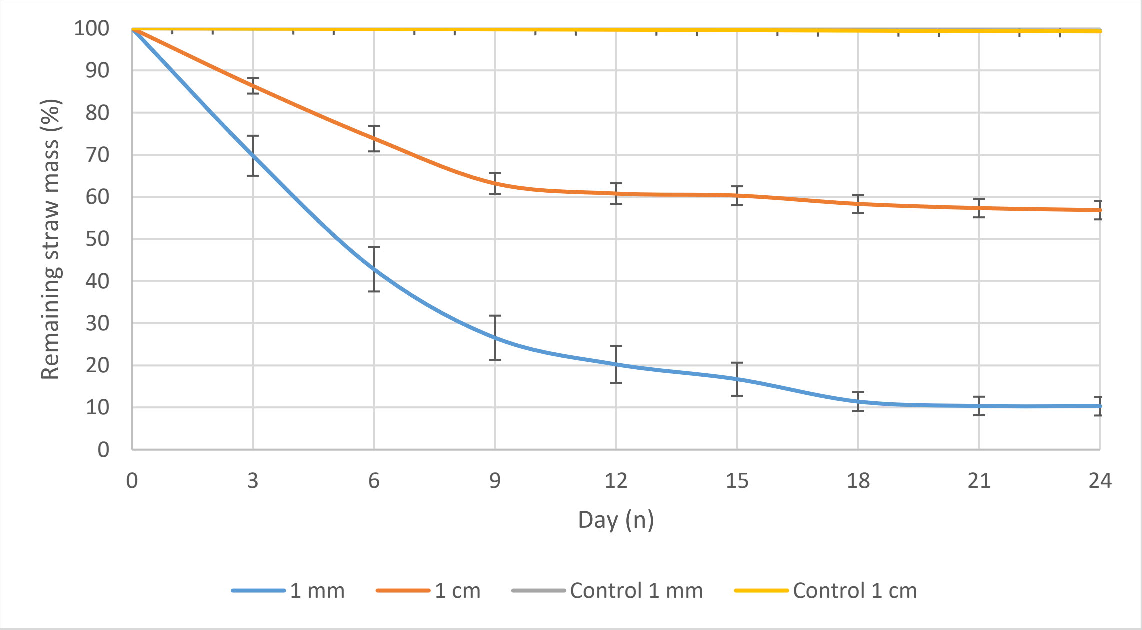


Figure 6.6. Mean straw residue selected (% ± S.E.) by adult *A. caliginosa* (N=8) over a period of 24 days with 1 cm and 1 mm particle size treatments. N=10.

Results for the control samples were very similar, such that the two lines for 1 mm and 1 cm particle size are indistinguishable in Figures 6.5 and 6.6 and remained at >99% throughout.

At 50% total removal of straw, there was a significant effect of particle size on the remaining straw mass (%) for both earthworm species, where milled straw was removed at a more rapid rate than 1 cm ($p < 0.05$).

Table 6.1. Mean (± S.E.) remaining straw mass (% from original wet mass) in choice chambers of earthworms at 50% total removal. Different letters in a row indicate significant differences ($p < 0.05$).

Earthworm Species	Days taken to remove 50% Straw	Particle Size	
		1 cm	1 mm
L. terrestris	6	12.4% ± 4.12 a	82.7% ± 1.68 b
A. caliginosa	9	26.5% ± 5.26 a	63.2% ± 2.47 b

As demonstrated in figures 6.5 and 6.6, there were differences in the rate of removal by the two earthworm species. *L. terrestris* removed 87.6% of milled material by day 6 and the rate of 1 cm straw selection began to increase after week 9 when most milled straw had been removed. With *A. caliginosa*, there was a significant difference in the mass of straw removed between particle size treatments, however the rate of removal followed a similar pattern and approximately 10% milled straw remained at experimental end.

6.4. EXPERIMENT 3: PARTICLE SIZE EXPERIMENT: UTILISATION OF WHEAT STRAW BY SELECTED EARTHWORM SPECIES.

6.4.1. INTRODUCTION

It was indicated in Experiment 2 that a particle size of 1 mm is preferred by *L. terrestris* and *A. caliginosa*, however, surface application of wheat residues does not occur in the field in no-till agroecosystems. A laboratory experiment was therefore designed to determine the rate of incorporation of straw residues by *L. terrestris* and *A. caliginosa,* in monocultures and mixed species combinations, when realistic particle sizes were offered, as determined from field examination post-harvest.

Based on previous results in this Chapter it was hypothesized that smaller organic matter particle sizes would be incorporated at a more rapid rate. A literature review also suggested that *L. terrestris* will incorporate a higher mass of residues than *A. caliginosa* due to the difference in burrowing and feeding behaviour (Bouché 1977; Curry and Schmidt, 2007). It was suggested in Section 4.4, that *L. terrestris* middens increased the population size of juvenile endogeic earthworms. An increased soil organic matter content in the belowground ecosystem engineered by *L. terrestris* could provide food for adult *A. caliginosa* populations. The facilitation effects of *L. terrestris* populations on adult endogeic *A. caliginosa* will be tested in this experiment.

Findings from Experiment 1 addressed organic matter removal from the soil surface. This experiment builds on these findings and conducts a detailed analysis on the effects of earthworm species and straw particle size on incorporation depth, where it is predicted that *L. terrestris* will incorporate organic matter to a greater depth than *A. caliginosa*. This will provide further information on the bioturbation effects of these earthworm species and how their interactions may impact organic matter dispersal in the belowground ecosystem.

6.4.1.1. Objectives:

- To measure straw removal by monocultures of *L. terrestris*, *A. caliginosa* and mixed species treatments when offered modal particle sizes.
- To analyse the nutrient concentration and C:N ratio of soil at experimental end, to determine any effects of earthworm treatment or straw application on soil nutrient dispersal.

6.4.1.2. Hypotheses:

- Total mass of straw removed (g) will be higher in *L. terrestris* treatments.
- Total *A. caliginosa* biomass will be higher in the mixed species treatment.
- There will be a reduction in the total mass of straw removed from the surface with increased particle size application.
- *L. terrestris* monocultures will incorporate straw to increased depths, due to the differences in earthworm burrowing and feeding behaviour within ecological groups (Bouché, 1977).

6.4.2. MATERIALS AND METHODS

6.4.2.1. Experimental design

Surface applied wheat straw was collected from Brook Lane farm, Preston, Lancashire in September 2018 (see Section 3.4) and left to air dry at room temperature for one week. From the collected straw, particles were randomly selected (N=1000) and maximum length measured to the nearest cm. Figure 6.7 shows that a tri-modal distribution was determined with peaks around 40 cm, 20 cm, and 1 cm lengths. These were therefore selected as the 3 straw treatments, alongside two controls, one with straw application and no earthworms and the second with earthworms and no organic matter application.

L. terrestris were purchased from Wigglywigglers.co.uk and *A. caliginosa* were collected from Walton Hall Farm, Preston Lancashire (see Section 3.3.2).

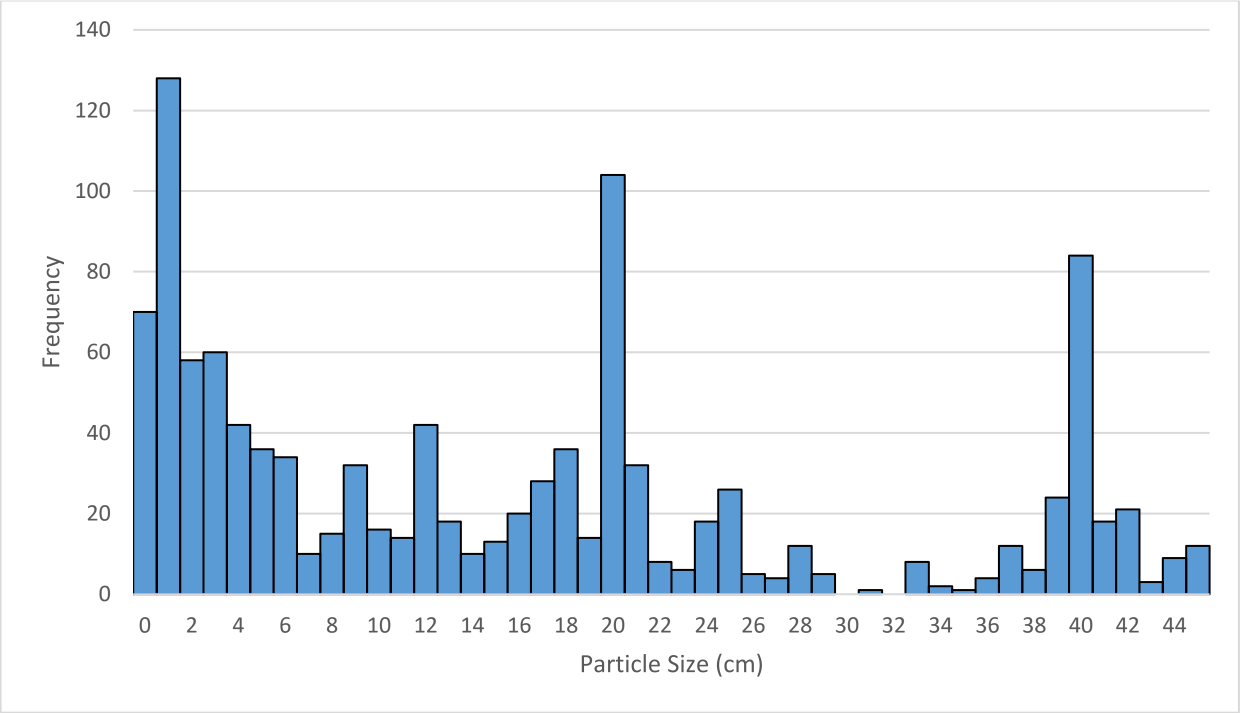


Figure 6.7. Distribution of wheat straw residue lengths (N = 1000) collected post-harvest from Brook Lane Farm, Farington Moss, Preston, UK.

Three adult earthworm treatment combinations were used:

1. *L. terrestris* monocultures (N = 2).
2. *A. caliginosa* monocultures (N = 3).
3. Mixed species (*L. terrestris* N = 1; *A. caliginosa* N = 3)

Two controls were used:

1. Control A: earthworm treatment without straw application
2. Control B: straw application without earthworms added.

The full experimental design is presented in figure 6.8.

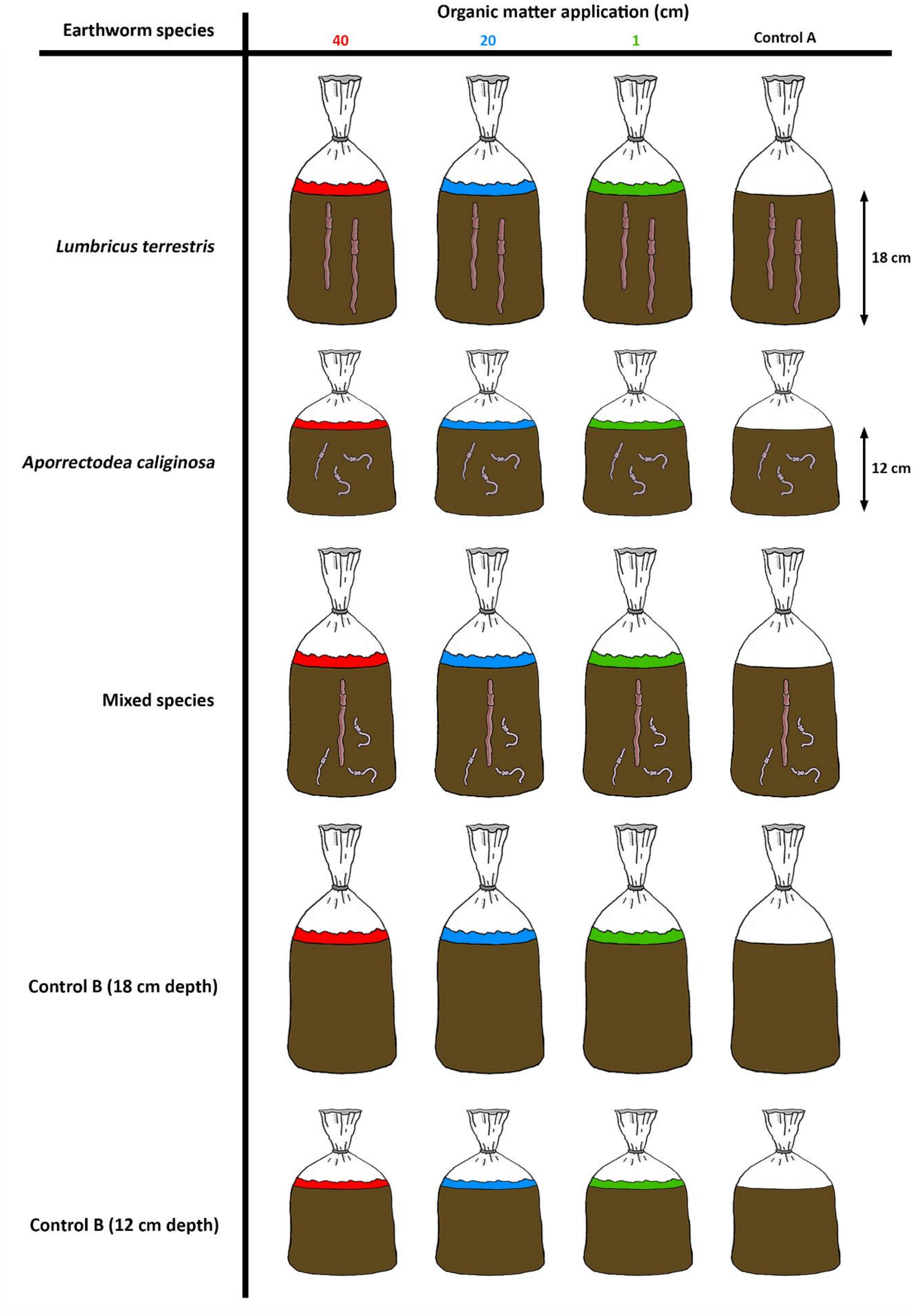


Figure 6.8. A diagram of the experimental design. Each treatment had 5 replicates (N=100).

To ensure equal mean biomass between samples, earthworm mean mass was determined per species in advance of allocation to treatment (*L. terrestris* = 6.44 ± 0.33 g; *A. caliginosa* = 0.65 ± 0.03 g). Kettering loam soil was prepared to 25% soil moisture content, of which 3 L was placed in labelled polyethylene envelopes (600 gauge; heavy plastic) for samples containing *L. terrestris* and 2 L for *A. caliginosa* monocultures. Earthworms were placed on the soil surface and left overnight to burrow.

The following day, 10 g of air-dried wheat straw (of the given length as per treatment) was mass determined and rewetted, then placed on the surface of the soil (Figure 6.9). The sample bag was tied off and pierced using a dissection needle to allow airflow. Samples were placed upright in a 15 °C incubator, where they were left in darkness for 8 weeks. Samples were monitored weekly and sprayed with equal amounts of water to retain soil moisture. Earthworms were not refed during this experiment.

Figure 6.9. An example of the mesocosm treatments for *L. terrestris* monocultures using 1 cm, 20 cm and 40 cm particle sizes.

6.4.2.2. Soil Sampling

At experimental end, the remaining surface straw was removed by hand using forceps, placed into an aluminium tray (mass determined) and then the sample was dried in an incubator at 105 °C for 8 hours. Dry mass was measured to calculate the surface straw removal and straw moisture content. Images of the remaining surface straw before and after removal were taken to ensure a consistent methodology.

To enable the analysis of depth, soil from within the plastic enveloped was carefully deconstructed and sampled in specific depths (0-60 cm; 60 – 120 cm; 120 – 180 cm (from the surface) for *L. terrestris* and mixed species samples; 0-60 cm; 60 – 120 cm (from the surface) for *A. caliginosa*). Earthworms were removed from the samples and masses determined. The depth at which each earthworm was located in the units was noted and soil was surveyed for cocoons by wet sieving. Straw found at each depth belowground had particle size and mass determined. At each sample depth, soil samples were collected for nutrient analysis, soil moisture content and organic elemental analysis (CHNS %). Details of these analysis methods are provided in Section 3.5 (page 97).

6.4.2.3. *Statistical Analysis*

Statistical tests were applied to determine effects of particle size and earthworm treatment on the mean total straw removal. Total straw removal was determined by subtracting the mean mass of straw remaining on the soil surface at experimental end from the starting mass. Following normality and homogeneity of variance testing, a Welch's one-way ANOVA was applied to determine whether there were any significant effects of earthworm treatment and particle size on the mean total straw removal. Post-hoc Tukey Kramer testing was used to determine the significance between groups.

At experimental end, straw mass incorporated was measured at 3 soil depths: 0-60 cm; 60 – 120 cm; 120 – 180 cm (from the surface). To test the effects of earthworm treatment and straw particle size on straw transport belowground, a General fit two-way ANOVA was applied within earthworm treatments to determine whether there were any interactions between straw depth and particle size for each earthworm treatment. Multiple comparisons tests (Tukey Kramer HSD) were applied to determine the significance between groups.

The effects of earthworm treatment and straw particle size on soil nutrient concentration was tested. Nutrient concentration results were analysed nonparametrically. To determine any significant effects of earthworm treatment on soil nutrient concentration, pairwise Mann-Whitney U tests were conducted between the earthworm treatments and the control B samples. To determine whether there were any significant effects of earthworm and straw particle size on soil nutrient concentration, Kruskal-Wallis tests were applied between treatments. Post-hoc Dunn-Bonferroni tests were conducted to locate the differences within groups.

To determine whether there were any effects of soil incorporation on soil nutrient concentration, Kruskal-Wallis tests were applied to compare the effect of mass incorporated at each depth with the nutrient concentration at each point. Post-hoc Dunn-Bonferroni tests were conducted to determine whether any significant differences were caused by the earthworm treatment or particle size.

6.4.3. RESULTS

6.4.3.1. Reduction in surface straw mass

The mean reduction in surface straw mass (g treatment^{-1} ± S.E.) by earthworm and particle size treatment is presented in Figure 6.10. A significant difference was determined in the surface straw mass reduction within particle size treatments in *L. terrestris* monocultures (one-way ANOVA; $p < 0.001$), where 1 cm particle size had the largest straw mass reduction (mean 6.3 ± 0.6 g treatment^{-1} ± S.E.). There were no significant differences in straw reduction between particle size treatments under *A. caliginosa* monocultures and mixed species treatments ($p > 0.05$). The mean straw mass incorporated within *A. caliginosa* species was within the range of 1.8-2.2 g regardless of particle size. The straw removal within mixed species treatments was low for all particle sizes.

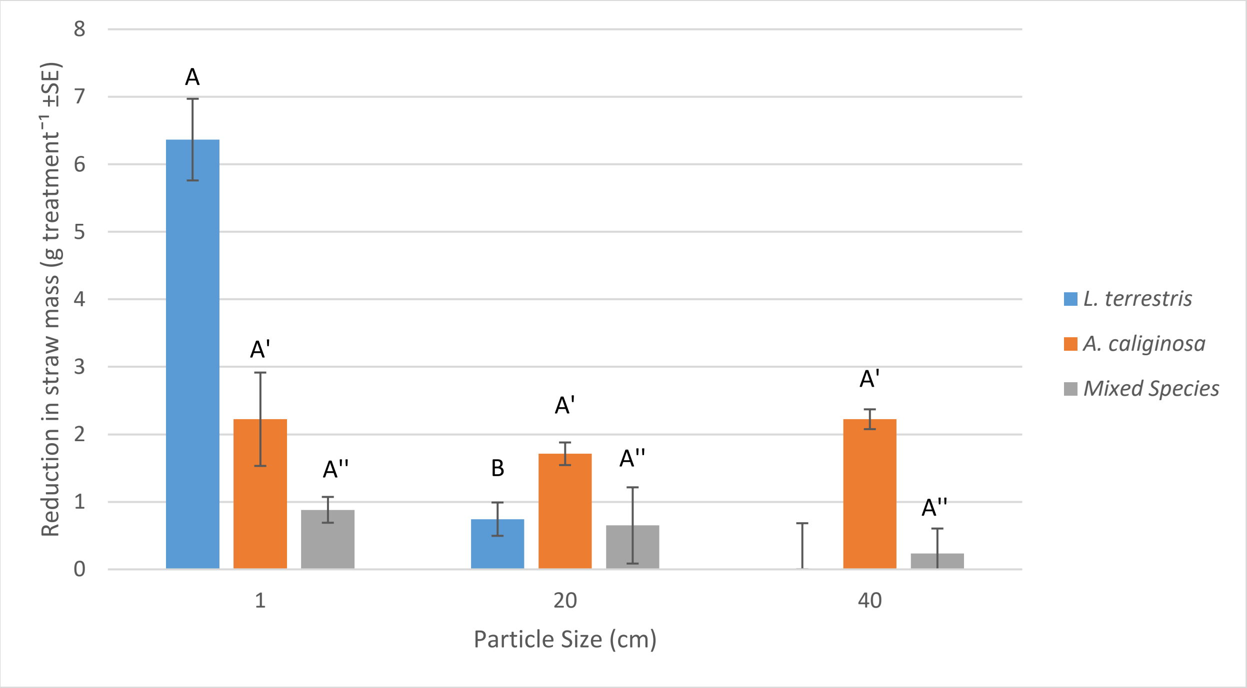


Figure 6.10. Mean reduction in wheat straw mass (g treatment^{-1} ± S.E.) by adult earthworms over 8 weeks with selected particle sizes (1 cm, 20 cm and 40 cm). Within earthworm treatments, samples that are statistically different between particle size are presented by different lettering (one-way ANOVA; $p < 0.001$).

L. terrestris monocultures incorporated straw to the lowest depths (range of 14-16 cm between particle size treatments), where maximum depth recorded was not significantly different between particle sizes (one-way ANOVA; $p > 0.05$). There were significant differences between the maximum depth of removal within *A. caliginosa* monocultures ($p < 0.05$), where post-hoc tests determined that

1 cm particle size was significantly different to the other treatments. Straw removal under the mixed species treatment was low, however there was a significant difference between the depth of incorporation between particle size treatments, where incorporation depth was higher under lower particle size.

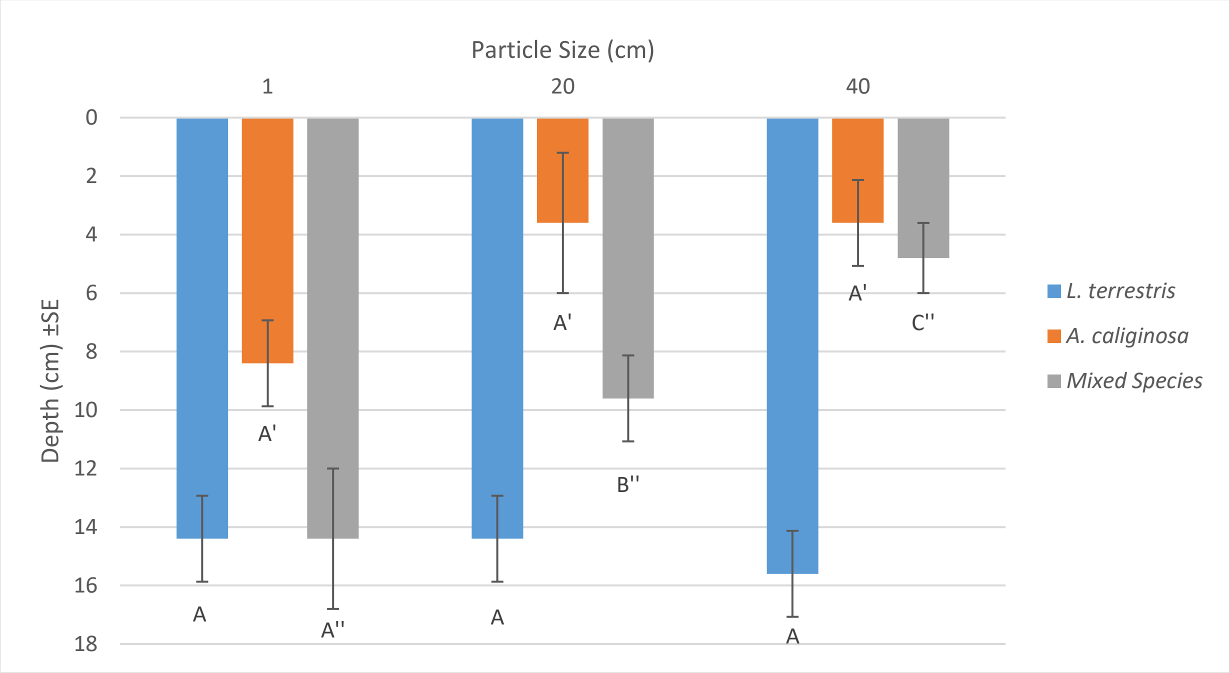


Figure 6.11. Effects of adult earthworm treatments and wheat straw particle size (1 cm, 20 cm and 40 cm) on mean depth (cm ± S.E.) of incorporation. Within earthworm treatments, samples that are statistically different between particle size are presented by different lettering (one-way ANOVA; p < 0.001).

Following destructive sampling, soil was separated into three depths a) 0-60 mm; b) 60-120 mm; c) 120-180 mm. For each depth, the mean mass of straw for each treatment was recorded and compared (Figure 6.12). Two-way ANOVA confirmed that there was an interaction between the effects of particle size and depth on the mean mass of straw recorded under *L. terrestris* monocultures (DF = 4; F = 5.41; p < 0.001) where multiple comparisons testing (Tukey HSD) confirmed that the maximum mass was contained within the upper soil depth (0-60 mm) and lowest particle size (Figure 6.12). Analysis of the single effects confirmed that there was a significant difference in the mean straw mass between depths under all earthworm treatments and particle sizes, where the significantly different depth was 0-60 mm, and where mean mass of straw recorded was higher (Figure 6.12).

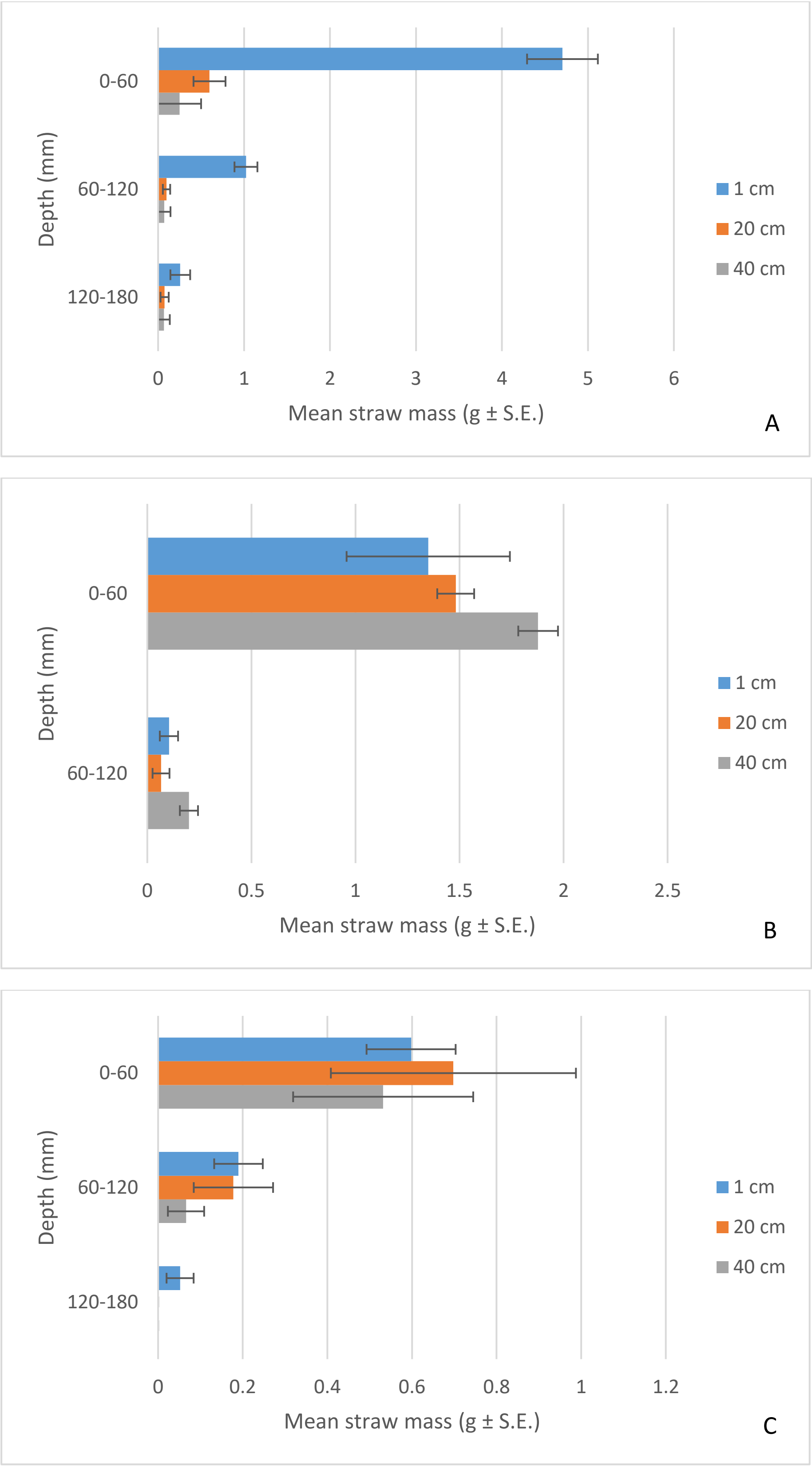


Figure 6.12A-C. Mean total straw mass (g ± S.E.) incorporated within 3 soil depths (0-60 mm; 60-120 mm; 120-180 mm) over 8 weeks under selected earthworm treatments (A = *L. terrestris*; B = *A. caliginosa*; C = Mixed species) and straw particle sizes. Note: *A. caliginosa* monoculture sample depth was 120 mm. N=5.

6.4.3.2. Earthworm survival and reproduction

At experimental end, a reduction in earthworm mass was recorded under all treatments (Figure 6.13.). Full survival (100%) was recorded under *L. terrestris* monoculture treatments, where cocoon production was recorded (1.4 ± 0.51 coc. ind.$^{-1}$). A one-way ANOVA confirmed that the % mass reduction was significantly different under *L. terrestris* monocultures ($p < 0.05$), where the mass reduction was higher in the control treatment (Figure 6.13.).

For *A. caliginosa* monocultures, there were no significant differences in the % mass reduction between particle size samples and the control (one-way ANOVA; $p > 0.05$). Again, there was 100% survival within monoculture treatments, but no cocoon production was recorded. Within mixed species treatments, one-way ANOVA confirmed a significant difference between the particle size and control treatments on the % mass reduction ($p < 0.05$), however, the significantly different treatment was 40 cm particle size (Figure 6.12.). There was 100% survival for *A. caliginosa*, however 90% under *L. terrestris*, where there was 40% mortality rate in the 40 cm treatments.

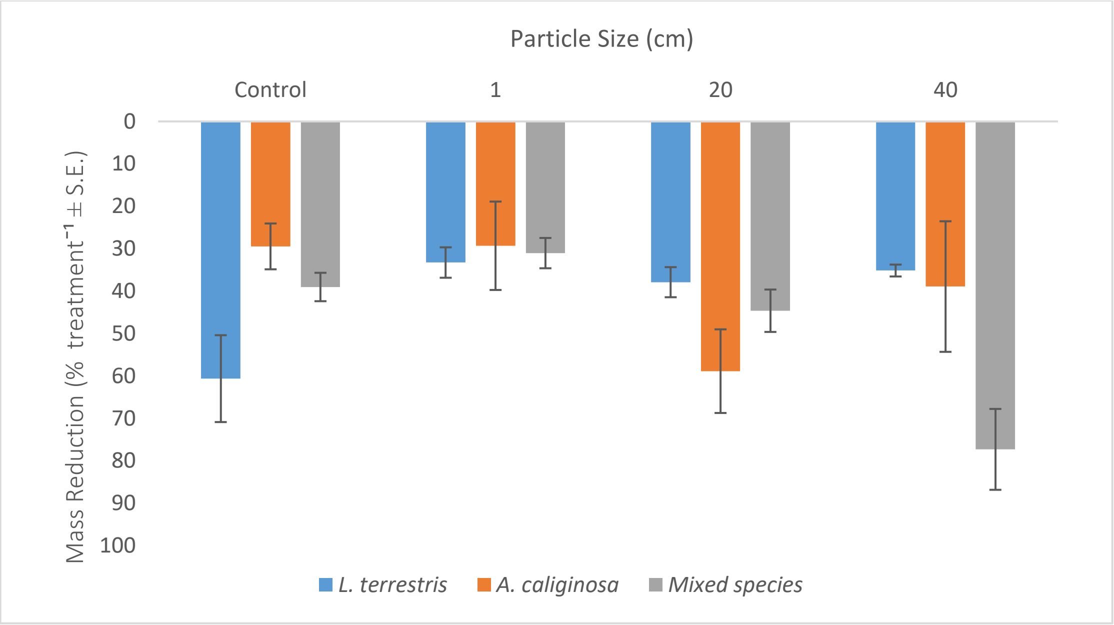


Figure 6.13. Mean total biomass reduction (% treatment^{-1} ± S.E.; N=5) of earthworms (*L. terrestris* N=2; *A. caliginosa* N=3; Mixed species N=4) under 1 cm, 20 cm and 40 cm particle size straw residue applications and control A.

Compared with monoculture treatments, there was a lower rate of *A. caliginosa* mass reduction in mixed species treatments than monocultures (Figure 6.14). *L. terrestris* decreased more than the control under mixed species than monoculture treatments (Figure 6.13).

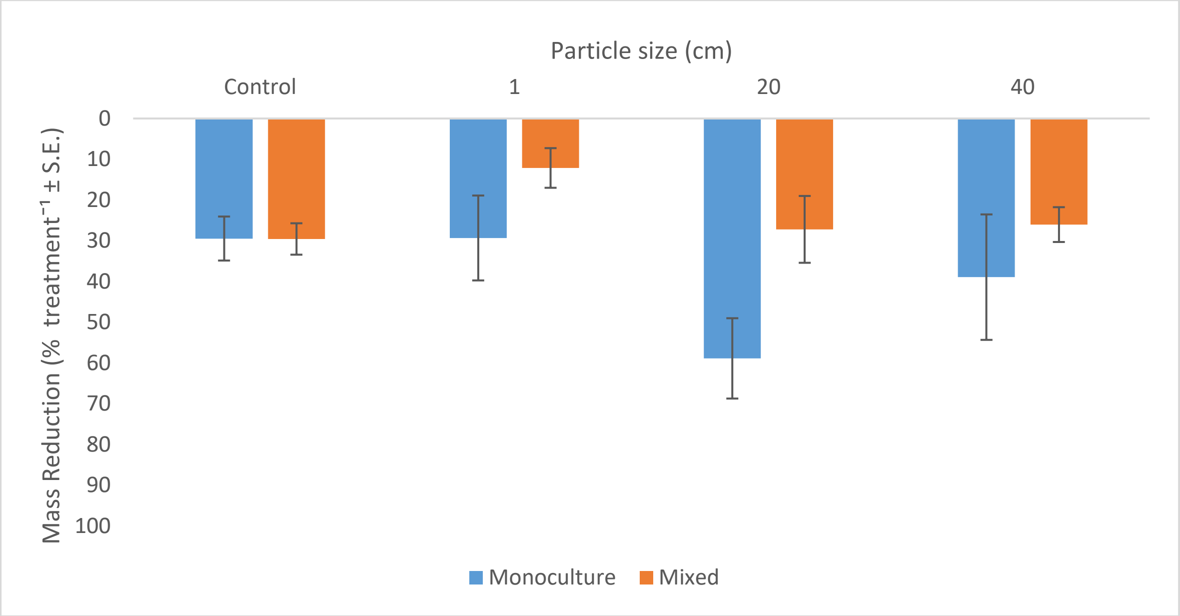


Figure 6.14. The mean mass reduction (% treatment^{-1} ± S.E.; N=5) of *A. caliginosa* monoculture and with *L. terrestris* (N=1) under 1 cm, 20 cm and 40 cm particle size straw residue applications and control A. *A. caliginosa* N=3 in both treatments.

6.4.3.3. Nutrient concentration

In this section the key findings of the analysis are presented, with a full set of elemental results in Appendix III. Total nutrient concentration and available nutrient concentration was determined to indicate whether the influence of earthworm species changed the nutrient levels in soil compared with a control treatment.

Table 6.2. Mean available and total nutrient concentration (mg kg^{-1} ± S.E.) of soil at experimental end. Concentrations of elements that are statistically different from the control soil B (straw application with no earthworms added) are presented by different lettering in the relevant sub-columns of the Table (Mann Whitney-U; $p < 0.05$).

Earthworm Treatment	Element	Mean available concentration (mg kg $^{-1}$ ± S.E.)			Mean total concentration (mg kg $^{-1}$ ± S.E.)		
		1 cm	20 cm	40 cm	1 cm	20 cm	40 cm
L. terrestris	P	47 ± 3^{a}	43 ± 1^{a}	51 ± 3^{a}	1534 ± 68^{a}	1858 ± 142^{a}	2017 ± 108^{a}
	Mg	177 ± 9^{a}	168 ± 6^{a}	175 ± 6^{a}	3609 ± 94^{a}	4842 ± 294^{a}	5462 ± 184^{a}
	K	266 ± 8^{a}	258 ± 4^{a}	255 ± 5^{a}	545 ± 17^{a}	695 ± 43^{a}	825 ± 29^{a}
	Mn	11 ± 1^{a}	9 ± 0.5^{a}	11 ± 1^{a}	854 ± 69^{a}	1292 ± 196^{a}	1413 ± 196^{b}
	Fe	2.7 ± 1.3^{b}	1.9 ± 0.6^{b}	3 ± 2^{b}	7822 ± 296^{a}	11293 ± 1177^{a}	14219 ± 811^{a}
	Ni	0.7 ± 0.2^{a}	0.5 ± 0.01^{a}	0.6 ± 0.1^{a}	38 ± 2^{a}	47 ± 3^{a}	71 ± 3^{a}
	Zn	0.9 ± 0.3^{a}	0.9 ± 0.2^{a}	0.6 ± 0.1^{b}	147 ± 4^{a}	178 ± 12^{a}	193 ± 8^{a}
	B	47 ± 3^{a}	43 ± 1^{a}	51 ± 3^{a}	104 ± 4^{a}	153 ± 14^{b}	336 ± 17^{a}
A. caliginosa	P	26 ± 6^{b}	20 ± 4^{a}	24 ± 5^{a}	1656 ± 49^{a}	1786 ± 128^{a}	1494 ± 97^{a}
	Mg	228 ± 21^{a}	162 ± 7^{a}	191 ± 11^{a}	3737 ± 158^{a}	4558 ± 193^{a}	4213 ± 277^{a}
	K	401 ± 23^{a}	328 ± 23^{a}	215 ± 40^{b}	682 ± 21^{a}	746 ± 29^{a}	709 ± 37^{a}
	Mn	12.8 ± 2^{a}	11.7 ± 1^{a}	12.7 ± 1^{a}	797 ± 103^{b}	995 ± 93^{a}	932 ± 91^{b}
	Fe	5.5 ± 0.8^{a}	7.9 ± 2.9^{a}	3.2 ± 0.2^{b}	8719 ± 509^{a}	11341 ± 856^{a}	9439 ± 761^{a}
	Ni	0.2 ± 0.02^{a}	0.1 ± 0.01^{a}	0.1 ± 0.02^{a}	44 ± 3^{a}	59 ± 4^{a}	53 ± 4^{a}
	Zn	2.1 ± 0.5^{a}	1.1 ± 0.2^{a}	1.6 ± 0.4^{a}	169 ± 10^{a}	196 ± 13^{a}	178 ± 5^{a}
	B	26 ± 6^{b}	20 ± 4^{a}	24 ± 5^{b}	238 ± 13^{a}	317 ± 20^{a}	285 ± 19^{a}
Mixed Species	P	43 ± 2^{a}	41 ± 1^{a}	47 ± 3^{a}	2592 ± 207^{a}	2252 ± 237^{a}	2089 ± 132^{a}
	Mg	120 ± 4^{a}	117 ± 2^{a}	122 ± 2^{a}	7080 ± 184^{a}	6498 ± 326^{a}	5510 ± 283^{a}
	K	241 ± 6^{a}	234 ± 6^{a}	246 ± 4^{a}	941 ± 34^{a}	889 ± 48^{a}	793 ± 20^{a}
	Mn	7.6 ± 0.3^{a}	7.6 ± 0.3^{b}	8.5 ± 0.5^{b}	1802 ± 291^{a}	1916 ± 437^{a}	2024 ± 587^{a}
	Fe	2.4 ± 1^{b}	3.5 ± 1^{b}	3.7 ± 1^{b}	18256 ± 1116^{a}	17534 ± 1683^{a}	15735 ± 1409^{a}
	Ni	0.1 ± 0.01^{a}	0.1 ± 0.01^{a}	0.1 ± 0.01^{a}	78 ± 5^{a}	60 ± 5^{a}	69 ± 6^{a}
	Zn	0.3 ± 0.01^{a}	0.3 ± 0.01^{a}	0.3 ± 0.01^{a}	254 ± 10^{a}	225 ± 11^{a}	209 ± 16^{a}
	B	43 ± 2^{a}	41 ± 1^{a}	47 ± 3^{a}	389 ± 22^{a}	374 ± 32^{a}	336 ± 27^{a}
Control B (no earthworms added)	P	36 ± 2^{b}	34 ± 2^{b}	31 ± 1^{b}	976 ± 134^{b}	853 ± 20^{b}	910 ± 31^{b}
	Mg	132 ± 4^{b}	135 ± 5^{b}	136 ± 4^{b}	3352 ± 54^{b}	3362 ± 66^{b}	3355 ± 119^{b}
	K	204 ± 6^{b}	162 ± 3^{b}	185 ± 3^{b}	578 ± 15^{b}	609 ± 7^{b}	594 ± 17^{b}
	Mn	9 ± 0.3^{b}	7.6 ± 0.2^{b}	8.8 ± 0.3^{b}	613 ± 75^{b}	632 ± 60^{b}	1077 ± 347^{b}
	Fe	3 ± 0.6^{b}	2.6 ± 0.2^{b}	2.8 ± 0.2^{b}	3957 ± 181^{b}	4015 ± 245^{b}	4310 ± 268^{b}
	Ni	0.3 ± 0.05^{b}	0.4 ± 0.03^{b}	0.4 ± 0.01^{b}	28 ± 1^{b}	27 ± 1^{b}	28 ± 1^{b}
	Zn	0.5 ± 0.01^{b}	0.5 ± 0.01^{b}	0.6 ± 0.01^{b}	88 ± 3^{b}	87 ± 2^{b}	93 ± 5^{b}
	B	31 ± 1^{b}	32 ± 3^{b}	28 ± 1^{b}	154 ± 6^{b}	153 ± 7^{b}	159 ± 8^{b}

6.4.3.3.1. *L. terrestris*

For the majority of elements, there was a significant difference in the mean elemental concentration (total and available) between straw treatments with *L. terrestris* populations present and straw controls with no earthworm addition ($p < 0.05$; Mann-Whitney U; Table 6.2).

Between earthworm treatments, *L. terrestris* monoculture samples had the highest mass of surface straw removal over the experimental period (Figure 6.9). Effects of particle size on the straw removed may have influenced the nutrient dynamics of the soil, where the total nutrient concentration was significantly different between particle size groups (Kruskal-Wallis; $p < 0.001$). Post-hoc tests (Dunn-Bonferroni) determined that the 1 cm particle size treatment (which had the highest rate of incorporation) was significantly different to other treatments. For all elements, the mean total nutrient concentration was highest at 40 cm, where 40 cm > 20 cm > 1 cm (Table 6.2). There were no significant effects of particle size on the mean available nutrient concentration (Kruskal-Wallis, $p > 0.05$).

Interval plots of the available and total nutrient concentration under particle size treatments and controls with no earthworms added (Appendix III) indicated that *L. terrestris* monocultures increased P Mg, Zn and B under both analysis types and decreased K.

Following analysis of organic matter incorporation depth, statistical analysis of total nutrient concentration between treatments indicated there were no significant differences between nutrient concentration and soil depth for all straw treatments ($p > 0.05$). Mean nutrient concentration results at depth for all earthworm treatments are presented in the Appendices (Appendix III).

6.4.3.3.2. *A. caliginosa*

For the majority of elements, there was a significant difference in the mean total and available elemental concentration between straw treatments with *A. caliginosa* populations present and straw controls with no earthworm addition ($p < 0.05$; Mann-Whitney U; Table 6.2).

The mean mass of straw removed under *A. caliginosa* monocultures was low, where there were no significant differences in the mass removed between particle sizes ($p > 0.05$; Figure 6.9). Unlike *L. terrestris* monocultures, there were no significant differences in the total nutrient concentration between particle size groups (Kruskal-Wallis; $p > 0.05$). There were no significant effects of particle size on the mean available nutrient concentration (Kruskal-Wallis, $p > 0.05$).

Interval plots of the available and total nutrient concentration under particle size treatments and controls with no earthworms added (Appendix III) indicated that *A. caliginosa* species increased Mg, Zn and B under both analysis types.

6.4.3.3.3. Mixed species

Earthworm mortality occurred during the experiment under this treatment. This could have had some effects on the nutrient concentration in these samples. For the majority of elements, there was a significant difference in the mean elemental concentration (total and available) between straw treatments with mixed species populations present and straw controls with no earthworm addition ($p < 0.05$; Mann-Whitney U; Table 6.2).

Section 6.4.3.3.1 indicated that the mean mass of straw removed under mixed species treatments was low, where there were no significant effects of particle size on the amount of straw removed ($p > 0.05$; Figure 6.8). Unlike *L. terrestris* monocultures, there were no significant differences in the total nutrient concentration between particle size groups (Kruskal-Wallis; $p > 0.05$). This could be due to the high range within sample means. Qualitatively, there are differences between particle size groups, where 1 cm particle size has higher total nutrient concentration (Table 6.2). There were no significant effects of particle size on the mean available nutrient concentration (Kruskal-Wallis, $p > 0.05$).

Interval plots of the available and total nutrient concentration under particle size treatments and controls with no earthworms added (Appendix III) indicated that mixed species treatments increased P, Mg and Mn concentration.

6.4.3.4. C:N Dynamics

For all treatments, there were significant differences in the %C concentration between the mean control soil sample (straw application without earthworms) and mean earthworm applications ($p > 0.05$), where %C was lower under *L. terrestris* monocultures and mixed species (Table 6.3.; Mann-Whitney U; $p < 0.05$). For *A. caliginosa* monocultures, the %C was higher under 1 cm particle size.

The effects of particle size on %C concentration within earthworm treatments indicated a significant difference under *A. caliginosa* monocultures (Kruskal-Wallis; $p < 0.05$). Post-hoc tests (Dunn-Bonferroni) confirmed that all particle size treatments (1, 20 and 40 cm) were statistically different from one another.

Analysis of %N indicated that there were significant differences between the %N concentration between earthworm treatments and control straw applications (Table 6.5; Mann-Whitney U; $p < 0.001$), where %N was 0.5% higher. There were no differences in %N concentration between particle size applications within all earthworm treatment groups ($p > 0.05$). Although *L. terrestris* applications had a slightly higher %N concentration for all particle size samples, the differences between earthworm applications were not statistically significant ($p > 0.05$).

The relationship between the effects of earthworm treatment and a control (straw + no earthworms) on the C:N ratio mirrored the %C results, where C:N ratio was lower under *L. terrestris* monocultures and mixed species samples (Mann-Whitney U; $p < 0.05$). C:N ratio was highest under *A. caliginosa* treatments, where a significant difference in the C:N ratio occurred between particle size treatments (Kruskal-Wallis; $p < 0.05$). Post-hoc tests (Dunn-Bonferroni) confirmed that the significantly particle size different group was 1 cm.

Table 6.3. Mean %C, %N and C:N ratio (± S.E.) for each earthworm treatment under the specified straw particle size (cm). Samples that are statistically different from the control soil B (straw application with no earthworms added) are presented by different lettering in the relevant sub-columns of the Table (Kruskal-Wallis, $p < 0.05$).

Earthworm Treatment	Analysis	Particle Size (cm)		
		1	20	40
L. terrestris	C (%)	1.78 ± 0.06^{a}	1.84 ± 0.10^{a}	2.07 ± 0.28^{a}
	N (%)	0.73 ± 0.03^{a}	0.73 ± 0.04^{a}	0.72 ± 0.12^{a}
	C:N ratio	2.82 ± 0.07^{a}	3.00 ± 0.10^{a}	2.93 ± 0.10^{a}
A. caliginosa	C (%)	3.03 ± 0.09^{a}	2 .00 ± 0.13^{a}	2.42 ± 0.13^{b}
	N (%)	0.63 ± 0.01^{a}	0.67 ± 0.02^{a}	0.66 ± 0.01^{a}
	C:N ratio	4.78 ± 0.15^{a}	3.32 ± 0.23^{a}	3.73 ± 0.23^{b}
Mixed Species	C (%)	1.80 ± 0.03^{a}	1.76 ± 0.04^{a}	1.89 ± 0.04^{a}
	N (%)	0.7 ± 0.01^{a}	0.67 ± 0.02^{a}	0.69 ± 0.01^{a}
	C:N ratio	2.85 ± 0.08^{a}	2.85 ± 0.08^{a}	3.08 ± 0.08^{a}
Control B (no earthworms added)	C (%)	2.55 ± 0.14^{b}	2.41 ± 0.06^{b}	2.44 ± 0.07^{b}
	N (%)	0.22 ± 0.01^{b}	0.21 ± 0.01^{b}	0.22 ± 0.01^{b}
	C:N ratio	4.27 ± 0.30^{b}	3.96 ± 0.17^{b}	3.87 ± 0.11^{b}

6.4.3.5. Observations

Analysis of the soil moisture was conducted at experimental start and end to determine whether the soil moisture conditions were equal between treatments throughout the experiment. One-way ANOVA confirmed that there were no significant differences between treatments on soil moisture ($p > 0.05$), however, it was noted that soil at the bottom of the envelopes had increased moisture (30%) at the end of the experiment.

At experimental end, images were taken of removed straw. The images of *L. terrestris* monoculture treatments (Figure 6.17A) were visually different to the *A. caliginosa* monoculture treatments (Figure 6.17B). In the larger particle size treatments (20 cm and 40 cm), the straw under *L. terrestris* monocultures was more decomposed, where it appeared that *L. terrestris* could strip and destruct the straw. This was not evident in the *A. caliginosa* monocultures and there was increased surface mould growth.

Under mixed species treatments, there was visual evidence of straw stripping (Figure 6.17C). At experimental end, *L. terrestris* was found within the surface straw for all replicates in mixed species treatments where *L. terrestris* survived (N=3). Although this experiment was conducted within full darkness so it could be possible *L. terrestris* was feeding, within all *L. terrestris* monoculture replicates the *L. terrestris* (N=2) were found in their burrows.

Figure 6.17. Images of surface straw (40 cm particle size) at experimental end under different earthworm treatments: A) *L. terrestris* monoculture (N=2) B) *A. caliginosa* monoculture (N=3) C) Mixed species (*L. terrestris* (N=1) and *A. caliginosa* (N=3)).

6.5. DISCUSSION

This Section discusses the key findings from the controlled laboratory experiments conducted within this Chapter and explores the effects of earthworm treatments and particle size of straw on the incorporation of organic matter and nutrient cycling.

6.5.1. Effects of earthworm species on organic matter incorporation

This Chapter highlights the positive effects of *L. terrestris* on the rate of wheat straw incorporation. All three experiments indicated a difference in the amount of organic matter incorporation between earthworm species, where *L. terrestris* had the highest removal and selection (Figures 6.3 and 6.10; Table 6.1). Organic matter type did not affect the rate of removal under milled particle size (Figure 6.3) but did influence earthworm growth rates (Figure 6.2), where horse manure was the most effective treatment under *L. terrestris* and *A. caliginosa* monocultures. The effects of organic matter type on earthworm growth was reduced under milled applications compared with results observed in Chapter 5, where particle size was larger. This suggests that field applications of milled waste organic matter may reduce the previously experienced effects of residue type on earthworm density in the field (Ashworth et al, 2017), however, the practical likelihood of milled applications of organic matter in the field are low. Future experiments could investigate the potential and implications of waste organic matter applications at lower particle size in the field.

The positive effects of *L. terrestris* on waste organic matter removal was expected, as it is a species with a higher biomass than *A. longa and A. caliginosa* (Sims and Gerard, 1999), with unique foraging behaviour more suited to surface applications (Bouché, 1977; Nuutinen and Butt, 2005). In Experiment 1, where the number of earthworms per vessel were the same (N = 2), increased removal rates of organic matter by *L. terrestris* was not surprising. Under treatments where equal masses of earthworms per vessel were applied and there was no bias in the feeding methods (Experiment 2), the removal of straw mass was still higher under *L. terrestris* (Table 6.1.). This highlights how *L. terrestris* has higher removal rates of fresh applied organic matter. This could be due to the feeding requirements of *L. terrestris*, which solely rely on relatively fresh organic matter to feed, compared with *A. caliginosa*, which can consume a higher rate of soil and feeds on organic matter at a more decomposed state in the soil profile. Therefore, under freshly applied organic matter, such as cereal straw residues, an increased density of *L. terrestris* may enhance the rate of organic matter removal compared with *A. caliginosa*, which is more abundant in arable soils.

Under straw applications more natural to field conditions (Experiment 3), *L. terrestris* reproduced under a 1 cm straw treatment, indicating that field applications of wheat straw could sustain field populations. It was noted in observations that *L. terrestris* have the ability to physically strip straw residues (Figure 6.17). Stripping straw could increase the surface area of straw exposed to decomposition, which may increase the decomposition rate. Added to the increased decomposition of straw residues mediated by microbial activity in surface casts, this indicates the ability of *L. terrestris* to utilise the material and increase survival compared with *A. caliginosa*, where surface straw appeared mouldy. The ability for wheat crop residue applications to solely maintain earthworm populations in no-till environments is still under review, where many field experiments suggest that applications of residues can improve earthworm populations (Fraser and Piercey, 1998; Fraser et al, 2003; Abhail and Whalen, 2018; Korucu et al, 2018), yet wheat straw alone could incur a population collapse (Stroud et al, 2016c), where the quality of residues have been identified as a key factor in enhancing earthworm abundances (Coq et al, 2007; Eriken-Hamel et al, 2009; Ashworth et al, 2017). These experiments have identified that the effects of residue quality and particle size on earthworm species differ, where *L. terrestris* is more resilient. To determine the effects of straw residues on *L. terrestris* population development, further investigations of the growth and development under wheat straw residues in the field is required.

Organic matter removal by *A. caliginosa* monocultures was lower than *L. terrestris* (Figures 6.3 and 6.10; Table 6.1) however small bioturbation effects by this species were recorded, where the mass removed was not limited by particle size under application of straw with lengths as found in the field post-harvest (Figure 6.10). The lower removal rate observed under *A. caliginosa* compared with anecic species concurred with observations by Rajapaksha et al, (2013), who confirmed in choice chamber experiments a preference and removal of smaller particle sizes at a slower rate than *L. terrestris* and *A. longa* populations. Responses of *A. caliginosa* to surface straw applications similar to field settings (Experiment 3) were negative, where a high rate of *A. caliginosa* mortality occurred (40 % for 20 cm particle size) and no reproduction. As expected, this suggests that surface applied wheat straw is an ineffective food source for *A. caliginosa.* In field settings, it could be that *A. caliginosa* populations benefit from other organic matter sources, such as cover crops and weeds (Roarty et al, 2017) where soils with a high OM content from ploughing can increase their survival (Crittenden et al, 2014; Frazão et al, 2019). No-till crop residue applications may negatively impact *A. caliginosa* populations and benefit *L. terrestris* populations; this change in numbers of given species may cause a shift in earthworm-mediated soil functioning.

The interactions between adult *A. caliginosa* and *L. terrestris* in mixed species treatments were inconclusive. A higher mass of straw was incorporated under monoculture treatments than mixed treatments (Experiment 3; Figure 6.10), indicating inter-specific competition. However, *A. caliginosa* survival was higher when present under mixture culture with surface applied wheat straw (Figure

6.14), suggesting some facilitative effects of *L. terrestris*. A potential mutualistic relationship between the species was not recorded, where increased *L. terrestris* mortality occurred under mixture. Observations at experimental end found all surviving *L. terrestris* within the surface straw (Figure 6.17C), which could suggest avoidance behaviour. Although previous field surveys have indicated that positive interactions could occur between the two species, where *L. terrestris* middens increased *A. caliginosa* juvenile population size (Chapter 5; Lowe and Butt, 2005), as adults competitive species interactions may cause dispersal. Field assessment of burrow distribution between *L. terrestris* and *A. caliginosa* populations indicated a heterogenous relationship (Pitkanen and Nuutinen, 1997), therefore no indication of a negative species interaction. Previous laboratory investigations into the interactions between *L. terrestris* and *A. caliginosa* have suggested that competitive interactions occur between *L. terrestris* and *A. caliginosa* in laboratory cultures under a population size of *A. caliginosa* (N=10) and greater (Eriksen-Hamel and Whalen, 2007a). The *A. caliginosa* density was much lower in this experiment to remove potential laboratory induced inter-specific interactions, where niche separation and migration are not possible (Eriksen-Hamel et al, 2009). However, even with a lower *A. caliginosa* population, interspecific interactions still occurred. The lack of SOM in Kettering loam meant that *A. caliginosa* relied upon *L. terrestris* incorporation to provide SOM into the soil profile to survive. In the field, it would be likely that the SOM content is higher. Soil with a high SOM content may regulate *A. caliginosa* populations as *L. terrestris* incorporate freshly applied residues. *L. terrestris* population density may also be a limiting factor on *A. caliginosa* survival. In this experiment, *A. caliginosa* survival was assessed by biomass change and reproductive rate. To reduce the inter-specific competition influencing *A. caliginosa* reproduction, one *L. terrestris* was used in every sample. To determine whether the incorporation rate by *L. terrestris* density limits adult *A. caliginosa* survival, future experiments should investigate *A. caliginosa* growth (N = 1) under increasing *L. terrestris* density.

Comparisons between the effects of organic matter type on the growth and rate of removal by *L. terrestris* and *A. longa* under milled treatments (Experiment 1) indicated that *L. terrestris* can incorporate at a more rapid rate (Figure 6.2). Observations indicated that *A. longa* fed and cast on the soil surface without incorporating organic matter, where organic matter was found within *L. terrestris* soil samples. Due to inter-specific competition, it is unlikely that *L. terrestris* and *A. longa* populations are found in abundance together in the field (Lowe and Butt, 1999; Uvarov, 2009; Hoeffner et al, 2019). However, the responses of *A. longa* and *L. terrestris* to organic matter applications were similar, where they increased in biomass at the same rate between feeds (Figure 6.3). The ‘free residue’ methodological approach was not effective under *A. longa* species, where their casts were layered within the organic matter, making residue sampling at experimental end difficult. This could be the reason for the high S.E. in the mean mass of straw removed by *A. longa* samples compared with *L. terrestris* (Figure 6.2). The difference in residue burial by these two species may change the transport and removal of organic matter down a soil profile, which could influence the rate of decomposition by microorganisms and nutrient dispersal through soil. Future experiments could compare the organic matter utilisation by these species to determine the implications on organic matter decomposition and nutrient mineralization.

Analysis of straw removal with depth indicated that *L. terrestris* species can incorporate surface applied residues to a depth of 18 cm, where the maximum depth recorded was not limited by *A. caliginosa* presence (experiment 3; Figure 6.13). The mean mass of straw recorded within increasing depth profiles indicated that there was a reduction in straw biomass incorporation with increasing depth, where 78.6% was found in the upper layers of the soil profile (0-60 mm). The relationship between decreasing straw mass within increasing depth was demonstrated in all treatments. A number of factors could influence the distribution of organic matter incorporated by *L. terrestris*. Straw held within the upper layers of the burrow could regulate burrow moisture and protect the burrow from predators (Butt and Nuutinen, 2005). Increased microbial activity within the upper soil layers (Aira et al, 2009; Hoang et al, 2016) could also enhance degradation of straw, which would further increase the nutrient mineralisation and palatability of the straw selected. It could also require more energy to incorporate organic matter to the lower soil depths and interfere with movement in the lower soil depths. Total straw mass incorporated at the lower sample depth (120-180 mm) was significantly reduced in mixed species treatments, possibly due to the impact of *A. caliginosa* on its burrowing behaviour. This suggests that the utilisation of organic matter by *L. terrestris* does not facilitate adult *A. caliginosa* and other geophagous earthworm populations.

6.5.2. Effects of particle size on straw incorporation.

There is significant evidence in this Chapter to suggest that the effect of particle size limits the ability of earthworm populations to incorporate organic matter into soil. This became evident when comparing *L. terrestris* results from Experiment 1 with Experiment 3, where 2 x *L. terrestris* removed on average 14 g straw at milled (1 mm) particle size (Figure 6.3), compared with 6.36 ± 0.6 g of 1 cm in Experiment 3 over the same temporal scale (8 weeks) (Figure 6.9). Choice chamber experiments indicated a significant difference between the selection of 1 mm particle size with 1 cm particle size under both *L. terrestris* and *A. caliginosa* species ($p < 0.05$), where *L. terrestris* removed smaller particle sized material at a faster rate (Figures 6.5 and 6.6; Table 6.1). This compared well with findings of Rajapaksha et al, (2013), where *A. caliginosa* removed forestry litter at a slower rate than *L. terrestris* but had similar organic matter preferences. In managed soils with low SOM content, it may be key to ensure that particle size of organic matter applications are low (< 1 cm) to sustain *A. caliginosa* and maximise organic matter decomposition. This indicates that particle size of organic matter applications is a limiting factor when utilising earthworm populations to increase the degradation rate in no-till agroecosystems.

The ability of *L. terrestris* to incorporate straw residues at particle sizes replicating the field was demonstrated in Experiment 3. The mass of organic matter incorporated by *L. terrestris* monocultures was significantly higher than other earthworm treatments and there was a statistically significant difference in the straw removal (g) between particle sizes within *L. terrestris* monocultures ($p < 0.05$), where 1 cm particle size had higher incorporation than 20 cm and 40 cm applications. Although the larger particle sizes were not incorporated, at experimental end visual observations indicated that *L. terrestris* had stripped the straw material (Figure 6.17), indicating that in the field earthworms could manipulate these particle sizes. This stripping action was also observed by Stroud et al, (2016a). However, whether *L. terrestris* was manipulating the straw for feeding or midden development could not be determined from this experimental design. Further investigations into the utilisation of straw residues (midden development or food) by *L. terrestris* species is required.

Although *A. caliginosa* showed a preference for milled wheat straw under choice chamber experiments (Figure 6.6; $p < 0.05$), under field applications no relationship was observed between the rate of removal on different particle size treatments under *A. caliginosa* monocultures (Figure 6.9; $p > 0.05$). Evidence from experiment 2 indicated that 1 cm particle size may be too large for *A. caliginosa* to feed upon, so in field environments these populations rely upon a high SOM content and other organic matter to maintain a high population density. When applied with milled wheat straw, the incorporation rate was higher (3 ± 0.5 g; Figure 6.3) than the field applications (range = 1.71-2.22 g; Figure 6.10.). The reason why *A. caliginosa* are the most abundant species could be due to their resilience and flexibility in feeding and burrowing behaviours compared to other UK species. Although the total mass of organic matter incorporated was significantly lower and slower than *L. terrestris* monocultures, the ability of *A. caliginosa* to remove lengths of 20 cm and 40 cm particle size should be noted (Figure 6.10.). In areas of high *A. caliginosa* populations and low *L. terrestris* populations this may provide valuable information on the rate of organic matter removal, which could inform decision making. It is noted that *A. caliginosa* populations can survive ploughing, where the increased soil organic matter content can enhance their survival (Crittenden et al, 2014). Further investigations could explore the rate of surface removal by *L. terrestris* and compare the soil organic matter dispersal in comparison with ploughed sites to determine any benefits provided by *L. terrestris* to *A. caliginosa* populations.

Under *L. terrestris* monoculture, there was no effect of particle size on the maximum depth of organic matter incorporation, where *L. terrestris* can incorporate this material up to 18 cm beneath the soil surface (Figure 6.11.). This indicated that bioturbation of organic matter by *L. terrestris* can increase SOM content at lower depths, regardless of particle size. However, there were differences in the mean mass incorporated at each depth, where most organic matter was recorded in the upper soil layer (Figure 6.12) and 1 cm particle size had a significantly higher mass of incorporated matter. Of the lowest soil layer (120-180 mm), there was a significant difference of the mass incorporated between particle sizes ($p < 0.05$), where post-hoc tests determined that 1 cm particle size was the significantly different group. An increase in SOM content at lower depths could mitigate losses through cultivation and prevent an organic matter layering in no-till agroecosystems. This experiment emphasizes that applications of crop residues with a lower particle size may enhance the rate of organic matter replenishment at lower depths and maintain soil health. Within *A. caliginosa* monocultures, there were also no effects of particle size on mean straw mass incorporation mass or depth. Upper layers of the soil profile have higher levels of microorganism activity (Mathew et al, 2012), which may enhance further decomposition of organic matter and benefit earthworm species (Aira et al, 2009). Incorporating material to lower depths may reduce the rate of degradation, which could be less beneficial to earthworms. Further investigations could investigate bioturbation depth by *L. terrestris* and *A. caliginosa* populations under different organic matter treatments and the impact of SOM decomposition at depth between organic matter types.

This Section highlights that if organic matter particle size on the field was applied at 1 cm particle sizes, more organic matter would be incorporated over time if *L. terrestris* populations were present in no-till agroecosystems. Due to the preference of horse manure over wheat straw, it might be beneficial in crop residue systems to apply applications of organic matter with a lower C:N ratio to sustain populations as wheat straw degrades. The differences in effects of particle size on earthworm population growth and utilisation of organic matter highlights the importance of an earthworm survey being conducted in agroecosystems, as the differences in organic matter utilisation between species will have different effects on the distribution of SOM, which may have implications on decomposition and mineralization. A high earthworm count and species diversity is considered a bioindicator for a healthy soil (Pérès et al, 2011; Pulleman et al, 2012; Fusaro et al, 2018); utilising the information gained in earthworm surveys on species diversity could assist decision making by estimating the rate of organic matter removal and transport through the soil system.

6.5.3. Impacts of earthworms on nutrient dispersal.

The impacts of earthworm populations on nutrient dispersal within soil under different particle size applications was explored in Experiment 3. Due to the relationship observed in many earthworm experiments between earthworm casts and a higher available nutrient concentration (van Groenigen et al, 2019) it was predicted that there would be a statistical relationship between earthworm activity and micronutrient concentration. General comparisons of the nutrient concentration data indicated that there were statistically significant differences between the nutrient concentration (total and available) between samples with earthworms and controls with straw applications and no earthworms under all earthworm treatments (*L. terrestris*, *A. caliginosa*, mixed species; Table 6.2), where P, Mg, Zn and B increased in concentration and K decreased. In the long-term, this may have implications for plant growth. For example, phosphorus is a highly immobile element where plant uptake depends on root efficiency and morphological traits (Fernandez and Rubio, 2015); an increase in P concentration in the presence of earthworms may reduce plant stress for crops that are P limited. The positive effect of *L. terrestris* on Zn concentrations has also been determined in other experimental studies (Zorn et al, 2005; Sizmur et al, 2011), where bioturbation increased Zn transport, and soil pore Zn concentration was increased through mobilisation of metalloids in casts. A decrease in elemental concentrations by earthworm populations has also been noted in research experiments (Resner et al, 2015), where earthworms may uptake nutrients during growth. The effect of earthworms on macro- and micronutrient concentrations in agroecosystems could be of significance in reduced tillage organic farming, where increased information on the elements they increase and decrease could enhance crop selection. This analysis was over a short period (8 weeks) and nutrient change mediated by earthworms is likely to be low. Future experiments could explore the temporal dynamics of nutrient release by earthworm populations and the interactions between elements over time.

One of the aims of organic matter residue application is enhanced nutrient mineralization by reducing nutrient losses following crop harvest by replenishing the soil with some nutrients from residues. Within straw particle size treatments, it was predicted that the effect of increased incorporation within smaller particle sizes would enhance nutrient concentrations caused by increased organic matter turnover (Wei et al, 2015). Results from this experiment indicated that the effects of straw particle size on nutrient concentration was only significant under *L. terrestris* monocultures ($p < 0.05$). Post-hoc tests confirmed that 1 cm particle size was the significantly different group. 1 cm particle size had the largest mass removed over the experimental period, however the nutrient concentration under this treatment was lower than other particle sizes (Table 6.2.). This could be due to *L. terrestris* bioaccumulating the nutrients from the straw in its body (or it is kept in its casts). Previous bioaccumulation studies of *L. terrestris* populations have focussed around heavy metals and ecotoxicological research, where all experiments indicated a bioaccumulation effect (Kizilkaya, 2005; Suthar et al, 2008; Leveque et al, 2013; Latifi et al, 2020). It is possible that there are temporal implications on micronutrient release under *L. terrestris* populations, where increased time after application could enhance nutrient concentrations. Further analysis of nutrient change over time by *L. terrestris* populations would determine these effects.

Unlike *L. terrestris* monoculture applications, there were no significant differences in the nutrient concentration within particle size treatments under *A. caliginosa* and mixed species treatments (Table 6.2), where there was a lower mean mass of straw incorporation (Figure 6.10). This could be due to the behavioural differences between the species and the reduced interactions with the organic matter, where *A. caliginosa* could not physically manipulate the straw (Figure 6.15), reducing decomposition rates. *A. caliginosa* feed at a slower rate than *L. terrestris* populations (Figure 6.6) but have similar organic matter preferences (Figure 6.9), so it could be that the earthworm mediated effects on the total nutrient concentration takes longer than 8 weeks to impact nutrient change. The differences in casting behaviour between the two species could also have significant implications for nutrient dispersal within a soil profile, where *A. caliginosa* casts on the soil surface and within the burrow system (Edwards, 2004). This could regulate nutrient concentration within soil depths, compared to *L. terrestris* casts on the soil surface, which could enhance microbial activity on the soil surface. Meta-analysis of 81 articles have highlighted the increased fertility of earthworm casts, where available P concentration is increased (van Groenigen et al 2019), yet research comparing the consistency of earthworm casts between species is still to be quantified. The cast age also determines its fate in the soil profile (erosion or aggregation) and the microbial activity within it, which will influence organic matter decomposition and nutrient mineralization (Aira et al, 2005, 2019; Agapit et al, 2018; Botinelli et al, 2010a, 2020b). To determine the effects of casts on nutrient concentration, future research could quantify the effects of earthworm casts age and location by species on the nutrient composition in soil.

The effects of straw incorporation depth and nutrient mineralization was explored. It was predicted that samples with a higher wheat straw incorporation mass would have a higher nutrient concentration, particularly at lower depths where SOM was increased. Although there were differences in the straw mass incorporated between earthworm treatments (Figure 6.10), no statistically significant differences occurred between soil nutrient concentration with depth under all particle size treatments ($p > 0.05$). It was hypothesized that increased soil organic matter removal to lower depths through bioturbation would result in increased nutrient concentration at lower depths through dispersal and leaching of cast material (Zorn et al, 2005; Sharpley et al, 2011, Massey et al, 2013; Botinelli et al, 2020). Sharpley et al, (2011) determined that *L. terrestris* increased P concentrations by reducing surface run off losses; it could be that the effects of *L. terrestris* burrowing behaviour on soil aeration and hydraulic activity has more of an impact than physical organic matter transport. Added to this, the amount of phosphorus in wheat straw residues is likely to be lower than poultry manure. This experiment was conducted with the presence of earthworms and no other soil fauna; it is possible that other trophic interactions belowground at these depths would further enhance the rate of decomposition of organic matter and assimilation of nutrients (Castro-Huerta et al, 2015). Also, the straw found at lower depths were not degraded. With increasing degradation nutrient mineralization should increase. Due to the slow degradation rate of straw and low nutrient concentration, the time taken to release nutrients is likely to be longer than 8 weeks (Gao et al, 2016). Future experiments could analyse the change in nutrient dynamics over a year (growing season) to determine changes over time and to provide recommendations on nutrient dispersal.

6.5.4. Impacts of earthworms on C:N dynamics

Analysis of the effects of particle size and earthworm treatment on the C:N ratio indicated that there were significant differences observed between the C:N ratio of soil between particle sizes and control samples under *L. terrestris* monocultures and mixed species samples. Within *L. terrestris* groups, carbon concentration was lower than the control (Table 6.3). This indicates that there is a higher amount of nitrogen within the soil, which may be beneficial to plants, if in the correct chemical form (e.g. NO_3^-). Samples with *A. caliginosa* had a higher C:N ratio than other treatments (Table 6.3) and at 1 cm particle size the C:N ratio was higher than the control. The effects of *A. caliginosa* on C:N ratio may be higher than *L. terrestris* due to their burrowing behaviour, where casting can occur within their burrows (Edwards, 2004). It has been indicated in laboratory experiments that *A. caliginosa* can increase carbon mineralization in their casts within the short term compared with bulk soil (Abail et al, 2017), therefore it is uncertain whether the increased C:N ratio will have lasting effects on crop development. *L. terrestris* mainly cast on the soil surface, therefore were less heterogeneously spread within the soil profile. Future experiments could determine the impact of earthworm behaviour under straw applications on the soil C:N ratio over a longer time period to determine the effects of earthworms over a growing season.

The C:N ratio of cereal straw residues are generally higher than other crops such as legumes, which can cause a longer rate of decomposition (Goh and Tuhuna, 2004). The mineralization of straw residues may be increased by the presence of earthworms, where feeding, casting and bioturbation can increase the rate of decomposition (Blouin et al, 2013; Bertrand et al, 2015a). Due to the decomposition rate of straw, even with the presence of earthworm species it may take longer than 8 weeks to impact nutrient cycling significantly. At 8 weeks, early effects of *A. caliginosa* and *L. terrestris* were noted, but, increased experimental time may provide further answers on the effects of earthworm species on soil C:N ratio. Future experiments could explore the effects of earthworm populations on the degradation of organic matter and assess the impacts of this through analysis of casts and C:N ratio changes in soil over time.

Although it is indicated that earthworm populations can increase soil carbon content (Zhang et al, 2013; Wachendorf et al, 2014), there were no significant differences in the %C results between treatments at experimental end (Table 6.3: $p > 0.05$). Although *L. terrestris* monocultures increased the SOM within the soil profile by incorporating 6.36 ± 0.6 g wheat straw (1 cm particle size), the length of the experiment only analysed a short-term change. Earthworm mediated effects on carbon turnover in soil is likely to be slower than this; future experiments could analyse carbon changeover over longer time periods and consider carbon losses aboveground during decomposition. Recent findings by Lubbers et al (2017) have indicated that earthworms have the ability to enhance CO_2 concentration simultaneously with belowground C, therefore there could be a neutral long-term effect of earthworm populations impact on soil C. To more accurately quantify the transport of carbon and nitrogen within the organic matter mediated by earthworm populations, future experiments could conduct isotopic analysis of straw cultivated with ^{13}C and ^{15}N (e.g. Giannopoulos et al, 2010; Heiner et al, 2011; Agapit et al, 2018).

The positive effects of earthworm populations on soil nitrogen concentration was indicated, where there was a higher %N under samples with earthworms compared to straw controls under all earthworm treatments (Table 6.3). Research experiments have indicated the effect of earthworm populations on nitrogen concentration, where an increased uptake of nitrogen within *Brachypodium distachyon* L. roots occurred in the presence of *A. caliginosa* casts (Agapit et al, 2018). There were no effects of particle size on the %N concentration under *L. terrestris* groups, where the %N ranged from 0.6-0.8% (Table 6.3). With straw application only, the %N was 0.5% higher than Kettering loam (control A) for all particle sizes, yet for earthworms and straw the %N was 5.5% higher. This highlights the short-term positive effects of *L. terrestris* and *A. caliginosa* populations on N mineralization in soil. This experiment did not quantify the type of nitrogen within the soil and whether this increase could be available to plants. There are also N losses (N_2O emissions; Giannopoulos et al, 2010) from earthworm populations when active, where the nitrogen cycling may change over time. Future experiments could assess the type of N (NO_3-N; NO_2-N; N_2O) emitted and transported through the soil profile to determine the effects on crop growth.

6.6. SUMMARY OF CHAPTER FINDINGS

- Earthworms prefer to feed on a smaller (1 mm) particle size. At a lower applied particle size, the effects of organic matter type are reduced.
- For surface applied organic matter*, L. terrestris* incorporated the largest amount of material regardless of particle size.
- Under straw residue applications, there were no inter-specific interactions between *L. terrestris* and *A. caliginosa.*
- *L. terrestris* can manipulate straw at larger particle sizes (> 20 cm length), which may enhance decomposition under untreated surface applications of wheat straw in no-till agroecosystems.

7: BEHAVIOURAL INVESTIGATIONS OF *LUMBRICUS TERRESTRIS* L.: EFFECTS OF SIZE AND LEVEL OF DEGRADATION ON STRAW SELECTION.

7.1. Introduction

Results from previous sections suggested that organic matter selection by earthworms was influenced by particle size, with smaller sizes (< 1 cm) preferred for feeding and incorporated into soil more rapidly (Chapter 6). The ability of earthworms to utilise organic matter as a feed is also influenced by C:N ratio (Bohlen et al, 1997; Curry and Schmidt, 2007), where degraded residues are more palatable. Straw particle size and decomposition rate may influence transportation and utilisation by *L. terrestris*. In the field, crop residues are not surface applied as a uniform small particle size, due to a high rate of surface run off and erosion. There is little evidence to suggest that milled material will increase the concentration of nitrogen (Ambus and Jensen, 1999), but there could be effects on decomposition rate and nitrogen mineralization of the residues (Redin et al, 2014). If organic matter was applied of a smaller particle size, milled organic matter would normally be incorporated within the upper soil layer (Christensen, 1987) to reduce surface run-off, erosion effects and enhance C and N mineralization. In sustainable agroecosystem practices, it is recommended to decrease ploughing frequency and intensity to reduce the effects of agriculture on greenhouse gas emissions (Section 2.3.3). How *L. terrestris* utilise organic matter aboveground and belowground may determine their impact on the mitigation of ploughing in no-tillage systems and consequently the rate of organic matter decomposition and nutrient mineralization.

Observations of remaining aboveground straw residues in the presence of *L. terrestris* indicated that this species has the physical ability to manipulate residues of larger particle sizes (20 cm and 40 cm) (Section 6.4). Cereal residue "stripping" behaviour by *L. terrestris* has previously been recorded by Stroud et al (2016a), but how *L. terrestris* manipulate the stripped straw and where they transport it (aboveground within their midden or incorporated into soil) could have unique effects on the decomposition rate of organic matter and nutrient mineralization within soil. This could also impact the soil community, where middens can improve the biodiversity of earthworms (Section 4.5; Nuutinen et al, 2017) and influence microorganism and fungal communities (Aira et al, 2009; Orazova et al, 2003). Further investigations of the behavioural interactions between *L. terrestris* and straw residues may provide an insight into how an increased population size may benefit agroecosystems.

Web cameras are often used in ecological experiments to determine animal behaviour and quantify factors, such as activity level (Rowcliffe et al, 2014). In earthworm ecology, web camera experiments have been conducted in the laboratory and the field to distinguish a number of *L. terrestris* behaviours, such as the impact of resource distribution on earthworm activity (Butt et al, 2003), the ability of *L.*

terrestris to relocate its burrow after fully exiting (Nuutinen and Butt, 2005), the impact of geographical location on earthworm behaviour with reference to difference in light conditions (Nuutinen et al, 2014), immigration behaviour (McTavish et al, 2013) and the impact of pesticides and vibration on *L. terrestris* dispersal (Ellis et al, 2010; Valckx et al, 2010). Imaging has also been used to determine the effect of anecic earthworms on soil displacement (Barnett et al, 2009), where findings suggested there was decreasing displacement with increased distance from burrow. The quantification of these activities have been used to model *L. terrestris* activity (e.g. Johnston et al, 2018), however there are gaps in datasets such as how aboveground utilisation of organic matter influenced by *L. terrestris* behaviour affects aboveground properties of agroecosystems such as the spatial heterogeneity of organic matter. Further investigations of the effects of land use change (e.g. different organic matter application) on *L. terrestris* behaviour may provide further insight to how replication of these changes in the field may impact earthworm mediated soil properties within the agroecosystem. The work described in this Chapter aims to investigate how *L. terrestris* utilise selected straw and determine if there are differences with particle size and level of degradation.

7.1.1. Objectives:

- To examine the effects of wheat straw particle size and state of degradation on selection by *L. terrestris* using infrared web camera recordings.
- To determine how *L. terrestris* utilises straw aboveground and belowground based on the straw particle size and level of decomposition.
- To analyse soil chemical changes caused by *L. terrestris* casting and midden properties.

Two linked web camera experiments were set to up to meet these objectives. Experiment 1 investigated the effects of straw particle size on utilisation by *L. terrestris* and Experiment 2 investigated the effects of straw utilisation at two levels of decay. At experimental end, destructive sampling of the midden-burrow complex was conducted which determined any soil chemical changes by *L. terrestris*. The methods and results for each experiment are presented separately, with combined findings discussed in Section 7.5.

7.1.2. Hypotheses:

- Selection of straw particles will be influenced by particle size, with a preference for smaller particle size when offered a mixture.

- *L. terrestris* will have preference for decomposed straw particles over freshly deposited straw, due to increased palatability and a lower C:N ratio.

7.2. Experiment 1: Effects of straw particle size on selection by L. terrestris.

7.2.1. Materials and Methods

7.2.1.1. Set-up of experimental arena

A first experiment analysed *L. terrestris* behaviour when presented with straw of mixed particle sizes. This was conducted over a period of 2 weeks during late November 2018. Four replicate units were prepared by placing 20 L of Kettering loam (25% moisture content) into 25 L containers (diameter, 335 mm; depth 600 mm) (Figure 7.1). Containers were placed in a controlled environment where lighting was set at 12:12 hr light:dark and room temperature was 15 °C. Potential burrow entrances were made with a pencil in the centre of the container (experimental arena), where a single adult *L. terrestris* (mass previously determined) was placed, encouraged to enter the burrow and left overnight to settle. In addition to the four experimental (worm) units, a control was prepared with no earthworm addition.

Figure 7.1. Experimental set up with web cameras. Image was taken during *L. terrestris* midden development, before the straw particle size experiment began.

The organic matter applied in this experiment were wheat (*Triticum aestivum*) straw residues, collected immediately after harvest (September 2018) from Brook Lane Farm, Farington, Lancashire (Section 3.2) and air-dried as described in Section 6.3. Straw residues were cut (using scissors and a ruler) to the following particle size treatments: 10, 5, 2.5 and 1 cm in length. The largest size was determined by restrictions of the diameter of the containers and the low incorporation rates of larger particle sizes observed in a previous experiment (Chapter 6). The given sizes were also easily distinguishable via the web camera.

Once the *L. terrestris* had settled in the containers overnight, equal amounts of the 4 straw particle sizes were randomly applied to the surface to allow midden formation, as described by Rajapaksha et al (2013). Mean mass of straw applied was 10 g per container (Figure 7.1). After 2 weeks, each *L. terrestris* had formed a midden. Remaining (non-midden) surface straw was removed and mass determined, enabling an initial calculation for wheat straw added to the midden, and ultimately total removal by each *L. terrestris* at the end of the experiment.

At the start of the experiment, straw particles of 10, 5, 2.5 and 1 cm (N = 5 of each) were randomly assigned on the surface of each container (Figure 7.2), rather than applied as randomised blocks or stratified, to replicate field conditions, where no sorting of organic matter occurs before application.

Figure 7.2. Random application of wheat straw particles (5 each of 10, 5, 2.5 and 1 cm) around a *L. terrestris* midden in Experiment 1 using a 25 litre container.

Following application of straw, photographic images were taken of each container (repeated daily). Activity of *L. terrestris* at the surface of each container was recorded via web cams (F19803EP, Foscam, Houston, USA) during the dark (12 hour) period over a total of 14 successive nights. The web cams had infrared recording, which allowed for night vision imaging without compromising *L. terrestris* behaviour (Nuutinen and Butt, 1997). Recordings were saved onto 32 GB SD cards and data was transferred to a hard drive every 2 days. To maintain soil moisture content, the surface soil was sprayed with approximately 100 ml water twice daily. Maximum and minimum temperature recordings were taken daily.

Images were taken of each container to compare straw movement, and any remaining surface straw (not taken to middens) was removed for further analysis, including mass determination. Middens and earthworms were left in the containers for further experimentation.

7.2.1.2. Data collection

Qualitative data was collected for the following:

1. Time spent active aboveground.

To determine whether there were any patterns in the activity times of *L. terrestris* under controlled conditions, the mean total activity time aboveground was recorded. Activity time (hr:min:sec night^{-1}) was calculated by subtracting activity end time (the time *L. terrestris* re-entered its burrow and could no longer be observed) from activity starting time (the time *L. terrestris* was first observed exiting the burrow).

Within the periods of 'total activity time,' specific behaviours were recorded (2-4):

2. Location of activity within arena.

L. terrestris activity occurred in the burrow, midden and in the outer experimental arena (Figure 7.3.). The location of where *L. terrestris* activity occurred was used to separate total time active in the outer experimental arena (C) from total time spent active in midden (B) and burrow (A). This was used to separate two activities: 'foraging' and 'midden reconstruction'. All activity in the outer arena was recorded as foraging. Although in field conditions *L. terrestris* may exit the burrow to mate or migrate to new areas, these activities were not promoted in this experiment (per arena, *L. terrestris* N=1; environmental conditions were controlled). The mean activity time (hr:min:sec night^{-1}) spent foraging and reconstructing the midden were compared to determine the energy used in organic matter selection and utilisation.

At experimental end, the midden was deconstructed (method in Section 7.4). Experimental straw was identified aboveground within the midden and belowground in the burrow. Combining these results with the qualitative data from the web camera recordings confirmed whether these activities were correct.

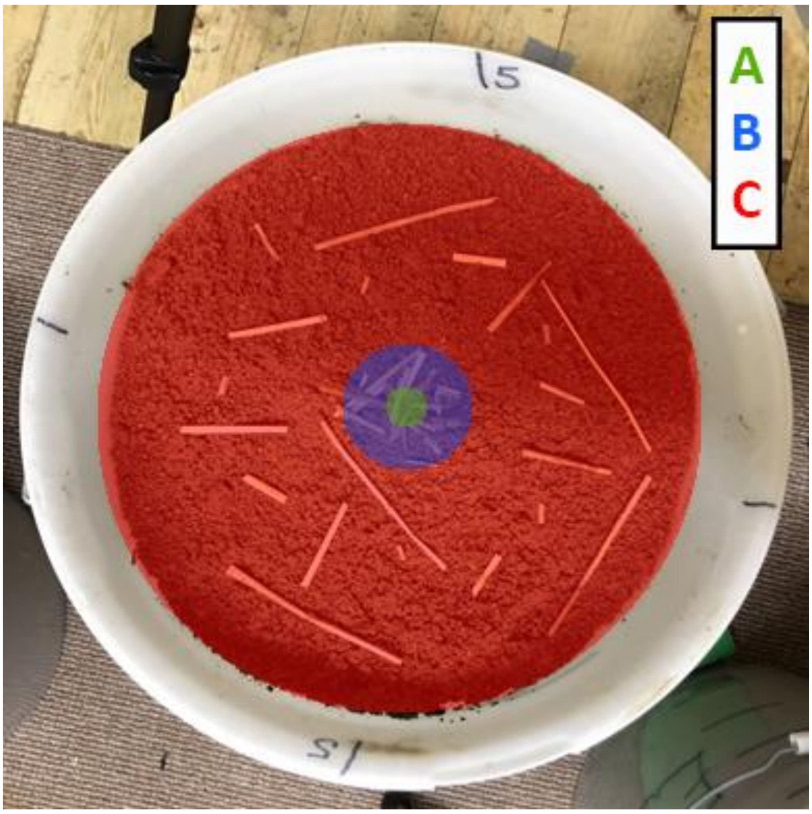


Figure 7.3. Demarcation of the experimental arena, outlining where ‘burrow’ (A), ‘midden’ (B) and ‘outer experimental arena’ (C) were located.

3. Straw selection.

When *L. terrestris* was surface active, every physical interaction with experimental straw were recorded. Each time it was observed that *L. terrestris* touched a piece of straw with its prostomium, it was recorded as an ‘interaction.’ The particle size during each interaction was recorded. At experimental end, the total number of interactions per night was quantified and averaged (N. ind.$^{-1}$ night $^{-1}$). An increased number of interactions with a given particle size indicated a preference.

If an interaction with experimental straw resulted in a piece being selected and moved into the midden, this was recorded as a ‘selection’. To assess straw removal and preference, the remaining number of straw particles within each vessel was recorded each night and averaged (mean night^{-1}). The rate of selection was used to determine particle size preference.

For each selection event, the distance of selection was also recorded (mm). For *L. terrestris* to leave its burrow poses a number of risks (e.g. predation) and utilises more energy. A relationship between distance and selection could suggest that straw particle size preference is not as important as the risks posed in increased travel distance.

4. Manipulation

To test whether *L. terrestris* utilise straw of larger particle size (5 cm and 10 cm), webcam recordings were analysed for any utilisation behaviours, such as "straw stripping," previously recorded by Stroud et al, (2016a). "Straw stripping" refers to the peeling of the outer epidermal layers of straw by *L. terrestris* using their mouthparts. If an event occurred, the time of the event was noted, the area in the experimental arena where it occurred (A, B or C from Figure 7.3) and the particle size of the straw manipulated. This information was used to determine whether *L. terrestris* receive any benefits from straw applications at larger particle sizes.

7.2.1.3. Statistical Analysis

To determine a preference of particle size, the difference in mean number of particles selected by *L. terrestris* were analysed (SPSS v.27). The results displayed a normal distribution with unequal variance. Therefore, Welch's one-way analysis of variance (ANOVA) was used to compare the effect of particle size on mean number of selections by *L. terrestris*. Where significant, post-hoc tests (Games-Howell) identified the significantly different groups.

When *L. terrestris* was active, the location of *L. terrestris* activity was recorded and the mean length of time spent within the midden or outside of the midden (foraging) was compared. To determine whether there were statistical differences between the time spent active in the midden and foraging, a 2-sample t-test was applied.

7.2.2. Results

Straw selection by *L. terrestris* was highest for 10 cm particle size, where 13 of 20 units were taken to the midden by experimental end (Figure 7.4). There was a high range between the samples due to the low number of replicates (N=4), and there were no statistical differences in mean number of straw particles removed by *L. terrestris* between straw particle sizes (Welch's one-way ANOVA; df = 3, F = 2.10, $p > 0.05$).

The rate of straw particles selected reduced over time, as the majority of straw selection occurred within the first week of the experiment (Figure 7.4). Of the 31 particles selected, 61.3% were selected within the first 7 days.

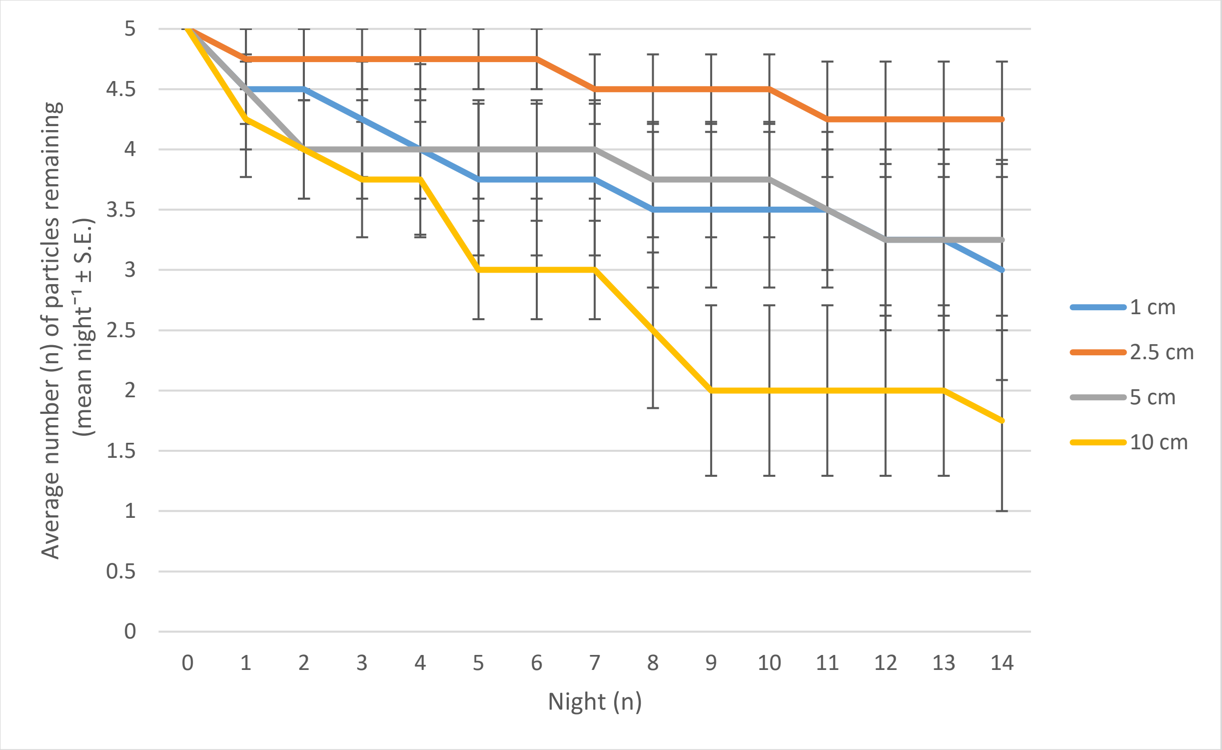


Figure 7.4. Effects of particle size on mean selection (± S.E.) by *L. terrestris* over time (14 nights) (N=4).

L. terrestrs were surface active on 13 ± 0.6 nights out of 14, where the total mean activity time was 04:33:03 ± 00:25:33 (hr:min:sec night^{-1}). Containers 2 and 3 were inactive on two of the 14 experimental nights. On the nights where there was a reduction in surface activity, the temperature dropped (e.g. to 10.6 °C on night 3; Table 7.1). Information on the activity within individual containers can be found in Appendix IV.

The amount of time spent active within the midden was significantly different to the time spent foraging (paired t-test; $p < 0.05$; Figure 7.5), where the majority of activity occurred within the midden (mean 03:34:38 ± 00:20:58 hr:min:sec night^{-1}).

Table 7.1. Maximum and minimum daily temperatures recorded. Due to the outdoor temperatures dropping below 10 °C, a heater was used to maintain night temperatures indoors.

Date	Indoor Temperature (°C)		Outdoor Temperature (°C)	
	Max	Min	Max (outside)	Min (outside)
19.11.18	15.3	13.6	12.4	8.2
20.11.18	14.8	13.5	10.8	7.8
21.11.18	12.8	10.8	8.7	4.2
22.11.18	15.9	13.8	11.3	7.5
23.11.18	16.3	14.7	12.1	8.6
24.11.18	15.7	14.5	11.2	7.3
25.11.18	14.8	13.3	13.6	8.4
26.11.18	13.6	12.8	10.6	6.8
27.11.18	14.2	13.8	11.4	7.2
28.11.18	16.1	13.4	13.3	6.7
29.11.18	15.4	14.1	10.6	8.6
30.11.18	15.3	13.6	9.8	7.3
01.12.18	14.5	13.5	9.6	6.4
02.12.18	14.7	13.0	10.7	6.8

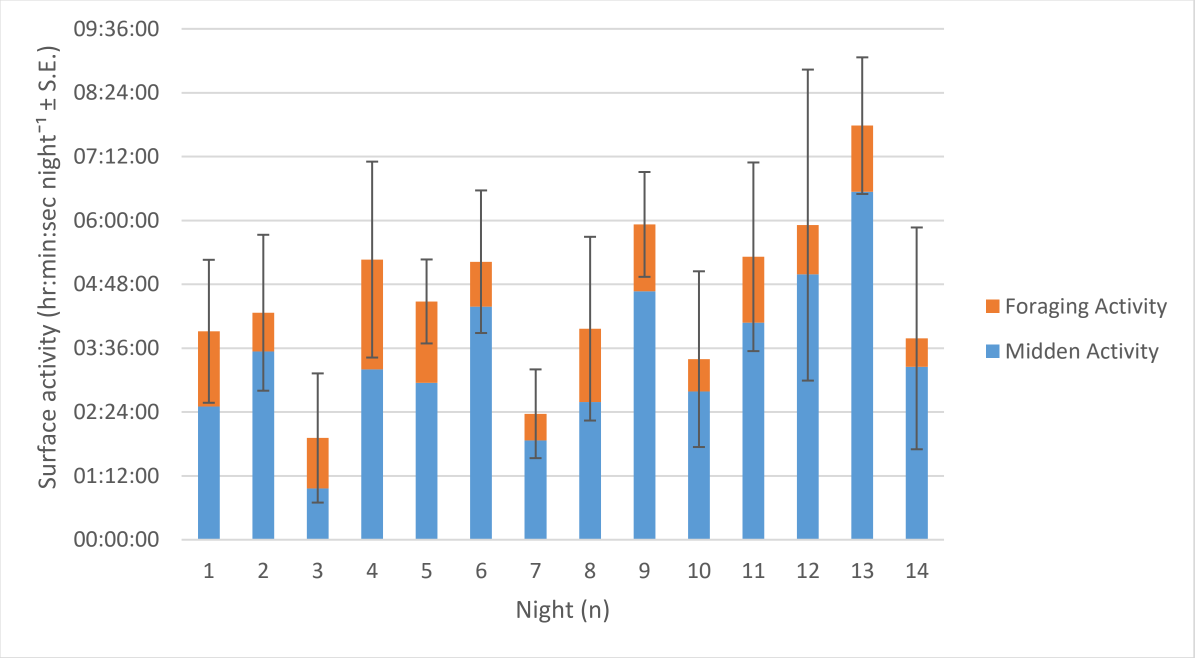


Figure 7.5. Mean total surface activity (± S.E.) of *L. terrestris* over 14 nights, separated by activity location (orange = foraging; blue = within midden).

L. terrestris had increased surface interactions with large particle sizes (10 cm and 5 cm) than smaller particle sizes (1 cm and 2.5 cm) (Figure 7.6). One-way ANOVA indicated that there were no significant differences between the mean number of straw interactions with particle size (df = 3; F = 1.59; $p > 0.05$).

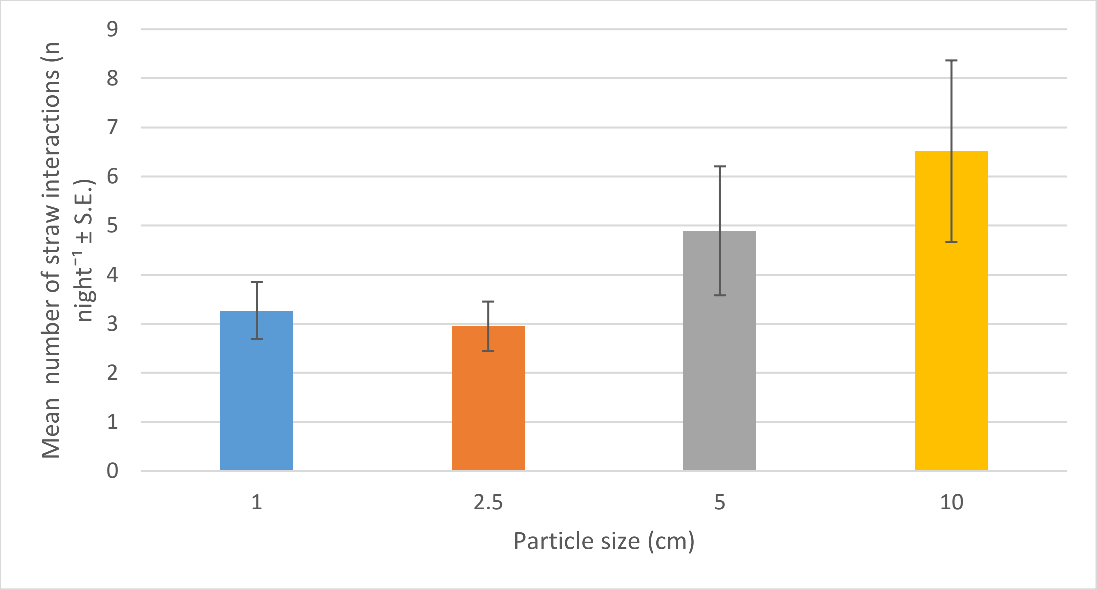


Figure 7.6. The mean number of wheat straw particles selected by *L. terrestris* (n night $^{-1}$ ± S.E.) of each particle size treatment (1, 2.5, 5 and 10 cm).

A relationship between the order of straw selection and distance of the straw from the *L. terrestris* burrow was observed, where, despite size, the particles closer to the burrow were selected first (Figure 7.7). *L. terrestris* did not fully leave the burrow during the experiment but did have extensive periods of time where it was on the surface foraging (For example, earthworm in container 2 spent 35:21 min:sec selecting 2.5 cm experimental straw on day 12). Of a total of 769 recorded activities (foraging, midden reconstruction, particle selection, incorporation of straw), the average time *L. terrestris* (N=4) spent active aboveground was 23:17 ± 02:03 min:sec^{-1}. Although there was a reduction in the mean rate of particles selected by *L. terrestris* in week 2 (Figure 7.4) the mean time spent foraging was similar (Figure 7.5).

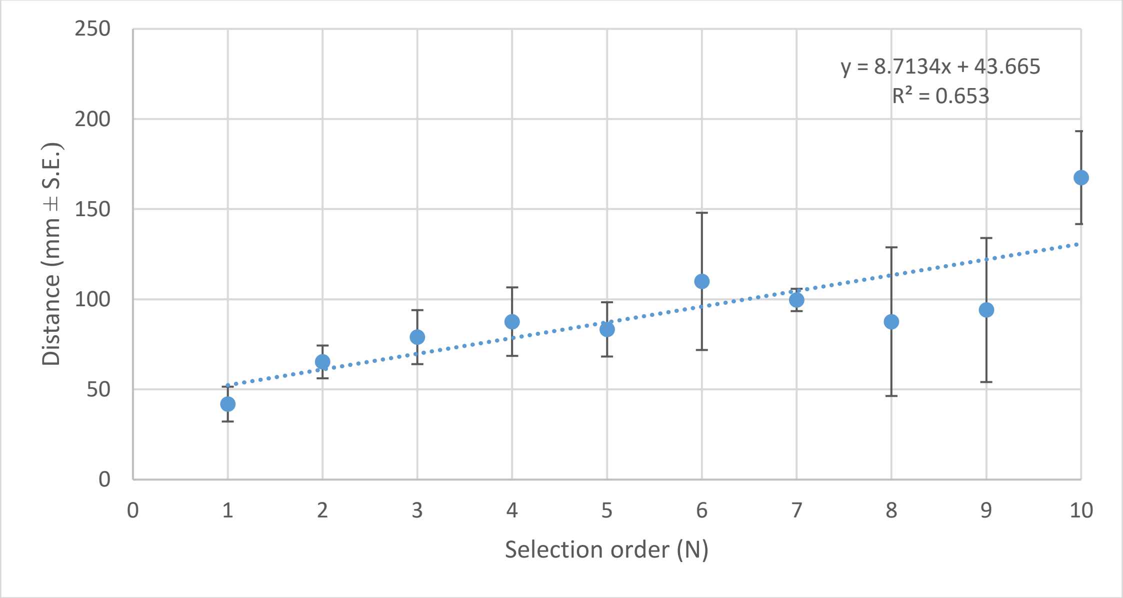
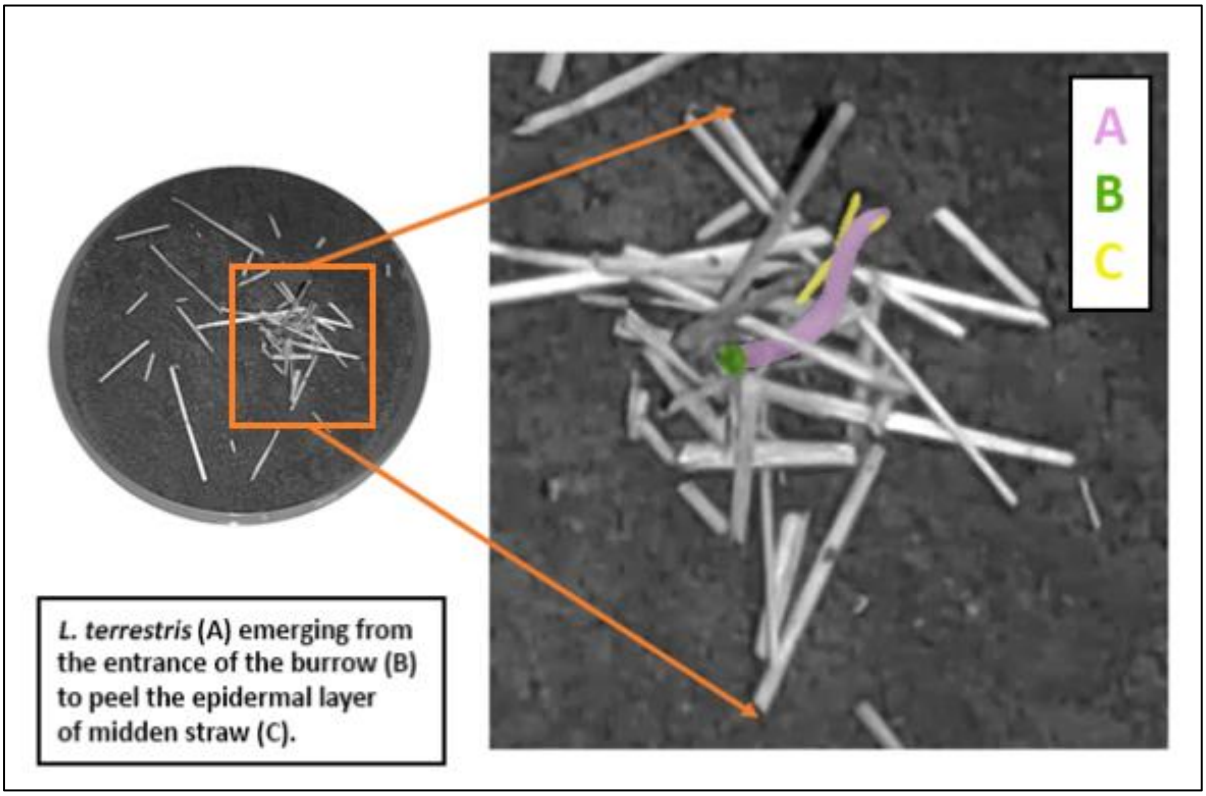


Figure 7.7. Mean distance (mm ± S.E.) travelled by *L. terrestris* (N=4) to select straw in order of particle selection (1 = first piece selected; 10 = final piece selected).

7.2.3. Behavioural observations recorded

Analysis of webcam footage revealed repeated behaviours where *L. terrestris* stripped the straw, pulling away the epidermis of the straw with the mouth (Figure 7.8). At experimental end, images of the midden and soil cores indicated that stripped material was utilised within the midden and incorporated. “Straw stripping” was mainly observed with 5 and 10 cm particle sizes.

Overall, *L. terrestris* was recorded stripping straw within its midden 110 times during Experiment 1. This activity was demonstrated across all replicates. The mean time spent stripping straw at each event was 05:33 ± 02:45 hr:min^{-1}. *L. terrestris* only stripped one particle at a time.



L. terrestris (A) emerging from the entrance of the burrow (B) to peel the epidermal layer of midden straw (C).

Figure 7.8. An annotated image of *L. terrestris* “stripping” a piece of straw. Image taken from Container 4 (Day 5: 03.30 am).

In addition to repeated behaviours in straw manipulation, some observed behaviours were influenced by soil surface structure. Aggregation of the soil surface impacted selection, where through time, soil compaction caused an uneven soil surface. This impacted straw selection and earthworm exploration of the experimental arena, where *L. terrestris* could navigate to areas easier to forage. Increased aggregation on the soil surface made larger straw particle sizes (5 and 10 cm) more challenging for *L. terrestris* to select and move, increasing the time taken to utilise the straw. For example, in container 4, *L. terrestris* made 19 attempts to select a 10 cm straw particle between day 1 and day 5, which was obstructed by a large macroaggregate (> 10 mm diameter). The *L. terrestris* eventually selected and made this straw part of its midden, however more physical effort and time was required than other attempts where the soil surface was homogenous.

7.3. Experiment 2: Effects of wheat straw degradation on selection by L. terrestris.

7.3.1. Materials and methods

This experiment recorded the selection of straw by *L. terrestris* when provided with fresh and naturally degraded wheat (*Triticum aestivum*) straw residues. Wheat straw for this study (used as fresh) was collected immediately after harvest (September 2018) from Brook Lane Farm, Farington, Lancashire (Section 3.2) and air-dried as described in Section 6.3. Further material (used as degraded) was left on the soil surface in the field to naturally degrade over the month of October 2018.

Prior to the experiment, chemical analysis was conducted on the “fresh” and “degraded” straw to determine whether there were any effects of one month’s decay on the organic (C:N ratio) and inorganic (macro- and micronutrient) concentration.

Initial results from Experiment 1 indicated that *L. terrestris* collected 5 cm and 10 cm experimental straw more rapidly than other sizes, so this determined that 5 cm lengths were used. To distinguish “fresh” from “degraded” due to equal particle size, natural food colouring dyes (red and green) were used. Food dyes were tested beforehand to ensure that they were not toxic for earthworm consumption and there was no preference of food dye. Degraded particles were dyed red and fresh particles were dyed green.

Using the set-up remaining at the end of Experiment 1, the pre-prepared containers with *L. terrestris* and developed middens were used for the determination of straw degradation preference testing. The experiment was conducted in early 2019, after a pause in experimental recording to reset the arena and experimental conditions. During this time, 20 g of straw was applied to each container to feed the earthworms, as 20 particles over 2 weeks was a low amount of food. Soil moisture was regulated by spraying the soil with approximately 100 ml water twice daily. Due to the evening temperature often being below 0 °C in January, a heater was used to regulate mean evening temperature above 10 °C.

Figure 7.9. Surface application of fresh (green) and degraded (red) wheat straw around a *L. terrestris* midden using a 25 litre container.

Figure 7.9 indicates the orientation of 5 cm wheat straw fragments surface applied in each of the four containers. Straw samples were applied stratified in pairs and of equal distance from the burrow. This removed any bias of distance with the two straw types applied in pairs. This experiment ran for 14 nights and was monitored under the same conditions as Experiment 1.

7.3.2. Data collection

Qualitative data was collected for the following:

1. Time spent active aboveground.

To determine whether there were any patterns in the activity times of *L. terrestris* under controlled conditions, the mean total activity time aboveground was recorded. Total time spent active (hr:min:sec night^{-1}) was calculated by subtracting activity end time (the time *L. terrestris* re-entered its burrow and could no longer be observed) from activity starting time (the time *L. terrestris* was first observed exiting the burrow).

Within the periods of 'total activity time,' specific behaviours were recorded (2-4):

2. Location of activity within arena.

L. terrestris activity occurred in the burrow, midden and in the outer experimental arena (Figure 7.10.). The location *L. terrestris* activity occurred was used to separate total time active in the outer experimental arena (C) from total time spent active in midden (B) and burrow (A). This was used to separate two activities: 'foraging' and 'midden reconstruction'. All activity in the outer arena was recorded as foraging. Although in field conditions *L. terrestris* may exit the burrow to mate or migrate to new areas, these activities were not promoted in this experiment (per arena, *L. terrestris* N=1; environmental conditions were controlled). The mean active time spent foraging (hr:min:sec night^{-1}) and reconstructing the midden was compared. Findings may determine the energy used in organic matter selection and utilisation.

At experimental end, the midden was deconstructed (method in Section 7.4). Experimental straw was identified aboveground within the midden and belowground in the burrow. Combining these results with the qualitative data from the web camera recordings confirmed whether these activities were correct.

Figure 7.10. Demarcation of the experimental arena, outlining where 'burrow' (A), 'midden' (B) and 'outer experimental arena' (C) were located.

3. Straw selection.

When *L. terrestris* was surface active, every physical interaction with experimental straw were recorded. Each time it was observed that *L. terrestris* touched a piece of straw with its prostomium, it was recorded as an ‘interaction.’ The type of straw (“degraded” or “fresh”) of each interaction was recorded. At experimental end, the total number of interactions per night was quantified and averaged (N. ind.$^{-1}$ night $^{-1}$). An increased number of interactions with a given particle size indicated a preference.

If an interaction with experimental straw resulted in a piece being selected and moved into the midden, this was recorded as a ‘selection’. To assess straw removal and preference, the remaining number of straw particles within each vessel was recorded each night and averaged (mean n^{-1} night^{-1}). The rate of selection was used to determine particle size preference.

Distance (mm) of selection was not recorded in this experiment, due to samples being applied stratified and of equal distance to the burrow.

4. Manipulation

To test whether *L. terrestris* utilise “degraded” straw differently to “fresh”, webcam recordings were analysed for any utilisation behaviours, such as “straw stripping,” previously recorded by Stroud et al, (2016a). “Straw stripping” refers to the peeling of the outer epidermal layers of straw by *L. terrestris* using their mouthparts. If an event occurred, the time of the event was noted, the area in the experimental arena where it occurred (A, B or C from Figure 7.8) and the level of decomposition manipulated. This information will be used to determine whether *L. terrestris* receive any benefits from straw applications that are decomposed.

At the end, contents of the container were destructively sampled to determine any impact of *L. terrestris* on the nutrient dynamics of the soil and how the incorporated material from experiments 1 and 2 had been utilised. Deconstruction of soil samples and chemical analysis of middens is defined in Section 7.4.

7.3.3. Statistical Analysis

To determine a preference of straw decay, the difference in mean selection of straw (degraded or undegraded) by *L. terrestris* were analysed (SPSS v.27). Due to the low number of selected particles, statistical analysis of *L. terrestris* selection was not applied.

When *L. terrestris* was active, location of activity was recorded and the mean length of time spent within the midden or outside of the midden (foraging) was compared. To determine whether there were statistical differences between the time spent active in the midden and foraging, a 2-sample t-test was applied.

To determine whether there were any effects of surface degradation on straw chemical composition, a 2 sample t-test was applied to the mean chemical results for fresh and degraded straw samples.

7.3.4. Results

Chemical analyses of fresh and degraded straw residues are presented in Tables 7.2 and 7.3. Following 1 month of surface degradation, the C:N ratio reduced from 56 to 53. The differences in C:N ratio were not statistically significant ($P > 0.05$; 2 sample t-test).

Table 7.2. Differences in the total Carbon and Nitrogen (% ± S.E.) and C:N ratio of wheat straw between matter collected at harvest (Fresh straw) and after one month left on the soil surface (Degraded straw). Significant differences ($p < 0.05$; 2 sample t-test) are shown where letters differ in the same row.

Analysis Type	Fresh Straw	Degraded Straw
C (%)	44.21 ± 0.21^{a}	43.61 ± 0.11^{b}
N (%)	0.47 ± 0.03^{a}	0.57 ± 0.04^{b}
C:N Ratio	56.00 ± 1.99^{a}	52.91 ± 2.57^{a}

Table 7.3. Differences in the total nutrient concentration (mg kg^{-1} ± S.E.) between straw collected post-harvest (Fresh) and after one month on the soil surface (Degraded). Significant differences ($p < 0.05$; 2 sample t-test) are shown where letters differ in the same row.

Element	Concentration (mg kg^{-1} ± S.E.)	
	Fresh Straw	Degraded Straw
Ca	3935 ± 139^{a}	3722 ± 133^{a}
Mg	426 ± 16^{a}	620 ± 13^{b}
K	5656 ± 236^{a}	1059 ± 52^{b}
Mn	4 ± 0.3^{a}	15 ± 0.7^{b}
Fe	26 ± 2^{a}	417 ± 45^{b}
Zn	303 ± 41^{a}	662 ± 90^{b}
Na	237 ± 10^{a}	1005 ± 44^{b}
P	495 ± 41^{a}	1098 ± 25^{b}

Of the four earthworms recorded, three were surface active during the whole experiment, therefore calculated means are based on three replicates. Of the three containers, two *L. terrestris* selected and utilised the experimental straw and all three interacted with it. Degraded straw was selected more frequently than fresh straw (Figure 7.11), but the difference between samples was low (N=2) and there were no significant differences between the treatments ($p > 0.05$). Interactions between *L. terrestris* and the experimental particles indicated no preference between degraded and fresh straw (2 sample t-test; $p > 0.05$; Figure 7.12).

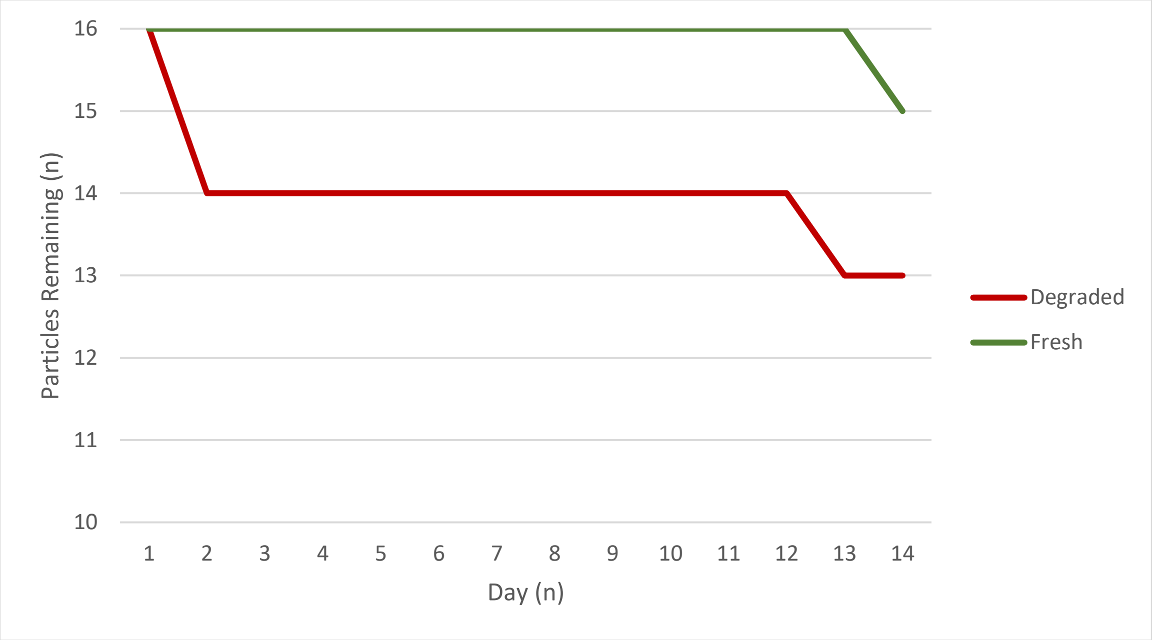


Figure 7.11. Total straw particle selection (n) by *L. terrestris* when provided straw at different stages of decomposition (Fresh = selected at harvest; Degraded = 1 month of surface degradation).

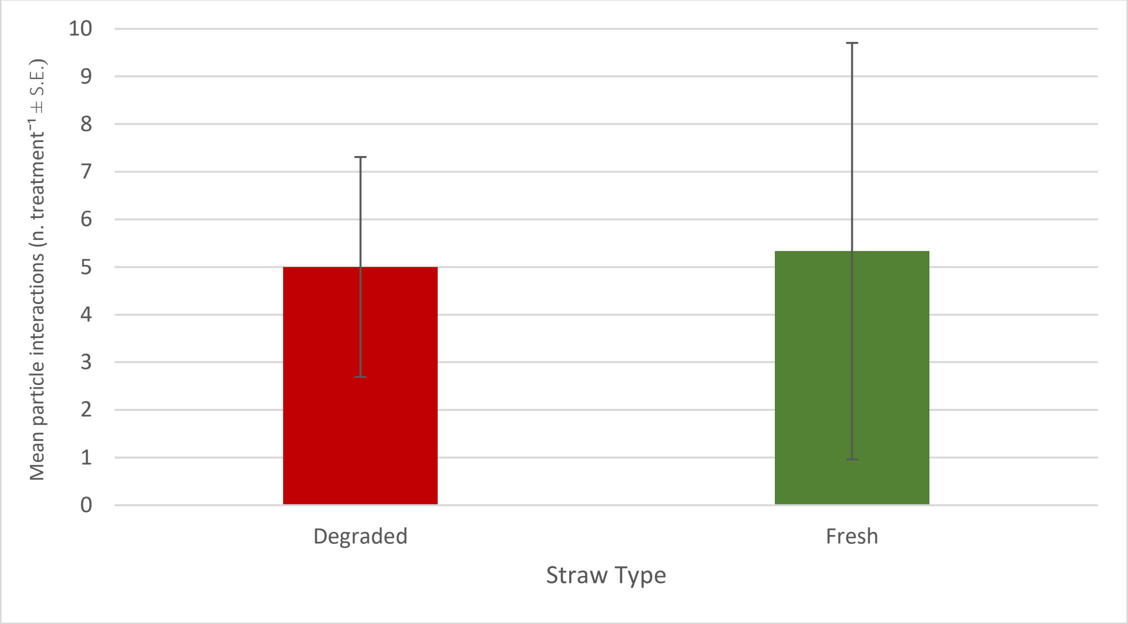


Figure 7.12. Mean number of interactions by *L. terrestris* with particles when supplied with fresh or degraded wheat straw residues.

Table 7.4. Maximum and minimum daily temperatures recorded. Due to the outdoor temperatures dropping below 10 °C, a heater was used to increase evening temperatures indoors.

Day (n)	Indoor Temperature (°C)		Outdoor Temperature (°C)	
	Max	Min	Max	Min
17.01.19	15.4	12.8	10.8	4.8
18.01.19	15.2	13.4	11.4	4.8
20.01.19	13.8	12.8	8.8	3.6
21.01.19	13.8	12.6	8.6	3.8
22.01.19	14.6	13.4	8.8	3.4
23.01.19	14.2	12.6	8.2	2.8
24.01.19	13.6	9.2	6.8	2.4
25.01.19	12.8	9.6	6.8	2.6
26.01.19	12.8	9.4	7.4	2.8
27.01.19	14.0	13.4	10.2	5.2
28.01.19	14.8	13.8	12.4	5.6
29.01.19	14.6	14.6	11.8	4.8
30.01.19	13.8	14.4	13.6	5.4
31.01.19	14.2	13.8	10.4	5.8

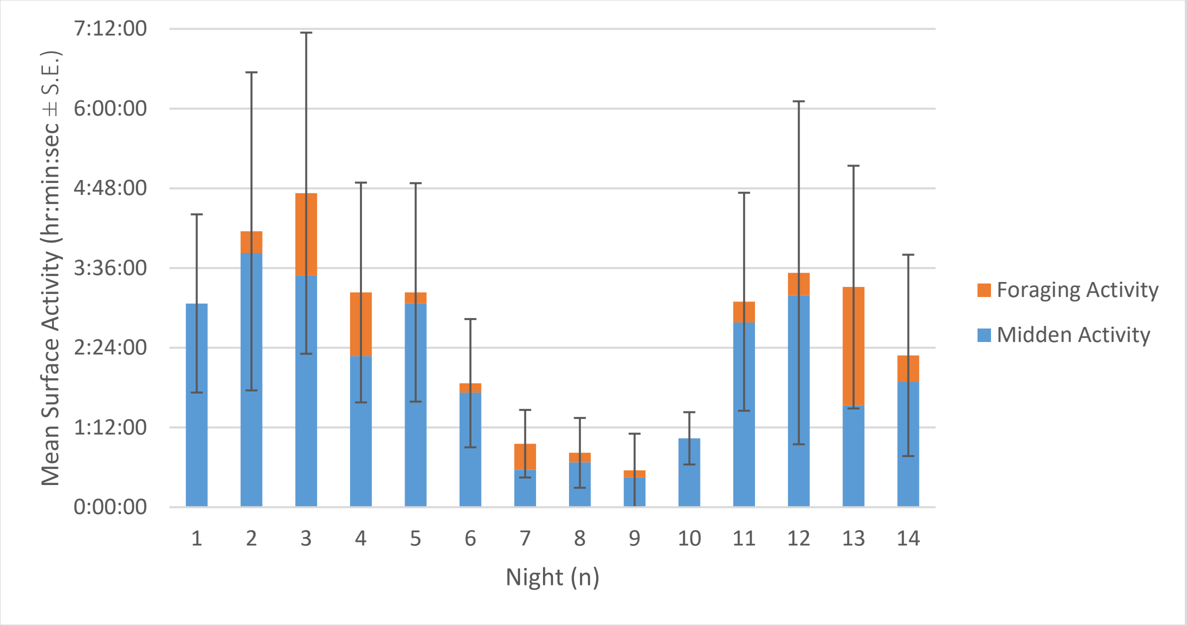


Figure 7.13. Mean total surface activity (hr: min:sec^{-1} ± S.E.) in Experiment 2 by *L. terrestris* (N=3) over 14 nights, separated by activity location (orange = foraging; blue = within midden).

Mean surface activity per container is presented in Figure 7.13. Mean activity time was lower than that observed in Experiment 1 (Figure 7.3), where the minimum room temperature recorded was also lower (Table 7.4). *L. terrestris* middens were larger at the start of this experiment, following 10 g straw applied between Experiments 1 and 2. *L. terrestris* travelled up to 16 cm during foraging events, however most prolonged activity events occurred within the midden (Figure 7.13).

7.4. Destructive sampling of soil: straw utilisation and chemical analysis.

At the end of Experiment 2, the soil within each container was destructively sampled. The objective was to determine how straw particles had been used within the midden-burrow complex and to analyse the chemical concentration of the midden area (casts and burrow) to determine any effects on nutrient dispersal.

7.4.1. Materials and methods

7.4.1.1. Soil and midden sampling

First, the midden (straw, cast material and mineral content) was carefully removed and bagged. Following midden removal, the soil immediately below the midden (midden soil) was sampled at increasing depth using an auger (75 mm diameter x 60 mm depth).

The destructive sampling of a container is indicated in Figure 7.14. In addition to midden soil, areas of the container that had no visual *L. terrestris* activity were determined using the webcam analysis. The selected area of “no earthworm activity” was also cored (representing ‘control soil’) and then stored for laboratory analysis. This was repeated throughout the full depth of the container, with 10 sub-samples for burrow soil (by depth) and 10 samples for non-burrow (control) soil (total soil samples per container N = 20). The soil was hand sorted to find the *L. terrestris*, which had mass determined.

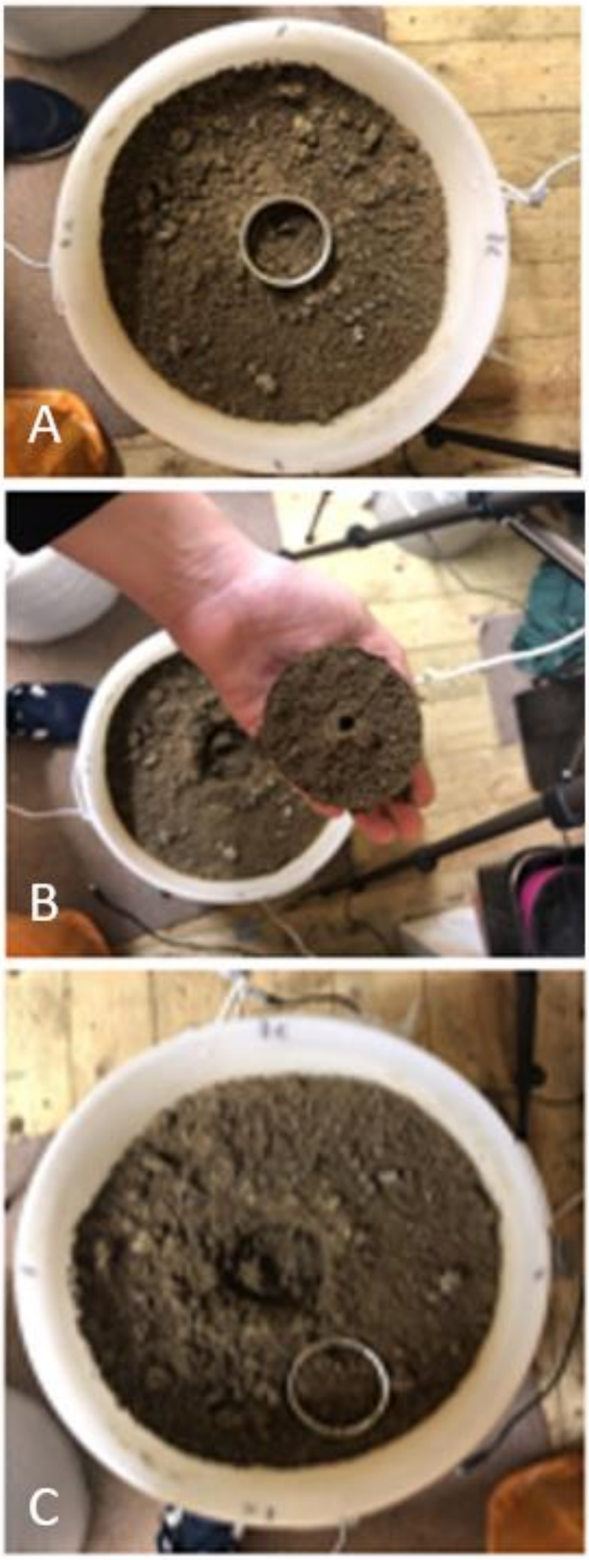


Figure 7.14. Sequence for destructive sampling of soil in containers. Following removal of *L. terrestris* and remaining surface straw, the soil was sampled using an auger (75 mm diameter x 60 mm depth); (A) around the centre of the *L. terrestris* burrow (B). A second sample was taken of equal distance between the arena edge and the *L. terrestris* burrow (C), which represented control soil. This process was continued at depth until the bottom of the vessel was reached (600 mm).

7.4.1.2. Midden analysis

Pre sampling images were taken of the remaining middens. Bagged middens were taken to the laboratory, where they were destructively sampled. First, middens were placed on medical roll and an image was taken of the sample. Wet cast material was carefully removed from the midden with a spatula and stored in plastic sample bags for chemical analysis. Straw from within the midden was carefully separated with forceps to avoid destruction of the utilised pieces. Particles where the size (Experiment 1) or colour (Experiment 2) was distinguishable was recorded. Once separated, images were taken of the matter found within the midden (Appendix IV).

7.4.1.3. Straw incorporation

The 'actual' dry mass of straw added to each container throughout Experiments 1 and 2 was recorded using a 3-decimal place balance. At experimental end and between experiments, straw unused by *L. terrestris* was removed, dried at 105 °C overnight and had its mass determined.

Once particle size analysis of the midden had been conducted (Section 7.4.1.2.), straw utilised within each midden sample was dried at 105 °C overnight. The following day, the midden straw (dry mass) recorded.

Of the 10 soil sub-samples taken at increasing depth around the burrow-midden complex (B; Figure 7.4), the core was destructively sampled for remaining straw particles. Collected straw found within the cores was removed using forceps and stored in plastic bags. The depth collected and particle size of the straw (if distinguishable) was recorded. For each sample, straw was dried at 105 °C overnight to remove moisture. The following day, the dry mass for incorporated straw at each depth was recorded.

7.4.1.4. Chemical analysis

Chemical analysis was conducted to determine the Loss on Ignition (LOI), C:N ratio and macro- and micronutrient concentration of two areas in each container: the soil underneath the midden (labelled in results as "midden" soil; image A; Figure 7.14) and of a control area which had low recorded *L. terrestris* activity and no evidence of burrowing (labelled in results as "non-midden" soil; image C; Figure 7.14). There were 10 subsamples for each midden and non-midden area.

Prior to analysis, soil samples had their moisture determined and were dried at 105 °C overnight. Once dry, samples were ground with a pestle and mortar and sieved (0.1 mm). For each sample (N=4), three replicates were analysed for each technique.

L. terrestris casts from middens were also analysed for LOI, C:N ratio and macro- and micronutrient concentration. For each sample casts from middens were pooled and dried at 105 °C overnight. Once dry, they were ground with a pestle and mortar and sieved (0.1 mm). For each sample (N=4), three replicates were analysed on each technique.

C:N ratio, %C and %N was analysed on a ThermoScientific Flash 2000 CHNS analyser at JBF Analytical Suite, UCLan. Details of this procedure and LOI is in Sections 3.5.2 and 3.5.3. Macro- and micronutrient concentration was analysed using a ThermoScientific ICE 7000 ICP-OES at JBF Analytical Suite, UCLan. Soils were analysed for total nutrient concentration and available nutrient concentration. Details of ICP-OES analysis is in Section 3.5.1.

7.4.1.5 Statistical Analysis

Statistical analysis was conducted on the midden and non-midden soil samples, to determine whether there were any significant differences between the nutrient concentration of soil containing a *L. terrestris* burrow and increased surface organic matter from middens, with nearby control 'non-midden' soil, at increasing depths. Where data displayed a normal distribution, a two-sample t-test was used to compare the difference in the mean nutrient concentration between midden and non-midden soil. Mann-Whitney U tests were applied to data which did not display a normal distribution.

To analyse the effect of depth, a one-way ANOVA was used to determine whether there was a difference in nutrient concentration at selected depths. Post-hoc Tukey pairwise comparisons were conducted to locate the difference between depths. Where data was not normally distributed, a nonparametric Kruskal Wallis test was used, followed by Dunn-Bonferroni post-hoc tests to determine grouping information.

7.4.2. Results

7.4.2.1. Resource Utilisation

At experimental end, images of the middens were taken from each container before they were deconstructed and analysed (Figure 7.15.).

Container 1 Container 2 Container 3 Container 4

Figure 7.15. Images of *L. terrestris* middens, taken at experimental end.

Of midden mass, an average 85 ± 3% of straw material was aboveground as part of the midden, with the remainder incorporated belowground. Of the total mass aboveground, this differed between containers dependent upon their activity within the experiments. For example, the lower surface activity in container 3 resulted in a lower midden mass compared with the other containers. The mean above- and belowground straw mass is presented in Figure 7.16.

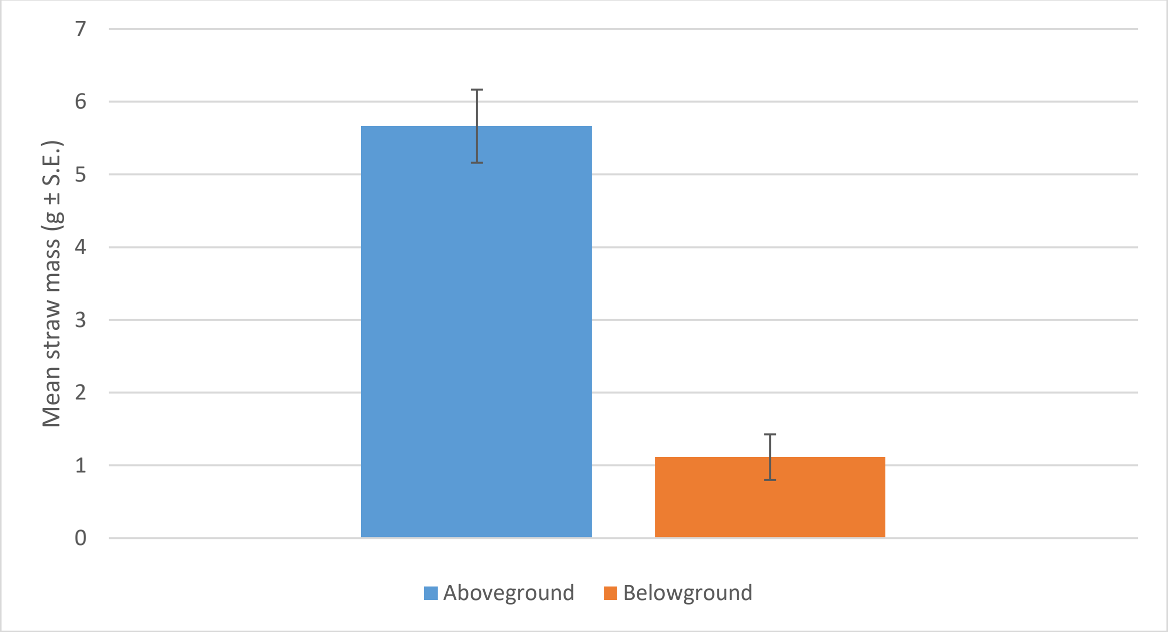


Figure 7.16. Mean total mass (g ± S.E.) of straw selected during 12 weeks by *L. terrestris*, subdivided into aboveground and belowground masses.

Although there were differences between total straw masses within the middens (aboveground straw), the mean component parts of middens were similar across replicates (Figure 7.17) Cast material represented a large proportion by mass, however this was due to the casts having a higher mass:volume ratio than straw.

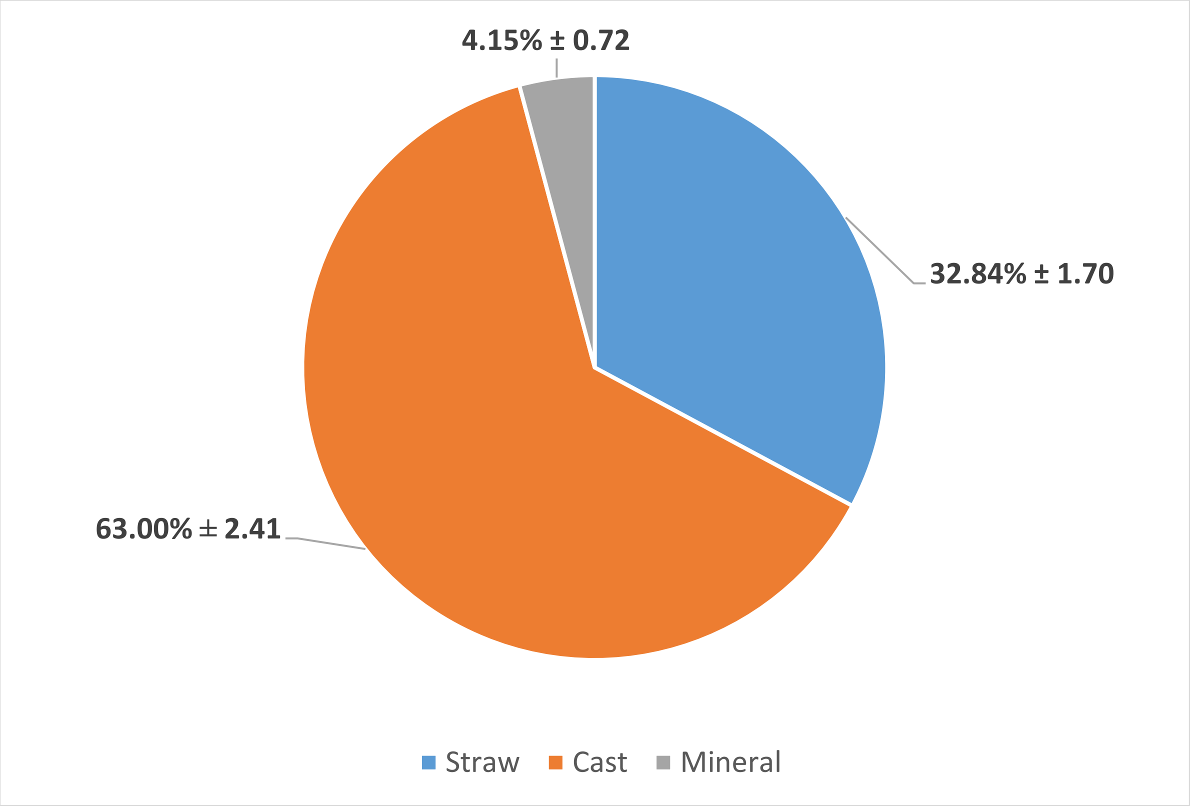


Figure 7.17. Component parts of middens separated into organic matter (straw), casting and mineral content (% by dry mass (g) ± S.E.): N=4.

Of the selected straw, the majority was utilised for midden construction. The mean mass incorporated into soil was 1.1 ± 0.3 g.

Following midden deconstruction, the number of straw particles for each experimental particle size contained within the middens (aboveground) and incorporated into the burrow soil (belowground) were recorded (Figure 7.18.). Within the aboveground and belowground matter, some of the particles were degraded or manipulated by *L. terrestris*. These particles were not included in this comparison but were included in the overall above- and belowground organic matter mass calculations.

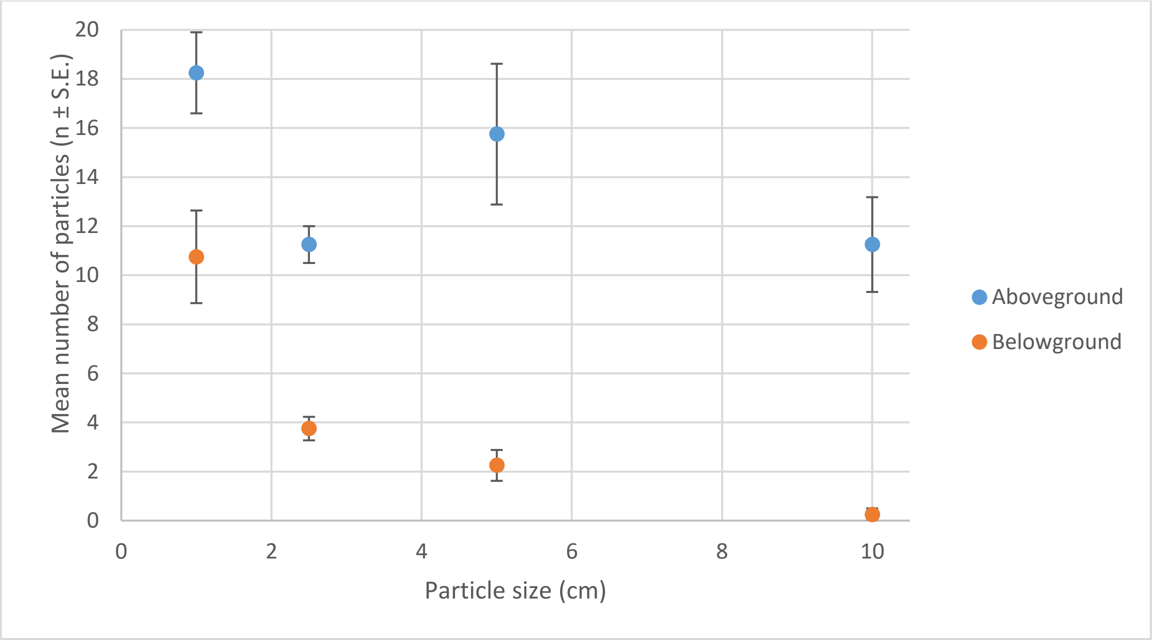


Figure 7.18. Mean number of straw particles (± S.E.) determined aboveground (within midden) and belowground (in soil) at experimental end, separated by particle size treatment (10 cm, 5 cm, 2.5 cm and 1 cm): N=4.

Of the belowground material, much of the straw mass was contained within the first 60 mm depth and amount gradually declined with increasing depth from the surface. The maximum incorporation depth was 240 mm. Often, these organic matter cores consisted of one or two pieces of degraded 10 cm straw.

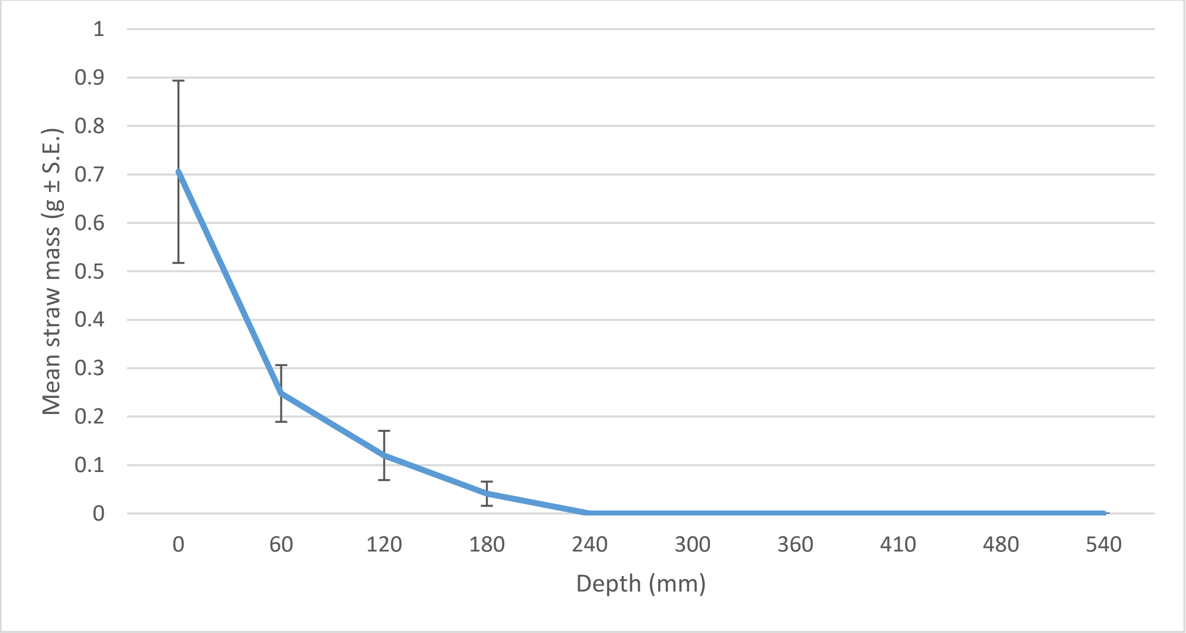


Figure 7.19. Mean mass of straw incorporated belowground (g ± S.E.) at increasing depth (mm). N=4.

Of the incorporated straw, it was difficult to determine particle size. Much of the particle sizes did not relate to the experimental straw sizes selected for the experiment due to the pieces being manipulated by the earthworm (possibly for food) before incorporating.

7.4.2.2. Nutrient Concentration

For all elements analysed, there were no significant differences between the mean nutrient concentration within soil occupied by an *L. terrestris* burrow and nearby soil with no recorded activity (control) (Mann-Whitney-U, $p < 0.05$). Within the *L. terrestris* burrow, there were no significant differences in the nutrient concentration with depth ($p > 0.05$) and no significant effect of straw incorporation mass on the nutrient concentration ($p > 0.05$). The nutrient data is presented in Appendix IV.

Significant differences between cast and control soil were determined for 6 elements (Test: $p < 0.05$; Figures 7.20 and 7.21), where cast concentration was higher.

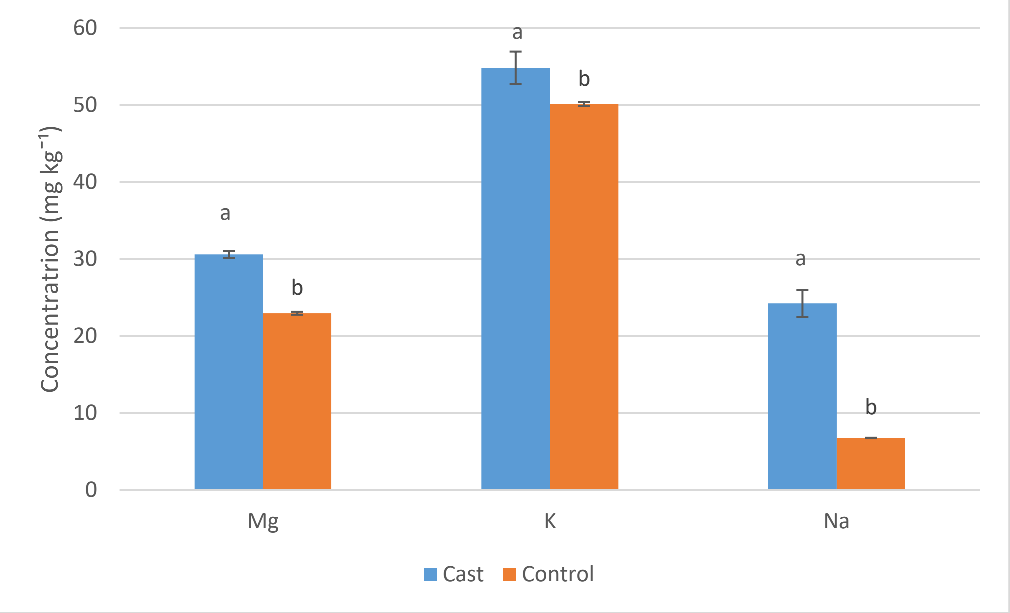


Figure 7.20. Difference in mean elemental concentration (mg kg^{-1} ± S.E.) between *L. terrestris* casts and control soil. Significant differences ($p < 0.05$; 2 sample t-test) are shown with different letters per element.

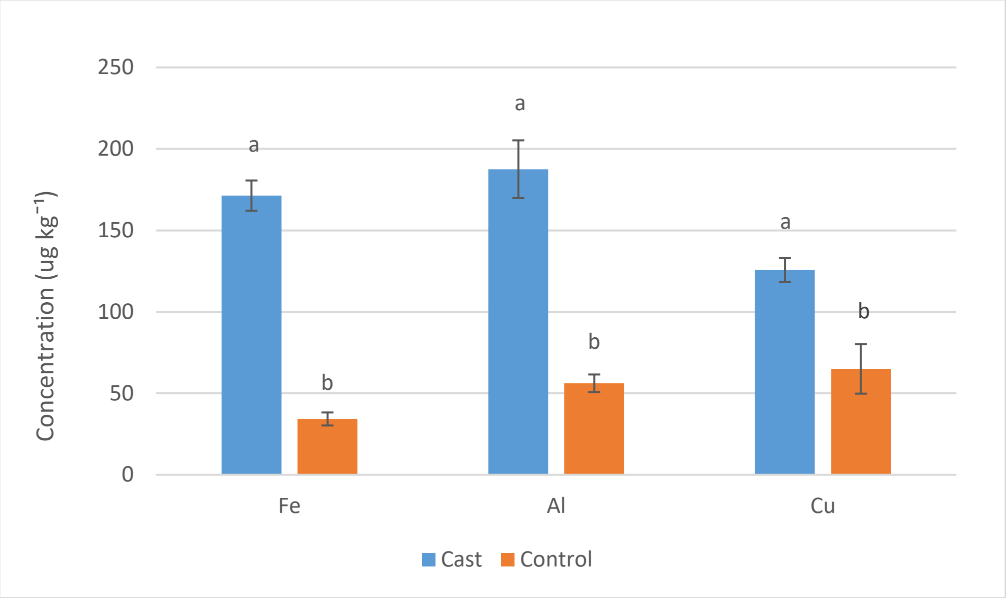


Figure 7.21. Difference in mean available nutrient concentration (ug kg^{-1} ± S.E.) between *L. terrestris* casts and control soil. Significant differences ($p < 0.05$; 2 sample t-test) are shown as different letters per element.

7.4.2.3. Carbon and Nitrogen Concentration

At experimental end there were no significant differences in the carbon and nitrogen concentration between soil mediated by *L. terrestris* ("midden soil") and control soil ("non-midden") from the unit which had not been manipulated by *L. terrestris*. There were no effects of *L. terrestris* burrow depth on C and N concentration (data not shown).

Table 7.5. Comparisons of mean carbon and nitrogen concentration (%C, %N, C:N ratio and LOI ± S.E.) between soil samples taken beneath the *L. terrestris* midden and a control (non-midden), recorded at experimental end: N = 40.

Analysis Type	**Midden**	**Non-Midden**
C (%)	2.26 ± 0.04	2.28 ± 0.06
N (%)	0.22 ± 0.004	0.21 ± 0.010
C:N ratio	3.62 ± 0.07	3.65 ± 0.11
LOI (%)	13.48 ± 0.34	12.84 ± 0.36

7.5. Discussion

7.5.1. Particle size selection

Experiment 1 indicated that there were no significant effects of particle size on straw selection by *L. terrestris*, as surface interactions occurred with straw of all particle sizes offered (Figure 7.4.). Quantitative assessment of the data suggested that there were a larger number of interactions with increasing particle size (Figure 7.6), however this was not statistically significant, possibly due to the low number of replicates (N=4). These findings do not agree with previous research (Lowe and Butt, 2005; Sizmur et al, 2017) and experiments within this thesis (Section 6.3) where a smaller particle size preference occurred. However, within controlled laboratory experiments in Chapter 6, homogenous particle sizes were applied. Although particle size may have an impact on the rate of incorporation by *L. terrestris* belowground (Chapter 6), findings from this experiment indicated that the selection of organic matter aboveground was not influenced by particle size. This supports findings that larger particle sizes provide benefits to *L. terrestris*, where they select and utilise multiple sizes.

The effect of organic matter placement may have influenced straw selection by *L. terrestris*. A relationship was identified between order of selection and distance from burrow, where straw closest to the burrow was selected first (Figure 7.7). Selecting material closest to its burrow first is likely to be beneficial to *L. terrestris* because it would require lower amounts of energy to forage and reduce predation threat. Although research suggests that *L. terrestris* can sometimes fully exit its burrow and has a homing ability (Nuutinen and Butt, 2005), *L. terrestris* did not fully exit during this experiment and preferred to be in contact with its midden, where observed surface activity from below the midden structure was more common than foraging activity (Figures 7.5 and 7.13). *L. terrestris* may only be prepared to leave its burrow when resources are scarce (Guy et al, 2018) or unevenly distributed (Butt et al, 2003), or if they have a specific food preference (Rajapaksha et al, 2013). None of which was explored in this experiment. Future experiments could explore waste organic matter mixtures (e.g. straw mixed with manure) to determine whether this influences the utilisation by *L. terrestris*.

A decrease in straw selection occurred between weeks 1 and 2 (63.7% straw selected: Figure 7.4). This could be due to a reduction in surface matter, due to surface activity remaining high (Figure 7.5). There is evidence to suggest that *L. terrestris* foraging behaviour is influenced by starvation, where more risky behaviours such as exiting the burrow or foraging in daylight may occur under increasing starvation (Guy et al, 2018). *L. terrestris* in this experiment were not under starvation conditions. It could be possible that they did not select as much straw in the second week of the experiment due to not requiring food. Future experiments could replenish selected straw particles and extend the length of the experiment to determine whether *L. terrestris* utilisation behaviour is influenced by time, risk, or preference.

There is a possibility that a 10 cm particle size was selected more frequently because its length led to an increased chance of interception over the arena, making it easier for *L. terrestris* to locate (i.e. the probability of selection was increased). Although there were equal numbers of particles (N=5), 10 cm covered double the surface area of 5 cm particle sizes and ten times the surface area of 1 cm particle size. Equal numbers of straw particle sizes based on the surface area (for example: 5 x 10 cm; 10 x 5 cm; 20 x 2.5 cm; 50 x 1 cm) would have made the overall number of straw particles difficult to track over the study and covered too much arena space. Preference experiments can be designed so that the arena was divided into quadrants separated by particle size, such as the food preference choice chamber experiments used by Rajapaksha et al, (2013), Ashwood et al, (2017b) and in Chapter 6. However, the experimental design of this experiment was to explore the utilisation of particle size as applied in the field, which is often mixed and not separated. Although there are limitations to this experimental design, findings clearly defined a difference between surface selection and utilisation aboveground and belowground, which was influenced by particle size. Future experiments could compare the benefits and limitations of preference testing methods.

7.5.2. Behavioural Observations

It was visualized in the recordings that *L. terrestris* stripped the larger pieces of straw, agreeing with finding of Stroud et al, (2016a). These stripped pieces were utilised in the midden but also manipulated pieces of straw with a narrow width (< 1 mm) were found in the belowground soil profile. The amount of stripped material was visibly less than observed within Chapter 6 (Figure 6.14A), where homogenous applications of larger straw particle sizes (20 cm and 40 cm) were used and contained a higher density of *L. terrestris* per unit (N=2). Stripping of straw was only observed on 5 cm and 10 cm particle sizes. At this particle size, *L. terrestris* could stabilise the straw with its body, enabling it to strip the straw. *L. terrestris* incorporated 1 cm particle size, but the material found belowground was not physically manipulated or decomposed. The size seemed difficult for them to utilise, which could have resulted in their lack of selection. This indicated that *L. terrestris* either may use larger particle sizes for midden construction and smaller particle sizes for incorporation belowground, or that to feed on larger particle sizes *L. terrestris* uses this stripping action to make the straw more suitable to feed upon. Added to organic matter length, the width of organic matter could limit feeding ability. This experiment was conducted over a short-time scale. Over increasing time, straw decomposition rate was increased under larger particle size, where after 17 days, 10 cm particle size straw decomposed more rapidly than 0.1 cm (Angers and Recous, 1997). Further research could explore the effects of decomposition rate at different particle size on behaviour and utilisation by *L. terrestris*.

Exploration of the homing ability of *L. terrestris* (Nuutinen and Butt, 2005) indicated that during foraging events, *L. terrestris* will exit its burrow and navigate back. During both experiments here, *L. terrestris* did not exit its burrow. There are numerous reasons why *L. terrestris* may want to remain in its burrow (discussed in Section 7.4.1). In areas where resources are patchily distributed *L. terrestris* will leave its burrow and increase its activity around resource rich areas (Butt et al, 2003). *L. terrestris* also may leave its burrow to find a mate (Nuutinen and Butt, 2005) and to colonise new territory when under starvation (Guy et al, 2018), neither of which were explored in this experiment. Webcam recordings by Rajapaksha et al, (2013) determined a clear selection preference of native tree leaf litter, such as alder over non-native chestnut and sycamore. In this experiment, organic matter type was homogenous, providing evidence of specific utilisation of organic matter based on particle size (e.g. 10 cm for midden and 1 cm for food). To determine whether this preference is strict to any particle size, future investigations could explore the effects of particle size and litter quality in situ. This would identify whether clear preferences for litter observed in choice chamber experiments are limited to particle size, utilisation requirements (e.g. midden construction food) or quality (e.g. C:N ratio, calorific content).

The utilisation of straw and the frequency of these behaviours may be limited by *L. terrestris* age and size. The earthworms used in this study were adult (mean mass = 6.8 ± 0.2 g; length 180 mm; diameter 10 mm). Behaviours such as straw stripping may be less common in juveniles due to the energy required to move and break down material. Added to this, when analysing the aboveground and belowground straw mass and particle size from *L. terrestris* middens, larger particle sizes were not present in belowground soil and stripped particle sizes were at a much smaller mass compared with 1 cm particle sizes. This supports the thought that smaller particle sizes are preferred for feeding, agreeing with Sizmur *et al* (2017) and findings from Chapter 5, yet larger particle sizes are required for midden generation and the support of aboveground ecosystem processes. Further investigations into juvenile earthworm behaviours may provide further information to how *L. terrestris* uses organic matter particle sizes over its life cycle.

7.5.3. Preference for Level of Degradation

Chemical analysis of straw indicated that there were significant differences in carbon and nutrient concentrations between straw collected following harvest and straw degraded on the soil surface for 1 month (Tables 7.2 and 7.3). Degraded straw had a significantly lower C:N ratio than fresh selected straw, suggesting that as a feed it would be preferred by *L. terrestris* (Curry and Schmidt, 2007). Added to a lower C:N ratio, degraded straw also consisted of a higher extractable nutrient concentration (Table 7.3). A preference of degraded organic matter could not be confirmed in this experiment. Although earthworms were active at the soil surface during this experiment (Figure 7.13), selection of experimental material was scarce, where 4 experimental straw particles were incorporated into the midden by the end of the two-week period between all containers (Figure 7.11). There were no significant differences in the rate of straw selection between degraded and fresh straw particles (Figure 7.12), which mirrored findings in Experiment 1. In Experiment 2, all straw particles were of equal distance from the *L. terrestris* burrow, so the effect of distance on selection was not an objective. Future experiments could explore the effects of organic matter placement on *L. terrestris* surface behaviour and selection.

Although the experiment was conducted under controlled conditions, evening outdoor temperatures dropped by the start of Experiment 2 (Table 7.4), which may have caused *L. terrestris* to be less active. A heater was used to keep the indoor temperature above 10 °C, but on several nights the temperature dropped and was lower than the optimum 15 °C. In addition, the experimental arena was reset between Experiment 1 and 2 when “experimental straw” was removed and 10 g straw was applied to feed *L. terrestris*. The midden size for all 4 samples increased during this period. Therefore, added to the effect of reduced temperatures, a reduction in experimental straw selection could have been caused by *L. terrestris* not requiring any food. Future web camera investigations need to consider whether the re-use of experimental arenas is effective. It could be argued that a new set of containers and experimental animals would have provided more valuable results. Further investigations into the selection of organic matter at different degradation levels would be useful to determine whether degrading crop residues before application would benefit *L. terrestris* and organic matter turnover in reduced tillage agroecosystems.

7.5.4. Particle Size Utilisation

Although there were no recorded effects of particle size preference on surface straw selection by *L. terrestris*, when soil was destructively sampled at experimental end, significant differences were identified in the particle utilisation between sizes. Of the experimental straw selected, a higher mass was utilised aboveground in the midden structure than incorporated belowground under all particle sizes (Figure 7.18). The difference between aboveground and belowground straw mass increased with increasing particle size, where incorporation of straw belowground decreased. There was a larger difference between the straw mass aboveground and belowground under 10 cm particle size (Figure 7.18), where most of the aboveground straw mass was of larger particle size. Belowground straw consisted of significantly higher density and mass of 1 cm straw particles (Figure 7.18), where 1 cm particle size was found at the highest recorded depths under all samples.

Analysis of straw incorporation and depth indicated how most selected material was concentrated within the upper soil profile (5.7 ± 0.4 g; Figure 7.17), where the maximum straw incorporation depth at the end of the experimental period was 240 mm (Fig 7.19). There could be many explanations for why organic matter was not incorporated to the full length of the burrow. There are many benefits to maintaining a mass of organic matter at the surface, such as a maintenance of soil moisture, a reduction of infiltration during heavy rainfall events, camouflage from predators, food storage (Hamilton and Sillman, 1989; Schrader and Seibel, 2001; Butt and Nuutinen, 2005; Butt and Lowe, 2007). Increased concentrations of microbial and fungal activity in the upper layers of the soil profile could increase the level of organic matter degradation, which could improve the palatability of the straw and the nutrient availability (Nevins et al, 2018; Schlatter et al, 2018; Hoeffner et al, 2018; Medina-Sauza et al, 2019). As the depth of soil increases, the soil profile often contains more mineral content and less oxygen concentration, causing a reduction in microbial activity (Tate, 1979). Therefore, conditions at the surface may be more beneficial for the earthworm to utilise. Increased surface area provided to microorganisms by smaller particle sizes may increase the rate of decomposition. Further investigation into organic matter incorporation depth by *L. terrestris* would be required to determine why most of the collected organic matter is stored in the upper soil layers.

This experiment suggested that organic matter selection by *L. terrestris* is not limited by particle size, but the utilisation of the straw is influenced by particle size to meet specific behaviours. Therefore, *L. terrestris* will select any type of straw it encounters on the surface then utilise the selected straw for different behaviours based on its particle size and its needs (food or midden construction). This indicates that although smaller particle sizes may increase earthworm mass (Lowe and Butt, 2003, 2005), in the field, mixed material may provide significant habitat benefits, which would be altered if particle size applications were homogenous. These results also highlight that larger particle sizes of organic matter are still of benefit to *L. terrestris*, where selection of 10 cm particle size was utilised for aboveground midden structure, vital for *L. terrestris* survival and a potential organic matter resource for epigeic populations in agricultural ecosystems, which may further enhance organic matter degradation alongside organic matter incorporation into the belowground ecosystem. By altering the aboveground ecosystem of *L. terrestris* populations, applications of homogenous milled particle size may limit the rate of waste incorporation into belowground ecosystems over the long-term and decrease SOM content of belowground soil. Future experiments could investigate the long-term effects of homogenous and heterogeneous particle size applications on *L. terrestris* populations in the field.

7.5.5. Impacts on soil chemistry

No significant differences were observed between the available nutrient concentration at soil below a midden and control soil and there were no changes in nutrient concentration with increasing burrow depth. The total length of the study was 12 weeks; a longer experimental time period may have provided further indications of the impacts of organic matter degradation on nutrient changes in a soil profile. These results confirm the findings of Chapter 6, where increased incorporation over time did not result in increased nutrient concentration. This suggests that nutrient changes mediated by earthworms may be contained within cast material and not within the drilosphere soil. Analysis of cast material indicated some significant differences by comparison with control soil (Figures 7.20 and 7.21.). Cast material by mass comprised up to 63% of the midden (Figure 7.17), which may enhance the total surface nutrient concentration compared with surrounding soil. The relationship between casts and higher nutrient availability has been acknowledged in numerous field and experimental studies (Devliegher and Verstraete, 1996; Clause et al, 2014; Vos et al, 2019). In this experiment, surface casts were compacted into a strong macroaggregate structure. There were no specific 'weather events' in this study. The only water application was surface spray to maintain soil moisture. In the field, it is possible that casts may degrade during rainfall events and through feeding by other soil organisms (Görres et al, 2001); variables which were not present in the current experiment. The interactions between cast development and decomposition on the transport of nutrients down a soil profile could be explored further.

Of the analysis of the straw material (degraded vs undegraded), it was evident that straw left on the soil surface for one month decreased the C:N ratio and increased available nutrients (ICP-OES extractions). As discussed in Section 7.5.3., this did not enhance earthworm selection in this experiment and was similar to observations of Bohlen et al, (1997), but further investigations could be developed to address this. Even with one month's degradation, the C:N ratio of straw was high in comparison to other organic matter applications such as horse manure or legumes (Persson and Kirchmann, 1994; Triberti et al, 2016; Wang et al, 2019). Although the *L. terrestris* survived the experimental period of 12 weeks, they lost mass (means of: 6.8 g at week 0; 5.2 g at week 12). This could be due to the difficulty digesting straw. It is evident that *L. terrestris* can survive on a diet of straw and lost comparatively less mass than control samples from growth experiments when not fed (Chapter 4). It would be useful to further explore applications of mixtures of organic matter to utilise the benefits of straw decomposition and other organic matter types, such as horse manure, to maintain *L. terrestris* population development.

7.5.6. Recommendations for future web camera experiments

Findings from this experiment have highlighted how the unique behavioural characteristics of *L. terrestris* could impact the selection and spatial distribution of organic matter aboveground, which may alter the decomposition rate of organic matter and the faunal community distribution in agroecosystem soils. The use of natural dyes was a cheap and effective method to locate organic matter within a soil profile without compromising *L. terrestris* selection behaviour.

To build on the findings from this research, this experiment could be extended with increased replication to confirm the interactions between *L. terrestris* and straw utilisation. The conditions in this experiment were environmentally controlled (15°C; 12:12 light:darkness; 25% soil moisture): recording this experiment outside in environmental conditions could determine how *L. terrestris* utilisation is limited by changes in weather. This could allow scientists to model how changes in weather conditions in agroecosystems might influence the decomposition of organic matter by earthworms and improve the quantification of SOM formation mediated by *L. terrestris*.

Future behavioural studies using web cameras could focus on increased levels of degradation to determine how straw decomposition over time influences the nutrient dynamics of the material and palatability for *L. terrestris*. As the physical structure of straw changes with decomposition, *L. terrestris* might utilise straw differently within the midden. It could also be extended to include other common anecic and epigeic earthworms, which feed on organic matter.

Recommendations from this experiment suggest that future laboratory experiments should consider the aboveground changes caused by *L. terrestris* on decomposition alongside belowground effects to get a full representation of this species on an agroecosystem, particularly in no-till settings where the aboveground is less disturbed. In addition to recommendations for *L. terrestris* culture in laboratory outlined in Lowe and Butt (2005), this experiment highlights the importance of vessel size and depth to allow the development of a midden within laboratory experiments. Although in growth experiments *L. terrestris* can feed and grow under a small vessel size (e.g. 1 L container), when recording decomposition, it is necessary that there is space for *L. terrestris* to develop a midden aboveground. Without this, there could be unrealistic effects on organic matter aboveground as the interactions with microbial and fungal species may differ, influencing decomposition.

7.6. Summary of Chapter Findings

- There were no significant effects of particle size or rate of decomposition on the selection of randomly positioned straw by *L. terrestris*.
- Utilisation of selected straw by *L. terrestris* differed between particle size, where belowground consisted of increased mass of lower particle sizes.
- *L. terrestris* can strip large particle sizes and utilise them, highlighting their importance in the initial stages of residue decomposition (bioturbation and casting) in no-till agroecosystems.
- In the short-term, soil nutrient changes mediated by *L. terrestris* are limited to the effects of casting.

8. A FIELD INVESTIGATION EXPLORING THE EFFECT OF *LUMBRICUS TERRESTRIS* POPULATION SIZE ON THE INCORPORATION OF CEREAL STRAW RESIDUES INTO AGROECOSYSTEM SOILS.

8.1. INTRODUCTION

Controlled laboratory experiments (Chapters 5, 6 and 7) indicated that *Lumbricus terrestris* can incorporate straw residues into the belowground ecosystem at a faster rate than other British earthworms. In an optimum laboratory setting, adult *L. terrestris* can incorporate up to 6.8 ± 0.6 g 8 weeks^{-1} of straw residues (44.2 g yr^{-1}), where the rate is impacted by particle size (Chapter 6). However, behavioural investigations indicated that the presence of residues of mixed particle sizes may benefit *L. terrestris* behaviour, where larger particle sizes are used to make a midden, which could be vital for the development of *L. terrestris* populations (Chapter 7) and juveniles of other species, which benefit from the food supply (Section 4.5). Therefore, to access the bioturbation benefits provided by *L. terrestris*, it is suggested that if controllable, applications of organic residues in a field setting should be of mixed particle size.

As earlier experimental Chapters were conducted under controlled laboratory conditions favourable for *L. terrestris* activity, the residue incorporation masses determined were potentially at the maximum rate for *L. terrestris*. In agroecosystem settings, a number of environmental and anthropogenic factors can influence the development and behaviour of *L. terrestris* populations (Section 2.3.5). The effect of climate has a significant influence on *L. terrestris* activity, where temperature, light conditions and precipitation cause increased activity in autumn and spring and reduced activities in winter and summer (Berry and Jordan, 2001; Nuutinen et al, 2014). In direct drilled systems, the presence of *L. terrestris* populations could be a key driver in the replenishment of SOM stocks post-harvest, where it has been estimated that 100 individuals m^{-2} of *L. terrestris* could consume 840 kg ha^{-1} year^{-1} of surface litter (Bohlen et al, 1997). This increase in belowground SOM could enhance decomposition and nutrient mineralization, vital for crop growth (Bertrand et al, 2015a). Although *L. terrestris* populations can increase the rate of surface organic matter removal, the rate of acceleration and the impact on direct drilled systems under different climatic conditions is still to be determined. An experiment was therefore designed to determine the effects of *L. terrestris* population density on the incorporation of cereal straw residues at 3 direct-drilled agricultural sites in south-western Finland.

8.1.1. Aims

To investigate the importance of earthworms in the incorporation of harvest residues in no-till fields where natural bioturbation is a significant factor in the burial of surface residue.

To determine the rate of residue incorporation by *L. terrestris* populations outside the growing season, in order to measure the residue reduction from harvest to sowing.

8.1.2. Objectives

- To record the mass of cereal straw residues removed under high and low *L. terrestris* population density.

- To explore the effects of earthworm surface casting on residue burial aboveground.

- To investigate the effects of *L. terrestris* population density on endogeic and epigeic earthworm abundances.

8.1.3 Hypothesis

- Straw removal over time will be increased under areas with a higher *L. terrestris* density.

8.2. MATERIALS AND METHODS

This study was a collaborative project between Natural Resources Institute Finland (LUKE) and UCLan. It was conducted over the winter period of 2015-2016 on three direct drilled cereal field sites in south-western Finland.

8.2.1. Study Sites

Three field sites were selected: Kotkanoja, Ojainen and Kiikoinen (Figure 8.1; Table 8.1). The first two are LUKE's research sites in Jokioinen. Kiikoinen is a nearby no-till farm under practical cultivation. Kotkanoja was inoculated with *L. terrestris* using the Earthworm Inoculation Unit technique (Butt et al, 1997) in October 1996 (Nuutinen et al, 2011). The other two sites had natural *L. terrestris* populations.

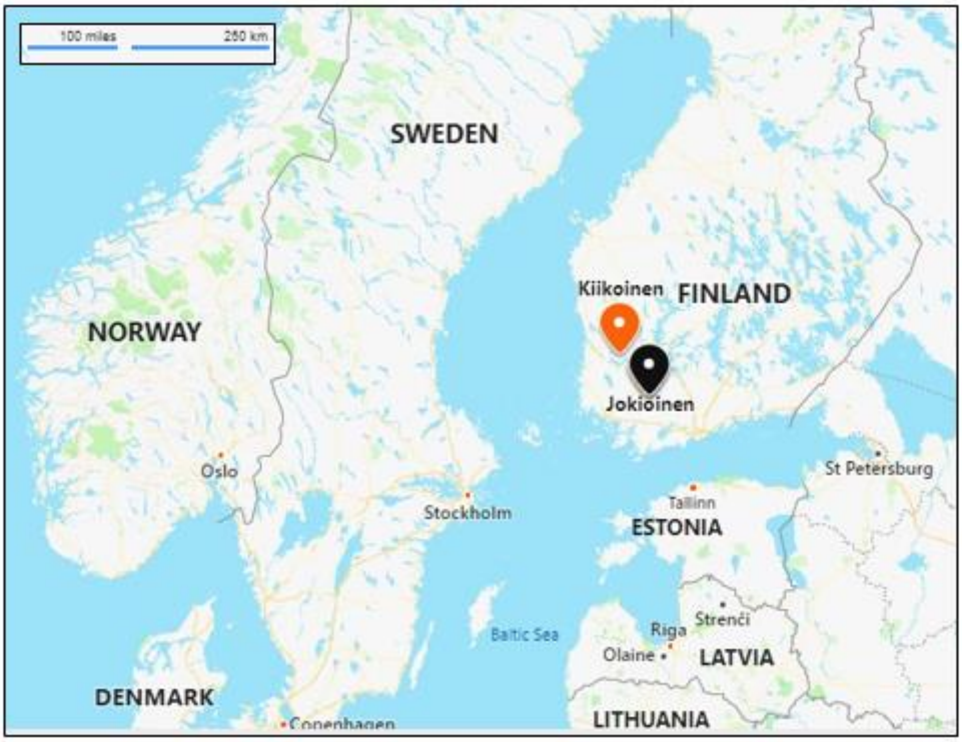


Figure 8.1. Map indicating the two experimental locations, Jokioinen and Kiikoinen, in south-west Finland.

In Ojainen, different tillage methods and no-till (direct drilling) have been compared from the year 1999, mainly under spring cereal cultivation. The soil is sandy clay and the individual study plots measure 18 m x 40 m. For this experiment, two direct drilled plots were chosen. Based on prior follow-up of earthworm and midden abundances over several years, one of the plots had *L. terrestris* (LT+) present while the species was entirely absent from the other plot (LT-). This difference was possibly due to initial spatial variation of the field and/or varying immigration rates of *L. terrestris* into the different parts of the field over the course of the experiment. The distance between the LT+ and LT- plots was approximately 55 m. Based on earlier observations, other earthworm species present were *Aporrectodea caliginosa* (Sav.), *A. rosea* (Sav.) and *Lumbricus rubellus* (Hoffm.) (LUKE, unpubl.).

The Kotkanoja site is an experimental leaching field established in 1975 on heavy clay soil with four adjacent 33 m x 140 m strips. *L. terrestris* was initially absent at the site and was introduced in the up-slope end of the field in autumn 1996 (Nuutinen et al, 2011). Through gradual population growth and spread, gradients of *L. terrestris* abundance have been formed in two direct drilled strips of the field (separated by a ploughed strip). Prior to this study, the whole site had been under spring cereal cultivation from 1996, except for a set-aside period during 2002-2008. Based on field observations of *L. terrestris* distribution in 2013 (Nuutinen et al, 2017), areas of high population density (LT+) and areas without *L. terrestris* (LT-; beyond the leading edge of dispersal) were chosen from both direct drilled strips. The distance between LT+ and LT- areas was 100 m. Other earthworm species of the field are *A. caliginosa*, *A. rosea*, *Lumbricus castaneus* (Sav.), *L. rubellus* and *Dendrobaena octaedra* (Sav.) (Nuutinen et al, 2017).

Kiikoinen is an arable field on loam soil under long term direct drilling of spring cereals. The field had been under direct drilling with some intermittent years of shallow disc harrowing (5 cm deep, the most recent disk harrowing conducted in 2013). After the harvest in late October 2015, the abundance of *L. terrestris* was surveyed in the field by observing the spatial variation of midden density on the soil surface. Based on these observations, areas of high (LT+) and low (LT-) were chosen. The distance between them was 50 m, with the LT- situated at a slightly sloping part of the field. There was no prior information on the species composition of the earthworm community in the field.

Weather data for the sites were obtained from the Finnish Meteorological Institute's (FMI) open data sources. For Ojainen and Kotkanoja sites data from of FMI's Jokioinen Observatory was used, for Kiikoinen data from FMI's Kokemäki/Tulkkila weather station. Weather and climate data for the experimental duration is presented in Table 8.1.

Table 8.1. Location of experimental sites, number of treatment replicates (N), quality and quantity of harvest residue used, duration of the experiment and selected variables describing weather conditions over the experiment. Weather data: Finnish Meteorological Institute (https://www.ilmatieteenlaitos.fi/avoin-data; Jokioinen observatory (Ojainen and Kotkanoja), Kokemäki/Tulkkila station (Kiikoinen).

Site	Latitude	Longitude	N	Residue	Residue DWT (g)	Experiment start date (2015)	Experiment end date (2016)	Duration of experiment (days)	Days minimum temp > 0 °C [1]	Snow cover (2016)[2]
Ojainen	60.80371068	23.4660112	5	barley	80.0	16. Oct	29. April	198	63	4. Jan -27. March
Kotkanoja	60.81627106	23.51131225	8	wheat	110.0	23. Oct	3. May	194	60	4. Jan -27. March
Kiikoinen	61.41992185	22.65285768	5	oats	70.0	3. Nov	9. May	189	70	4. Jan -24. March

[1] The days outside the period of snow cover are included.

[2] The period of snow cover excluding autumn periods of temporary snow cover.

8.2.2. Experimental set up

The experiment was set up as soon after the autumn harvest in 2015 as possible. Because of exceptional growth and harvest conditions in 2015, the harvest was unusually late on all three sites. For instance, at Ojainen and Kotkanoja experiments the harvest dates were 28th and 29th September, respectively, while the median harvest date during the preceding five years (2010-2014) had been 3rd and 4th September.

Experimental set-up was similar on all sites except that the number of treatment replicates varied. Experimental plot positions were first marked on LT+ and LT- areas. At Ojainen, five plots separated by 5 m were marked along linear transects through the two treatments. At Kotkanoja, four plots were marked in rectangular configuration (minimum distance of plots 3.5 m) in both LT+ and LT- areas on both strips (thus treatment total N=8). At Kiikoinen, five plots with a minimum distance of 5 m were marked in an irregular configuration on the two areas.

At each plot, a square of 0.25 m^2 was delineated with a wooden frame. The stubble (and weeds when present) were cut close to the soil surface and removed from the area. The harvest residue was then manually collected from the soil surface into paper bags using forceps, carefully avoiding the inclusion of soil. The collection was done so that only the smallest pieces of residues, such as awns and husks, remained on the soil surface (Figure 8.2). If moss was present on the soil surface, it was removed and discarded. After the soil surfaces were cleared, plots were covered with 50 cm x 50 cm metal mesh cages, with a height of 5 cm and mesh size of 8 mm (Figure 8.3). Cages were firmly attached to the ground by two metal rods pushed through the mesh at opposite corners. Simultaneously a large batch (appr. 2 kg) of residues was collected from areas close to the experimental plots.

Figure 8.2. Soil surface at the start of the experiment, following manual harvest straw removal from a 0.25 m^2 area.

Residue samples were taken to the laboratory and kept in open paper bags for four days at +60 °C. The additional residue collected was dried in the same way. The plot residue samples were then weighed, and the mean dry mass of residue (g dwt 0.25 m^{-2}) for each site was determined. This mass was used in the field experiment at a given site (Table 8.1). For each site, the corresponding amount of the separately collected and dried residue from the site was weighed in paper bags and taken to the field for the start of the experiment. An estimate for the ash free dry weight (AFDW) of this residue was determined from milled residue samples (first dried at 105 °C and subsequently at 550 °C).

In the field, the mesh cages were removed and the given amount of dried residue added evenly on the soil surface (Figure 8.3). The plot was then covered with a cage and a coded tag attached. The plot positions were marked with appr. 1 m tall pegs pushed into the soil adjacent to the plot to allow location of the plots during times of snow cover. The sites were visited 2-4 times during the experiment and plots were photographed from above (with cages on) in an attempt to document the change in residue cover (Figure 8.4).

Figure 8.3. Straw residue in cage at start of experiment (Ojainen, November 2015).

Figure 8.4. Straw residue in cage at end of experiment (Ojainen, May 2016).

8.2.3. Experimental end

The experiments were terminated in the spring of 2016, as closely as possible before the sowing of the fields (Table 8.1). In each plot, the mesh cage was removed and the residues remaining on the soil surface were collected carefully in paper bags. Basically, the technique and accuracy of collection was similar to the initial cleaning of the plots in the autumn. When pieces of straw were partially buried into *L. terrestris* burrows*,* only the above ground parts were collected by cutting. In the case of middens, "trimming" of the midden residue by scissors was necessary. The collected residue was placed in a paper bag. To guarantee equal efficiency of sampling, residue collection was done by the same three persons, simultaneously collecting from a particular plot (three persons in Ojainen and Kotkanoja, two of the three persons in Kiikoinen). To document consistency in residue collection at experimental end, images were taken of every plot at three points throughout the sampling process (1 - residue before removal; 2 - sample delineated with metal frame; 3 – soil surface after residue removal), which indicated that there was a standard protocol for residue removal and samples were treated equally between plots. Images of the straw removal process can be seen in Figure 8.5.

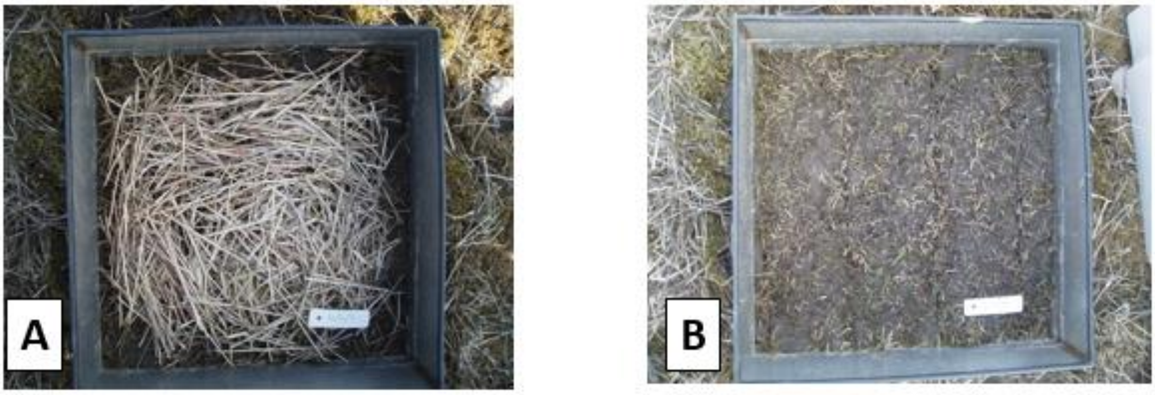


Figure 8.5. Residue removal from an experimental plot (A) Cereal residue cover at the end of the experiment before collection; (B) Soil surface of the plot after manual residue collection (LT-, plot 0/1/1, Ojainen, May 2016).

After residue collection, earthworms were sampled with mustard extraction from the experimental plot (Gunn, 1990). The plot was delineated by a metal frame (seen in Figure 8.5) hammered into the soil. After that, a mustard suspension (60 g mustard powder thoroughly mixed in 10 L of water) was poured on to the plot. The amount of suspension used depended on the infiltration rate of the plot, but most often approximately 20 L was applied. In many cases the emergence of *L. terrestris* – the main target of the sampling - was slow which is typical in local spring conditions and therefore collection of earthworms was continued for up to 50 minutes. Earthworms were put into fresh tap water and were separated into three classes: *L. terrestris* (juveniles identified by the unique pigment gradient on the dorsal side), epigeic earthworms (small to medium sized, uniformly darkly pigmented individuals) and endogeic earthworms (unpigmented specimens). The mass of *L. terrestris* individuals were determined with a field balance. For epigeic and endogeic earthworms, only the individual count was recorded. After the earthworm sampling, *L. terrestris* middens were counted from the plots.

Soil temperature (0-7 cm) and moisture (0-15 cm; TDR-measurement) during the earthworm sampling was recorded each day to verify that sampling was done in conditions favorable for earthworm activity in the top-soil. The ranges of temperatures and soil moisture percentages for the three sites were: Ojainen, 7.6-9.8 °C, 35.2-48.2%; Kotkanoja, 8.1-11.6 °C, 37.2-51.8%; Kiikoinen 14.0-15.2 °C, 24.9-38.4%. The conditions on all sites were therefore favorable for earthworm activity and suitable for successful earthworm extraction.

In the laboratory, the residue samples were first dried at 60 °C for four days. Soil crumbs attached to the residues were subsequently removed from the samples. This was done by gentle moving and shaking the residue on a 2 mm mesh and collecting the detached soil. Forceps were used to remove crumbs that remained attached to the residue after the procedure. The total mass of detached soil was determined for each sample. The dry mass of the cleaned residue was then determined. The ash-free dry-weight of the residue was determined for each plot from a milled residue sample (method as in 8.2.2) and the mass of the remaining residue in the plot converted to AFDW.

8.2.4. Weather conditions

The weather conditions at Jokioinen and Kiikoinen over the experimental period are presented in Figure 8.6 where a similar general pattern for temperature and rainfall was recorded. Differences in the experimental end date (Jokioinen 16/10/15; Kiikoinen 03/10/15) meant that there were some differences in temperature and precipitation towards the end of the experiment between the Jokioinen sites and Kiikoinen.

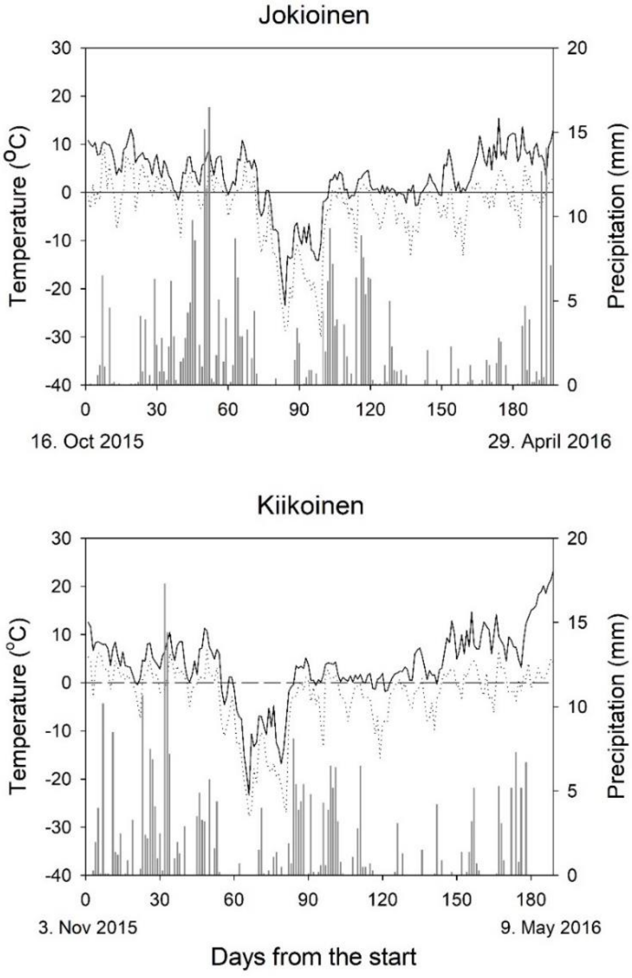


Figure 8.6. Weather conditions at Jokioinen and Kiikoinen from beginning to end of experiment. The bar chart represents precipitation (mm) and the line graph represents temperature (°C). Solid line: daily max temperature; dotted line: daily minimum temperature. Source: Finnish Meteorological Institute.

The average monthly weather conditions covering the experimental period was compared at Jokioinen with a 30 year reference period (Figure 8.7). Average winter temperatures were warmer during 2015, where there were 3 months spent below 0 °C compared with the 30 year average of 4 months. There was a cold spike in January 2016 with the average monthly temperature of - 11 °C. Total precipitation was exceptionally higher in the months November-February and April in 2015.

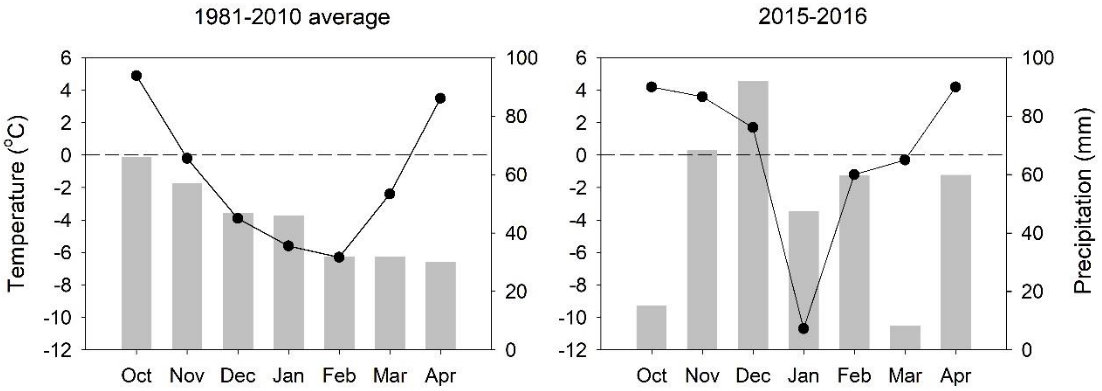


Figure 8.7. Average October-April temperature and rainfall conditions at Jokioinen during 1981-2010 (the present official reference period) compared with the average monthly temperature and total precipitation during the 2015-16 experimental period. Source: Finnish Meteorological Institute.

8.2.5. Statistical analysis

To evaluate how successfully the experimental plots were assigned to LT+ and LT- treatments and to compare the *L. terrestris* density and biomass between the sites, the abundances for each geographical location were compared with a one-way ANOVA with treatment as the factors, by first assuring that basic assumptions of normality and equal variance were met. Where data did not follow normal distribution, non-parametric Mann-Whitney U tests were applied.

The reduction in residue mass was converted into % for each sample. A one-way ANOVA was applied to determine the effects of *L. terrestris* density (LT+ and LT-) on the reduction of the mass at each geographical location (Ojainen, Kotkanoja and Kiikoinen). Where data did not follow a normal distribution, non-parametric Mann-Whitney U tests were applied. Results were also analysed as a continuous dataset, where a multilinear regression was applied to determine which recorded *L. terrestris* variables (total abundance, total biomass, adult abundance, juvenile abundance) had significant effects on the reduction in residue mass.

The effects of *L. terrestris* casting on residue burial was estimated using the mass of soil removed from surface straw at experimental end as a measure of casting activity. A one-way ANOVA was applied to determine whether there were any effects of *L. terrestris* density (LT+ and LT-) on the mass of soil removed (g) at each geographical location (Ojainen, Kotkanoja and Kiikoinen). Where data did not follow a normal distribution, Mann-Whitney U tests were applied. Results were also analysed as a continuous dataset, where a multilinear regression was applied to determine which recorded earthworm density variables (*L. terrestris* abundance, midden abundance, endogeic abundance, epigeic abundance) had significant effects on the soil removed.

The interactions between *L. terrestris* and the other ecological groups of earthworms was studied by correlation of *L. terrestris* abundance with endogeic and epigeic earthworm population densities. Spearman's correlation was applied to all *L. terrestris* variables (total density, total biomass, adult density, juvenile density) with endogeic and epigeic densities.

8.3. RESULTS

8.3.1. L. terrestris abundance in LT+ and LT- areas

For all sites combined, the mean (± S.E.) total density of *L. terrestris* in LT+ plots was 11 ± 2.1 ind. 0.25 m^{-2} and LT- plots 1 ± 0.5 ind. 0.25 m^{-2}. A statistically significant interaction was observed between sites and expected *L. terrestris* abundance (LT+ and LT-) on the *L. terrestris* density (ind. 0.25 m^{-2}) (Table 8.2). For *L. terrestris* biomass there was no statistically significant interaction between site and treatment and it was confirmed that the mean biomass at LT+ was significantly higher than in LT- (Table 8.2.).

The total earthworm density at Kiikoinen was higher than the other two sites (Table 8.2). The mean number of juveniles was significantly higher in Kiikoinen (21 ± 0.5 ind. 0.25m^{-2}) than Jokionen (Kotkanoja 4.5 ± 1.4 ind. 0.25m^{-2}; Ojainen 4.2 ± 1.0 ind. 0.25m^{-2}). The mean soil moisture at Kiikoinen was significantly lower than the sites at Jokioinen (Kiikoinen = 36 ± 0.5%; Kotkanoja = 44 ± 1.2%; Ojainen = 49 ± 0.9%), but at each field location, there was no significant difference in the mean soil moisture between LT+ and LT- samples (p > 0.05).

Table 8.2. Mean abundance (± S.E.) of *L. terrestris* at high (LT+) and low (LT-) treatments at each field site. For each *L. terrestris* factor, there was a significant difference in the means of LT+ and LT- at each location (one-way ANOVA; df = 1; p < 0.001).

L. terrestris	Ojainen		Kotkanoja		Kiikoinen	
	LT+	LT-	LT+	LT-	LT+	LT-
Total (ind. 0.25 m^{-2})	5.0 ± 1.4^{a}	0.0^{b}	6.5 ± 1.7^{a}	0.4 ± 0.3^{b}	22.8 ± 2.0^{a}	6.0 ± 1.3^{b}
Adults (ind. 0.25 m^{-2})	0.8 ± 0.5^{a}	0.0^{b}	2.0 ± 0.8^{a}	0.0^{b}	1.8 ± 0.5^{a}	0.6 ± 0.4^{b}
Juveniles (ind. 0.25 m^{-2})	4.2 ± 1.0^{a}	0.0^{b}	4.5 ± 1.4^{a}	0.4 ± 0.3^{b}	21.0 ± 0.5^{a}	5.4 ± 1.1^{b}
Biomass (g 0.25 m^{-2})	5.1 ± 2.4^{a}	0.0^{b}	9.0 ± 2.9^{a}	0.2 ± 0.1^{b}	11.1 ± 2.1^{a}	3.3 ± 1.3^{b}

Compared with the Jokioinen sites, there was less background information available on *L. terrestris* distribution and abundance in Kiikoinen, where LT+ and LT- areas were located based on qualitative midden observation. To study the reliability of midden counting in predicting *L. terrestris* abundance, *L. terrestris* abundance and midden counts (collected at experimental end) were compared. One-way ANOVA confirmed that there was a significant difference between the midden counts under *L. terrestris* density (df = 9; F = 3.302; p < 0.01) and biomass (df = 9; F = 5.328; p <0.001), where the mean midden counts between LT+ and LT- samples were significantly different (df = 9; F = 14.44; p < 0.001). There was no discernible relationship between midden counting and total *L. terrestris* density.

8.3.2. Rate of residue mass decline

There was a significant difference in the mean decline in residue between LT+ and LT- samples at Ojainen and Kotkanoja (one-way ANOVA; $p < 0.05$): the mass decline was lowest at Kiikoinen ($p > 0.05$) and clearly highest at the Jokioinen sites where the difference between the treatments was apparent (Figure 8.8).

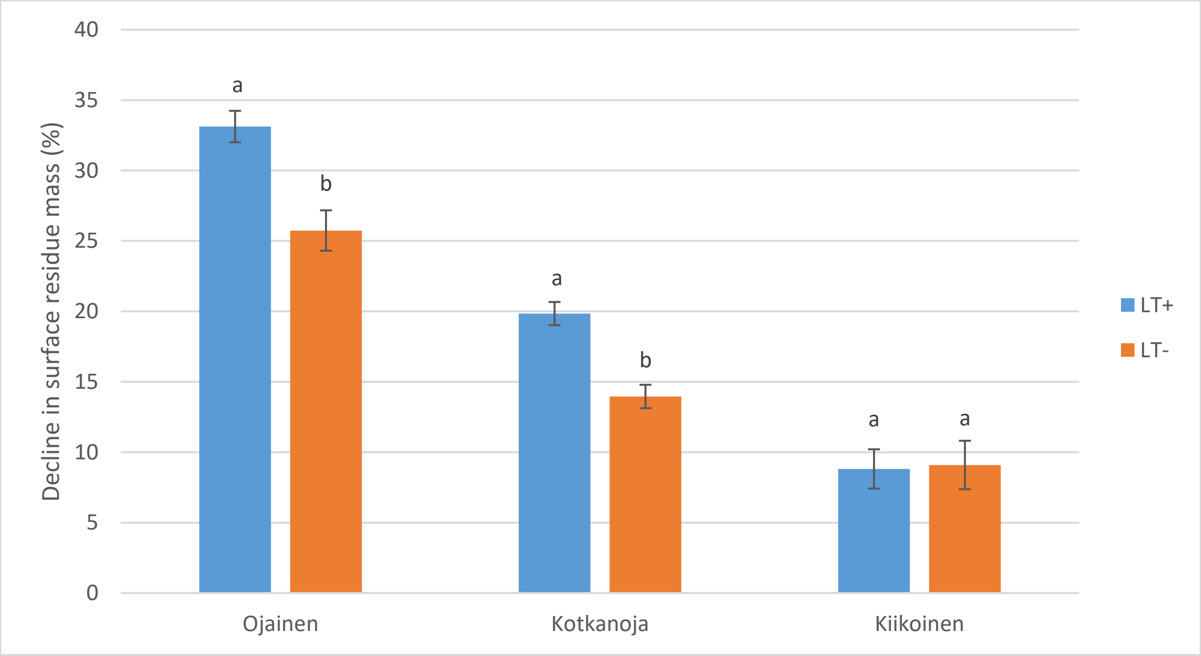


Figure 8.8. Mean (± S.E.) reduction in mass of surface residue (% mass ash free dry weight) at each site in LT+ and LT- areas. For Ojainen and Kiikoinen, N=5; for Kotkanoja, N=8. Statistically significant differences at sites ($p < 0.001$) are represented by different letters.

Linear regressions were conducted to determine the relationship between % residue decline and recorded *L. terrestris* total density at each site (Figure 8.9.) There was a positive relationship between *L. terrestris* density and the decline in residue mass (%) at Kotkanoja and Ojainen.

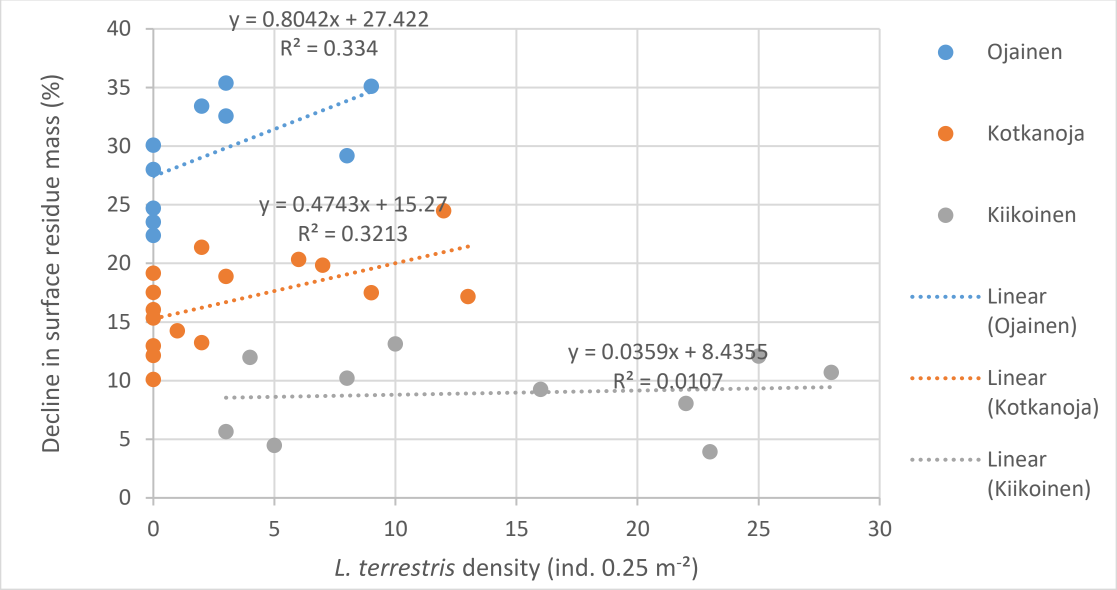


Figure 8.9. Relationships between the decline in surface residue mass (% AFDW) and *L. terrestris* density (ind. 0.25 m^{-2}) for each site.

8.3.3. Surface casting in residue incorporation

Within sites, LT+ and LT- treatments differed statistically only in Kiikoinen, where the dry mass of soil removed from residues was 31.5% ± 9 SE higher in LT+ compared with LT- (Figure 8.10). The total soil removed was higher than at Ojainen and Kotkanoja for both LT+ and LT- samples.

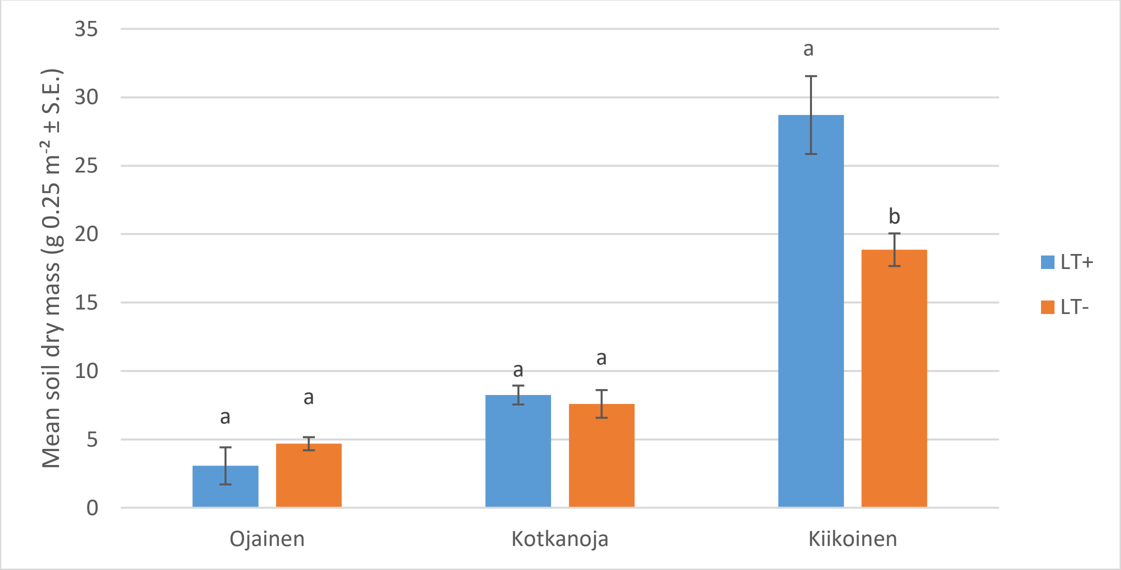


Figure 8.10. Mean (± S.E.) dry mass (g) of soil detached from surface residues at experimental end in high and low *L. terrestris* densities for each field. A statistical difference between LT+ and LT- at each location is indicated by different letters (one-way ANOVA; $p < 0.05$). N=5 (Ojainen and Kiikoinen); N=8 (Kotkanoja).

Species other than *L. terrestris*, particularly endogeic species, could have contributed to the casting on surface residues. In Kiikoinen in particular, there were abundant *A. caliginosa* casts on the soil surface and attached to the residues at the end of the experiment (Figure 8.11).

Figure 8.11. *A. caliginosa* casts on the soil surface and on oat residues at Kiikoinen at experimental end.

Linear regression was applied to study the relationship between *L. terrestris* density, endogeic species density and total earthworm density (ind. 0.25 m^{-2}) and the dry mass of soil detached from residues (Fig 8.12A-C.) There was a positive relationship for all three explanatory variables, where the strongest relationship was total earthworm density.

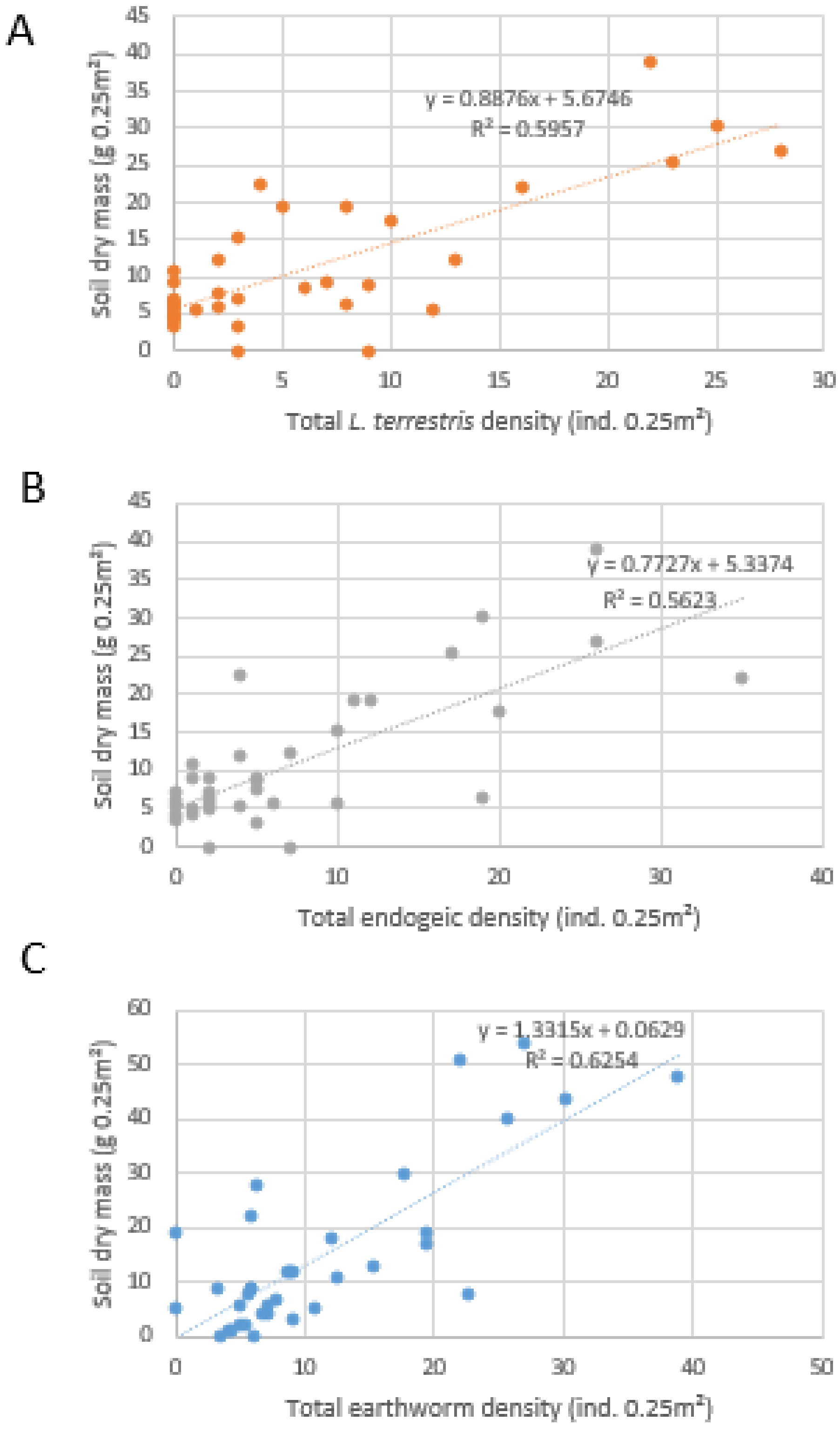


Figure 8.12. Relationships between total *L. terrestris* density (A), total endogeic density (B) and total earthworm density (C) with the dry mass of soil removed (g 0.25m^{-2}) from remaining surface residue at experimental end. N=36.

8.3.4. Species abundance relationships

In data combined from the experimental sites, there was a positive relationship between the density of endogeic earthworms and *L. terrestris* total density (Fig. 8.13A). On the contrary, there was a negative relationship between *L. terrestris* and epigeic earthworm total densities (Fig. 8.13B). When epigeic density in LT+ and LT- treatments were compared in the combined data, however, there was no statistically significant difference in the density ($p > 0.05$). This may have been caused by the overall low epigeic density across the field sites.

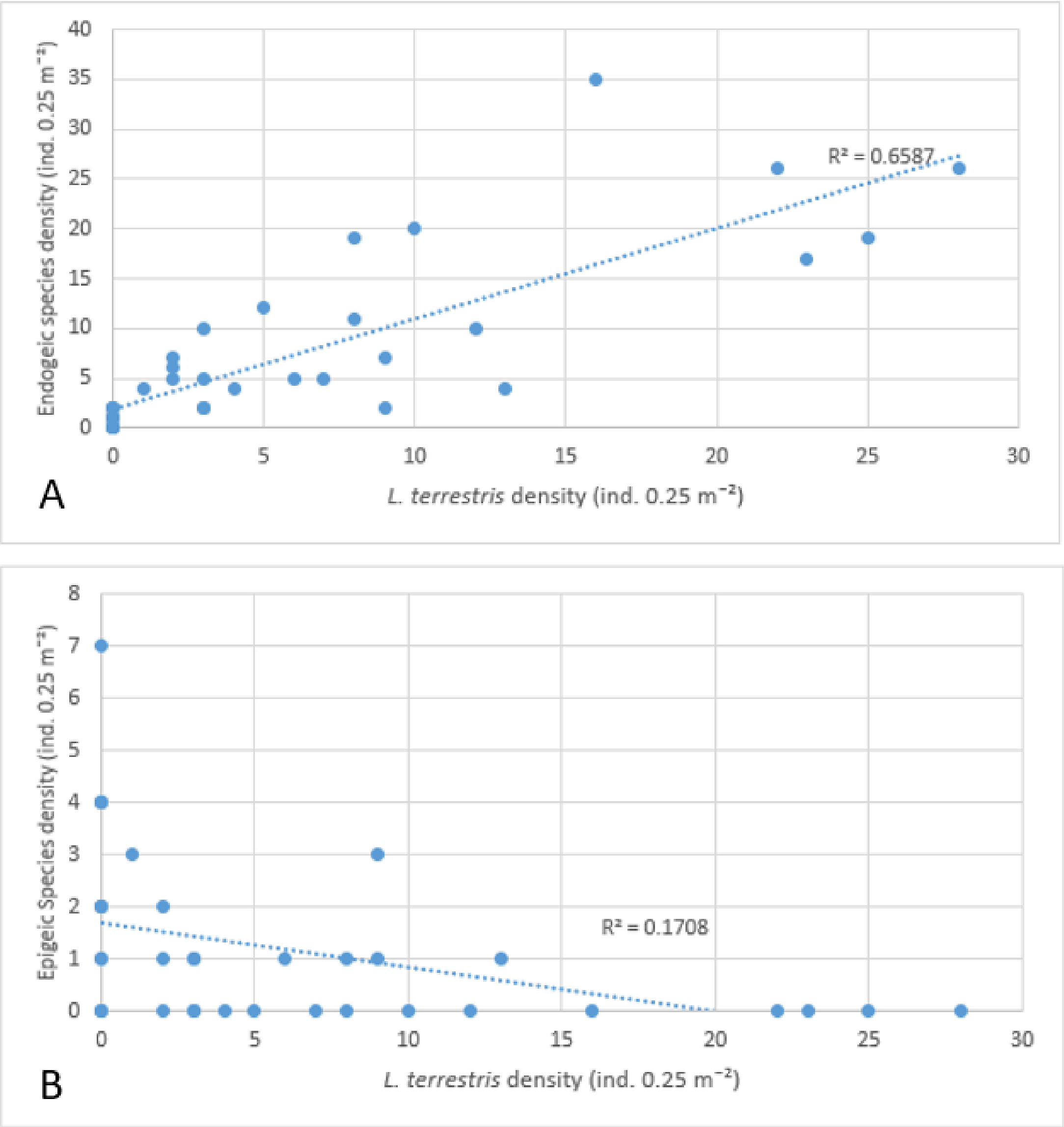


Figure 8.13. Relationships of *L. terrestris* density with (A) endogeic species density and (B) epigeic species density (ind. 0.25 m^{-2}) at experimental end.

Positive correlation occurred between endogeic earthworm total density and all variables describing *L. terrestris* abundance (Table 8.3). Negative correlation was observed between *L. terrestris* variables and epigeic species density, where the negative correlation was higher with juvenile *L. terrestris* than with adult individuals.

Table 8.3. Correlation (r_s) between different variables describing *L. terrestris* abundance and endogeic and epigeic earthworm total densities. There were no other anecic species recorded in the fields. N=36. Statistically significant correlations are marked by * ($p < 0.05$).

Earthworm Group	*Lumbricus terrestris* Factor			
	Total density (ind. 0.25m^{-2})	Biomass (g 0.25m^{-2})	Adult density (ind. 0.25m^{-2})	Juvenile density (ind. 0.25m^{-2})
Endogeic	0.849*	0.732*	0.56*	0.852*
Epigeic	-0.517	-0.388	-0.334	-0.512

8.4. DISCUSSION

8.4.1. The effect of **L. terrestris** *abundance on surface residue decline.*

This experiment provided preliminary evidence that under boreal field conditions, surface applied cereal residue can be incorporated into soil at a more rapid rate when *L. terrestris* are present (Figure 8.8). Between autumn harvest and spring sowing, the total mean reduction in straw residue mass was on average 4.6 ± 0.62% higher under LT+ treatments. Between sites, the rate of residue decline varied widely and was lowest on the Kiikoinen site. It was not possible to explain conclusively the differences between the sites, as the type of residue, *L. terrestris* abundance and many other environmental conditions varied across the sites. Also, due to differences in the experimental design between field sites, it was not possible to consider statistically the effects of *L. terrestris* density on straw removal across field sites. A factorial design with equal numbers of replicates between field sites would make this possible.

There were notable differences in the age composition of *L. terrestris* populations between field sites, with Kiikoinen having the largest number of juveniles. A lower density of adults and an increase in proportion of juveniles could have caused a reduction in the rate of straw mass removed, as it has been previously determined that juveniles with a biomass less than 1 g inhabit the soil sub-surface and may not incorporate organic matter (Lowe and Butt, 2002). The intraspecific relationship between *L. terrestris* adults and juveniles is complex. Research suggests that *L. terrestris* middens may facilitate juveniles by providing a food source and habitat (Butt and Lowe, 2007), yet in laboratory culture, it was indicated that the presence of an adult reduced the rate of growth to maturity of *L. terrestris* hatchlings (Lowe and Butt, 2003) and juvenile avoidance behaviour of the adult burrow area was also observed (Grigoropoulou et al, 2009). To determine an accurate removal rate of straw residue over time, further investigations on the effects of *L. terrestris* population development on residue removal are required.

L. terrestris behaviour is affected by light conditions, temperature and soil moisture (Edwards, 2004; Perrault and Whalen, 2006; Nuutinen et al, 2014) and in boreal climate there are specific periods of time, particularly in autumn, where maximum activity occurs. The present estimates of residue mass decline rates due to *L. terrestris* activity may have been considerably lower than in an average year, due to the harvest dates being exceptionally late. For instance, in Jokioinen sites, the harvest in 2015 occurred between 28-29th September, where previous years it had been 2-3rd September. This resulted in the experimental start date being three weeks late compared to that during a more typical year (Table 8.1) and which correspondingly reduced the *L. terrestris* population's time to incorporate the residue. Residue burial mediated by adult *L. terrestris* may have occurred later in the spring season in

2015 as the juveniles matured. This experiment could be extended to examine whether harvest time has an impact on residue removal by *L. terrestris* populations, where annual removal rates could be compared with harvest times.

No differences in the rate of straw decline between LT+ and LT- populations at Kiikoinen were noted. This could have been influenced by a presence of *L. terrestris* in LT- plots in clearly higher numbers than at the other sites (Table 8.2). The mean total population density in LT- was 6 ind. 0.25 m^{-2} at Kiikoinen, which indicated that the residue decline rate over the experimental period could have been influenced by *L. terrestris* activity. The differences in mean soil moisture concentrations between Kiikoinen (36%) and Jokioinen (43-47%) field sites – if indicative for consistent long term differences in soil moisture levels - could have influenced the difference in *L. terrestris* abundances (Table 8.2), where 36% is a more optimum condition for earthworm populations in clay fields (Perrault and Whalen, 2006). Higher *L. terrestris* population size at the LT- sites in Kiikoinen were obviously due to the inaccuracy of midden observations under the conditions which prevailed during the positioning of LT+ and LT- areas at the start of the experiment. Previous earthworm sampling data and site history available for the two Jokioinen sites increased the accuracy of *L. terrestris* sample density estimations. Although middens can be an accurate indicator of *L. terrestris* population presence (Simonsen et al, 2010; Stroud et al, 2016c, 2017), the weak correlation recorded between *L. terrestris* midden counts and total population density (r^2 = 0.146; data not shown), indicated the inaccuracy of midden counts as *L. terrestris* population density indicator in this experiment. Research has suggested that *L. terrestris* burrows can be utilised for up to 7 years, and that they may "recycled" within the population after the death of the resident individual. (Nuutinen, 2011; Potvin and Lilleskov, 2017). However, laboratory experiments investigating *L. terrestris* re-use of a previous midden and burrow indicated that only 50 % of middens were utilised (Grigoropoulou and Butt, 2012). Therefore, initial midden counts at Kiikoinen could have been an over-estimate of total *L. terrestris* population density and contributed to inaccuracies in LT- plot positioning. To ensure that *L. terrestris* population density is accurately predicted, future investigations could explore different methods of *L. terrestris* population density estimation (Butt and Grigoropoulou, 2010) and compare the accuracy of each method with the actual *L. terrestris* density.

This experiment was designed to assess the incorporation of residues by *L. terrestris* populations under different fields. Although the tillage management (no-till) was the same within LT+ and LT- plots, the cereal residue type was different between sites (Table 8.1). The controlled experimental conditions (cereal straw applied in monoculture; organic matter removed from surface at experimental start) meant that there were no effects of food selection on *L. terrestris* behaviour and dispersal. However, the difference in food type could have caused some differences in the rate of organic matter incorporation and decomposition, where factors such as residue C:N ratio, lignin concentration and particle size can have an impact on *L. terrestris* feeding (Lowe and Butt, 2003; Curry and Schmidt, 2007; Chapter 6). Differences in organic matter type have suggested a difference in the growth rate and reproductive capability of *L. terrestris* species (Chapter 5; Butt, 2011), where wheat residues have been suggested to improve growth and fecundity (Sizmur et al, 2017). However, analysis of *L. terrestris* feeding, growth and reproduction between the residue types in this experiment (barley, oats and wheat) have not been done *in-situ*. Future investigations could explore closely the differences in cereal residues and their effects on *L. terrestris* incorporation rate in the field.

The method used to measure residue mass reduction may have caused some inaccuracies where manual removal of straw left small amounts of residues on the surface at experimental end. The aim of this experiment was to determine the residue decline as influenced by *L. terrestris* density under a natural setting. Previous organic matter removal experiments have used litter bags to measure mass reduction of standard size litter, where earthworms can feed on organic matter without losing the sample (e.g. Suaréz et al, 2006; Valckx et al, 2011). Litter bags were not used in this experiment because it was considered that litter bags would interfere with *L. terrestris* foraging behaviour by hampering the collection of residue which was offered in its inherent size distribution (pieces up to > 10 cm in length included). Although this experiment had therefore uncontrolled features, it allowed natural foraging behaviour which otherwise may have been limited. This method was labour intensive, taking 3 researchers two weeks to conduct the residue sampling at experimental end. Using litter bags would be more rapid and possibly allowed for more replicates to be included, but might not have answered questions regarding surface residue burial, discussed below. Future experiments could explore the differences between litter bags and the "free residue" approach adopted in this field experiment.

8.4.2. Earthworm surface casting in residue burial.

Surface casting is a significant bioturbation effect of earthworms. Up to 90% of *L. terrestris* casts are deposited on the soil surface (Whalen et al, 2004) and the casting rate can be impacted by temperature conditions and soil bulk density (Joschko et al, 1989). Added to the physical transport of soil aboveground, casting on the soil surface increases residue burial and could increase the rate of residue degradation by enhancing microbial activity (Aira et al, 2009; Nechitalyo et al, 2009). It was indicated in this experiment that surface casting increased with *L. terrestris* density (Figure 8.12A), where there was a significantly higher average mass of casts at Kiikoinen compared with other sites (Figure 8.10). Kiikoinen had higher densities of *L. terrestris* compared with the other sites (Table 8.4), which could explain these differences. Further experimental studies could explore the effects of juvenile and adult *L. terrestris* casts on the dynamics of residue burial.

Initial earthworm surveys at Jokioinen sites indicated the presence of other earthworm species in addition to *L. terrestris* (Section 8.2) and the sampling at the end of the experiment confirmed the same at Kiikoinen. The other species could have also contributed to soil addition through surface casting, a notable species in this respect is the endogeic *A. caliginosa* (Whalen et al, 2004). Observations made at experimental end at the Kiikoinen site indicated heavy endogeic casting on the soil surface (Figure 8.11), where the mean mass of casts removed from surface residues were the highest (Figure 8.10). The effects of *L. terrestris* and endogeic population density on the mean mass of soil removed indicated that both earthworm groups increased the mass of soil removed (Figure 8.12A-B) and that the effects were higher under both populations combined (Figure 8.12C). Endogeic species such as *A. caliginosa* cast on the soil surface, but also commonly cast within their burrow system below the soil surface (Haynes et al, 2003; Capowiez et al, 2014a, 2014b). Casting belowground by endogeics can provide other benefits to soil and plant health, such as the positive effects of *A. caliginosa* casts on grassland growth (Haynes et al, 2003; Zaller et al, 2013). Further exploration of the differences between *L. terrestris* and *A. caliginosa* casting rate and behaviour could determine the effects of these species on organic matter incorporation and decomposition.

Alongside earthworm ecotype, environmental factors may have influenced the amount of surface casts present. Numerous physical characteristics can influence the consistency of casts produced, such as soil type and moisture content (Lavelle, 1988; Joquet et al, 2008, Clause et al, 2014). Earthworm casts are high in moisture and their structure can change over time, causing loss and transport back into the belowground ecosystem by erosion and decomposition (Andriuzzi et al, 2015a). The Jokionen and Kiikoinen sites had different soil types (Jokionen = clay; Kiikoinen = loam) and there were significant differences in the soil moisture between the sites where the mean soil moisture at Kiikoinen was lower (36%). Loam soil is a more favourable environment for earthworms than clays (Edwards and Bohlen, 1996), which may have contributed to increased casting at Kiikoinen. Increased soil moisture can have significant implications on the structure of casts, where annual variations in precipitation can impact erosion rates (Le Bayon and Binet, 2001; Le Bayon et al, 2002). The precipitation at Kiikoinen was lower prior to the collection of samples (Figure 8.6), therefore, Kiikoinen may have a higher density of earthworm casts due to a reduction in erosion. The physical structure of casts is also dependent upon the organic matter present and its decomposition stage. Temporal changes in cast composition by *L. terrestris* populations was investigated by Vidal et al, (2019), where it was identified that the organic components of casts were initially (8 weeks) determined by surface applied ryegrass residues. With increasing time (52 weeks) the cast components were dominated by mineral organic matter (MOM). The changes in cast physio-chemical condition could impact their degradability, transport and nutrient benefits to crops. Further investigations of straw residue decomposition over time could determine the physio-chemical components of casts and their effects on soil characteristics in agroecosystems.

8.4.3. Species abundance relationships

Interspecific relationships between *L. terrestris* and *A. caliginosa* were investigated in Chapter 6, where competition between adults was identified under laboratory conditions. Conclusions from that experiment indicated that the effects of *L. terrestris* population density could have been a limiting factor on the population development of endogeics in soils with limited SOM. Here, to test the relationship between *L. terrestris* with endogeic populations, the effects of *L. terrestris* abundance (LT+ and LT-) and endogeic population density was explored. All recorded *L. terrestris* factors had a positive correlation with endogeic population density (Table 8.5; Figure 8.13A), which could imply that there are positive interspecific interactions occurring between *L. terrestris* and endogeic populations. However, further experiments would be required to clarify this. It could, for instance, be possible that both populations benefitted from the environmental conditions within the soil. Contrasting information occurs on the inter-specific effects between *L. terrestris* populations with endogeic species, where in laboratory culture it was indicated that the presence of *L. terrestris* adults may facilitate *A. chlorotica* growth to maturity (Lowe and Butt, 2003) and reduce the growth rate of *A. caliginosa* (Butt and Lowe, 2007) with negative effects increasing with earthworm density (Eriksen-Hamel and Whalen, 2007a). Further exploration of the behavioural responses of endogeic earthworms with *L. terrestris* presence is required to determine the relationships between species.

Interactions between epigeic species density and *L. terrestris* density were also investigated. It is possible that *L. terrestris* populations can facilitate epigeics through the development of a midden, where previous studies have indicated their higher abundance in midden environments (Butt and Lowe, 2007; Section 4.5). Alternatively, the efficient residue collection and incorporation of *L. terrestris* may negatively affect epigeic earthworms by reducing the availability of their litter layer habitat. The results from this experiment suggested a negative correlation between *L. terrestris* and epigeic earthworm densities (Figure 8.13B). There could be many reasons for this. A high density of juvenile *L. terrestris* was recorded within LT+ treatments (average density was 9 ± 2 ind. 0.25 m^{-2}). This could have increased food competition, where both juvenile *L. terrestris* and epigeics species can gain from *L. terrestris* middens (Butt and Lowe, 2007). This potential competition between epigeic species and juvenile *L. terrestris* could cause epigeic migration to other areas not surrounding middens and juvenile species. Added to this, a high number of juveniles and low density of adults resulted in a low midden density at Kiikoinen LT+ sites. If epigeic populations are limited by the presence of *L. terrestris* middens, a low abundance of middens could have reduced the epigeic population size. Due to the low numbers of epigeic earthworms in the present study, a representative impact of *L. terrestris* populations on epigeic species would require a more comprehensive study. At present, it is still undetermined how earthworm population dispersal and development is affected by niche overlap and ecological group characteristics (Uvarov et al, 2019). Further investigations on population development and species interactions could indicate the effects of earthworm species diversity on residue incorporation and bioturbation in the field.

8.4.4. Effects of weather conditions

The differences in residue mass decline rates between Jokioinen and Kiikoinen field sites could be partly explained by a difference in experimental start times between the two areas and a slight variation in weather conditions (Table 8.1). In Kiikoinen, the experiment started later in the autumn season, which resulted in a shorter total experimental period than at Jokionen sites. This may have, however, been compensated by the higher number of experimental days with minimum temperature > 0 °C at Kiikoinen (Table 8.1). For all sites, the exceptionally late harvest of 2015 shortened the favourable period for *L. terrestris* activity before the onset of winter and reduced the time available for residue incorporation compared with average autumn conditions. In a Fennoscandian climate, which has shorter autumn and spring periods than the UK, a later harvest could reduce their positive bioturbation effects. This experiment indicated that *L. terrestris* population density had significant impacts on of the reduction rate of surface residues even under relatively short activity period, highlighting their importance in SOM addition belowground. Future investigations could expand the experimental time to measure the effects of *L. terrestris* foraging activity and rate of residue removal over a full season in no-till agroecosystems.

L. terrestris activity and performance is influenced by geographical location, where climate conditions and daylengths, for instance, differ (Nuutinen et al, 2014). In Finland, longer winter periods could reduce activity time compared with European countries at lower latitudes, such as the UK. In the experimental year, there was a warmer climate in October-December at Jokioinen compared with the reference period climate data (Figure 8.7). This could have increased earthworm activity during this time. Although the duration of time where the temperature was < 0 °C was shorter than the reference period average, there was an extreme cold spike in January 2016 where the average temperature was -11 °C (Figure 8.6). This could have influenced the resurgence of *L. terrestris* populations in spring. Earthworm populations are resilient to cold soil conditions and can survive frost conditions (Nuutinen and Butt, 2009; Berman and Meshcheryakova, 2013). Laboratory experiments have indicated that the survival in cold conditions depended on the species, where *A. caliginosa* can survive soil temperatures as low as -5 °C, yet *L. rubellus* cannot (Holmstrup and Overgaard, 2007). Although this indicates that earthworms can survive cold temperatures, it does not identify the behavioural responses to fluctuating temperature, which may impact the rate of bioturbation. Previous investigations on earthworm bioturbation in Scandinavian environments (Taylor et al, 2018), indicated effects of pH and organic matter type on bioturbation rate under average conditions. Further experiments could be designed to determine the effects of extreme weather events caused by climate change on earthworm population performance.

9. DISCUSSION AND FUTURE RESEARCH

9.1 Introduction

The aim of this research was to investigate the interactions between earthworms, waste organic matter and the effects of particle size on bioturbation by earthworms in agroecosystems; with the opportunity to identify benefits of specific applications on earthworm mediated bioturbation effects. Whilst earthworms have been the subject of investigation by agroecologists for decades, where they are known for their benefits to soil health, there are significant knowledge gaps in the understanding of earthworm population dynamics in response to waste organic matter addition, and the subsequent benefits their interactions may provide to soil quality and crop growth. A range of field and laboratory-based experiments and surveying were undertaken to address these knowledge gaps and the research objectives outlined in the early chapters of this thesis. The results of each study were individually discussed and summarised in Chapters 4 to 8. This final chapter provides a wider discussion according to the overall research aims of this thesis. Implications of this research for waste organic matter applications in no-till arable ecosystems are presented, followed by contributions to knowledge, the limitations of the research and finally, suggestions for future research.

9.2. General discussion

It was indicated in initial field surveys (Chapter 4) that waste organic matter applications can increase and sustain earthworm abundances and alter their community dynamics, where in arable settings the density of anecic species was increased. This added to previous knowledge that waste organic matter application in low resource areas can increase earthworm populations. Further field investigations of the soil underneath *L. terrestris* middens, where increased organic matter is found, indicated that this species can increase the population abundance of other juvenile earthworm species. This supported findings by Butt and Lowe, (2007) and Stroud et al, (2016a), yet highlighted how *L. terrestris* midden size could have a significant impact on the facilitation of epigeics aboveground. In low resource areas, the facilitative effects of *L. terrestris* on other earthworm species is suggested to be lower.

The effects of waste organic matter applications (horse manure, wheat straw, anaerobic digestate and CGW) had different implications for earthworm population growth and fecundity, where liquid anaerobic digestate and CGW were determined inefficient feeds (Chapter 5). Of the organic wastes tested, horse manure, anaerobic digestate (dry fraction) and wheat straw increased earthworm growth rates. The positive effects of horse manure and wheat straw have been demonstrated in other studies (Butt, 2011; Sizmur et al, 2017), however the utilisation of anaerobic digestate as an effective application requires further research, due to the positive effects of a dry fraction and negative effects of a liquid fraction. Application dosage could be an important factor limiting earthworm growth, where a lower N concentration resulted in a lower growth rate and a high metal or ammonia concentration could require application dilution. The consistency of the digestate could influence whether earthworms utilise liquid anaerobic digestate as a feed, where further experiments could analyse liquid anaerobic digestate as a mixture with other waste organic matter.

The effects of particle size on earthworm bioturbation was explored in Chapters 6 and 7, where it was determined that the rate of removal was not affected by organic matter type under milled applications (Section 6.2). Further analysis of the effect of particle size indicated that *A. caliginosa* and *L. terrestris* preferred milled particle size over 1 cm applications (Section 6.3). Following mesocosm applications of highest modal particle sizes from the field, there were significant effects on the surface removal of organic matter and bioturbation effects (Section 6.4). This supported knowledge that a lower particle size increased the growth rate and assimilation of organic matter by earthworms in an experimental setting (Lowe and Butt, 2003; Sizmur et al, 2017), however highlighted the importance of larger particle sizes in habitat development. When presented with a mixture of particle sizes, *L. terrestris* demonstrated specific utilisation of larger particle sizes (5 cm and 10 cm) for midden construction and smaller particles (1 cm) were incorporated belowground. Recommendations from these findings highlight that in a no-till agroecosystem, a mixture of organic matter particle size should be applied to support the development of *L. terrestris* populations.

The effects of earthworm and organic matter interactions on the macro and micronutrient dynamics in the drilosphere soil and within earthworm casts was explored in Sections 4.5, 6.4 and 7. Under controlled laboratory conditions there were no significant differences in the soil chemistry between soil surrounding the burrow and control soil. Previous experiments by Tiunov and Scheu (1999), Schmidt et al, (2001) and Andriuzzi et al, (2013) determined differences, however the total experimental time was longer. There were no differences in soil chemistry with increasing depth of the burrow, even though there were increased masses of surface organic matter located in the upper soil layers. Analysis of fresh *L. terrestris* casts indicated that there were increased concentrations of macro- and micronutrients compared with control and burrow soil, therefore it could be possible that casting has more of an effect on nutrient dispersal than burrowing effects. This supported findings by van Groenigen et al (2019) and Vos et al (2019) where an added number of micronutrients were analysed in this research. On average, the increased availability of nutrients within *L. terrestris* casts was higher for selected elements than van Groenigen et al (2019) (van Groenigen meta analysis: 40-241%; *L. terrestris* casts in Chapter 9: 9-500%) The experiments were conducted over a short-term period (8-12 weeks); future experiments could explore the effects of *L. terrestris* drilosphere on nutrient dispersal over longer time periods. The biogeochemical cycling of soil mediated by earthworm activity is a complex area of research which is still developing. Further analysis of casts in agroecosystems is required to model effects of organic matter type on earthworm decomposition. Further exploration of the effects of organic matter type, earthworm age, physical cast structure and the microbiological activity within earthworm casts may provide further insight into the dynamics of nutrient availability in earthworm casting and quantify the relative cast fertility (RCF).

These studies demonstrated that in no-till agroecosystems with waste organic matter applications, anecic earthworms such as *L. terrestris* can have significant benefits on the decomposition of organic matter. Effects of particle size and organic matter type can enhance the rate of bioturbation by earthworms, however the evidence presented in this thesis suggests that *L. terrestris* populations, which incorporated the most waste, utilised larger particle sizes for their middens, therefore a uniform particle size may inhibit their midden/burrow complex and bioturbation behaviour.

9.2.1. The effects of waste organic matter addition and earthworm activity on bioturbation

There were specific effects of waste organic matter type on the growth rate of earthworm populations, where horse manure was the most efficient feed (Chapter 5). This supported findings from other studies (Butt, 2011; Euteneuer et al, 2020; Butt et al, 2020). Horse manure, anaerobic digestate (dry fraction) and wheat straw all increased earthworm biomass, however there were significant differences between the three treatments, where horse manure led to the highest increase in growth and reproduction of all species. Applications of all three treatments within no-till agroecosystems could enhance earthworm populations and their impact on soil functioning. Further investigations into the application method of waste in agroecosystems could provide information to farmers in how to use the waste effectively to support earthworm populations, particularly for anaerobic digestate, where liquid and solid fractions had differing effects.

The mass of organic matter incorporated over time was significantly higher under *L. terrestris* populations (Sections 6.2 and 6.4), which encouraged later experiments to focus on the interactions between these species and straw residue applications (Chapters 7 and 8). The presence of *L. terrestris* populations in agroecosystems could enhance the removal of surface applied organic matter by up to 5% over a growing season and facilitate the growth of juvenile earthworm populations, enhancing bioturbation. However, straw applications may reduce the population growth rate and reproduction of these species, so it is recommended that mixtures of waste organic matter may better sustain populations in agroecosystems, as in Stroud et al, (2016a). Inter specific interactions between *L. terrestris* and *A. caliginosa* indicated a higher removal rate under monocultures, therefore the species richness in the field could impact the bioturbation in addition to the organic matter type. Prior to organic matter application, completion of an earthworm survey alongside an accurate soil assessment could enable applications to be further tailored towards soil functioning needs of agroecosystems is encouraged.

The removal of straw through residue burial by *L. terrestris* casting was considered (Chapter 8). Added to the accelerated removal of straw biomass to the belowground ecosystem under *L. terrestris*, increased casting caused a higher amount of soil in straw under high density *L. terrestris* populations (Chapter 8). Added to organic matter burial through casting, an associated increase in soil microorganisms present within *L. terrestris* casts could accelerate the degradation of surface organic matter (Aira et al, 2009). There was no observed effect of straw degradation level on selection and utilisation by *L. terrestris* in monocultures (Section 7.3), however due to experimental conditions further analysis is required to determine the palatability of residues on the selection by *L. terrestris*. Investigations of the interactions between microorganisms within *L. terrestris* casts and the decomposition of surface applied straw would provide evidence of how these species could influence aboveground decomposition.

Although *A. caliginosa* incorporated a lower mass of organic matter when compared with *L. terrestris*, there was evidence to indicate their effects on belowground incorporation of organic matter (Section 6.4; Chapter 8). Due to the increased density of *A. caliginosa* in agroecosystems (Chapter 4 and literature evidence) and the influence of casting on residue burial and decomposition (Chapter 8), straw removal by these species could be significant in agroecosystem settings. Although the interactions between *L. terrestris* and juvenile earthworm abundances was noted (Section 4.5), interspecific competition was observed between adult populations (Section 6.3). In low organic matter soils, the density of *L. terrestris* populations could increase the SOM concentration in the belowground ecosystem, which may facilitate *A. caliginosa*. This relationship could benefit from further exploration with a higher density of *L. terrestris*.

9.2.2. The effects of particle size and earthworm utilisation

This research indicated that there were significant differences in the selection, utilisation, feeding and bioturbation of organic matter with different particle sizes. The main waste organic matter utilised to determine the effects of particle size was cereal straw, due to residues being a popular organic matter application, a resource which is easily available, with uncertain effects on earthworm growth and utilisation. A selection of laboratory investigations highlighted how cereal residue particle size had a significant effect on utilisation by earthworm populations, where it was confirmed that monocultures of *L. terrestris* and *A. caliginosa* preferred 1 mm particle size of straw compared with 1 cm (Section 6.3). Under modal particle sizes of wheat straw applied in the field (1 cm, 20 cm and 40 cm) there were significant differences on rate of removal by *L. terrestris*, where 1 cm particle size was incorporated most rapidly. The low mass incorporated (< 1 g) under multi-species treatments (*L. terrestris* N=1; *A. caliginosa* N=3) indicated that inter-specific competition may be a limiting factor in organic matter incorporation, regardless of particle size application. With the effect of particle size being accelerated under smaller biomass individuals, 1 cm particle size and larger is not a recommended treatment for *A. caliginosa*.

Further investigations of organic matter utilisation by *L. terrestris* determined that larger particle sizes (10 cm and 5 cm) were utilised for midden development and smaller particle sizes (1 cm) for incorporation belowground (Chapter 7). Analysis of the interactions between *L. terrestris* populations and organic matter particle size on the depth of straw incorporation belowground indicated that smaller particle sizes can be incorporated deeper into the burrow (Section 6.4; Chapter 7). Further analysis of organic matter biomass with increasing depth under *L. terrestris* indicated that 63.3 % of incorporated straw was deposited within the upper (0-60 mm) layer of the soil profile, with a reduction in mass with increasing burrow depth (Chapter 7). Presence of organic matter within the upper layer of a soil profile could stimulate bacterial communities, which could further increase decomposition and mineralisation rates. Future investigations could extend the exploration of organic matter particle size on the aboveground utilisation by earthworms, which is less widely studied and could have unique effects on organic matter decomposition and the soil biological community within agroecosystems.

These experiments have highlighted that for *L. terrestris*, mixed particle size was the best application to benefit both their growth and habitat development. *L. terrestris* can utilise all particle sizes of straw, by manipulation of larger particle sizes as feed and midden development. Although milled and 1 cm applications may increase the growth rate and fecundity of *L. terrestris* populations, the benefits of their burrow-midden complex to other earthworm populations (Section 4.5) and the regulation of moisture within the burrow indicated that organic material that is larger is also useful to this species.

9.2.3. The effects of interactions on soil nutrient quality

The effects of earthworm bioturbation on the soil macro and micronutrient concentration were explored in field surveys (Section 4.5) and laboratory experiments (Chapters 6 and 7). In the field, analysis of the total nutrient concentration between drilosphere, soil below the midden and non-midden soil indicated that lower concentrations of K and P were found under earthworm mediated soil. However, the concentration of available Mg and K were higher in soil under middens compared with non-midden soil. It was suggested that the presence of casts within middens may have increased available nutrient concentration, where previous research had indicated the increase of soil organic matter held within *L. terrestris* casts.

Analysis of *L. terrestris* casts conducted in Chapter 7 showed the available nutrient concentration was significantly higher in *L. terrestris* casts for 6 elements (Mg, K, Na, Fe, Al and Cu). There were no significant differences in nutrient or carbon concentration between the burrow soil with non-burrow soil, which highlighted that the effect of earthworms on the nutrient concentration in soil is cast-related. The burrowing behaviour of earthworms and where their casts are deposited (e.g. *L. terrestris* deposit on the surface, *A. caliginosa* within the burrow and the soil surface) could determine the nutrient dispersal within the soil profile. Further characterization of the physical, biological and chemical properties of earthworm casts under different waste organic matter applications could determine the effects of their transport and decomposition on the nutrient availability in agroecosystems, which could be used to support agroecosystem management.

Effects of earthworm burrow depth and soil nutrient concentration, analysed in Chapters 6 and 7, showed there were significant differences in the mass of organic matter at different depths, where 63% straw was located in the first 60 mm, but there were no differences in the nutrient concentrations in the burrow-related soil. Laboratory experiments were short-term and further analysis of nutrient dynamics following long-term bioturbation and assimilation by *L. terrestris* under more controlled conditions may provide further valuable information on drilosphere effects.

9.3. Implications for no-till agroecosystems

Results have provided evidence that applications of horse manure, anaerobic digestate (dry fraction) and cereal straw residues will sustain earthworm populations, where *A. longa*, *L. terrestris*, *A. caliginosa* and *A. chlorotica* increased in biomass under these feeds (Chapter 5). Further investigations are required into the applications of CGW and anaerobic digestate (liquid fraction), as although previous investigations highlighted their utilisation as a soil conditioner (enhancing crop growth), when applied to *A. longa* populations there was a significant reduction in biomass. This is supported in the literature (Section 2.3), where mixed effects of these conditioners on earthworm populations were determined. This could affect future soil health in no-till agroecosystems, which may rely on the soil fauna to decompose organic matter.

Applications under a milled particle size organic matter could accelerate earthworm growth, where the differences between organic matter types (AD and HM) observed in growth experiments were not replicated under milled treatments and the impact on bioturbation was not significant (Section 6.1). However, surface applications of milled organic matter in the field could be prone to erosion, which would result in organic matter and nutrient losses. The energy required to environmentally mill organic matter on a large scale may not be beneficial. *L. terrestris* populations can use organic matter of larger particle sizes, so their presence in no-till agroecosystems is important to begin the utilisation of residues and to make these bioavailable to other species. Future laboratory experiments of organic matter effects on *L. terrestris* should consider application of mixed particle size residues to provide a more accurate representation of their habitat requirements.

Increased abundance of *L. terrestris* populations in no-till agroecosystems will have a significant effect on the increase in SOM addition belowground through accelerated bioturbation and the increase in soil addition aboveground through increased casting. This could increase macro- and micronutrient concentration on the soil surface and facilitate decomposition of residues. Although milled applications of organic matter may enhance the growth of these populations, a mixed particle size should be applied to encourage midden development. The increased bioturbation over time by high density *L. terrestris* population could be limited by factors such as organic matter nutrition, where cereal residues reduced earthworm growth and reproductive rates compared to horse manures. Mixtures of residues could be applied *in situ* to sustain earthworm biomass and maximise bioturbation effects.

The temporal effects of organic matter application can have significant effects on earthworm bioturbation rates, e.g. in Finland a later harvest caused by a wetter summer period caused later application of residues in autumn, which reduced the potential earthworm activity period. The effects of climate change causing wetter summers e.g. in the UK could have implications on future harvest times and organic matter applications, which may have significant effects on the SOM incorporated mediated by earthworms. This may also cause a reduction in earthworm populations in future seasons. Harvest times vary dependent upon crop and weather conditions, however future experiments could explore the effects of harvest timing on earthworm population development and organic matter removal rates.

Findings from this research project have provided unique observations of the behaviour of *L. terrestris* aboveground, a concept of organic matter decomposition which is often overlooked in laboratory investigations of earthworm effects on soil properties. *L. terrestris* behaviour aboveground could support further earthworm and biological activity, which may stimulate soil development. The spatial patchiness of organic matter aboveground created by increased midden presence may also enhance soil resilience, which may be vital to mitigate future environmental challenges caused by climate change and soil degradation. Evidence of *L. terrestris* behaviour presented in this thesis could be used to support further conceptual modelling of *L. terrestris*, as by Johnston et al, (2018) (Figure 9.1), to improve understanding of how changes to agroecosystem management might influence a change in *L. terrestris* community dynamics.

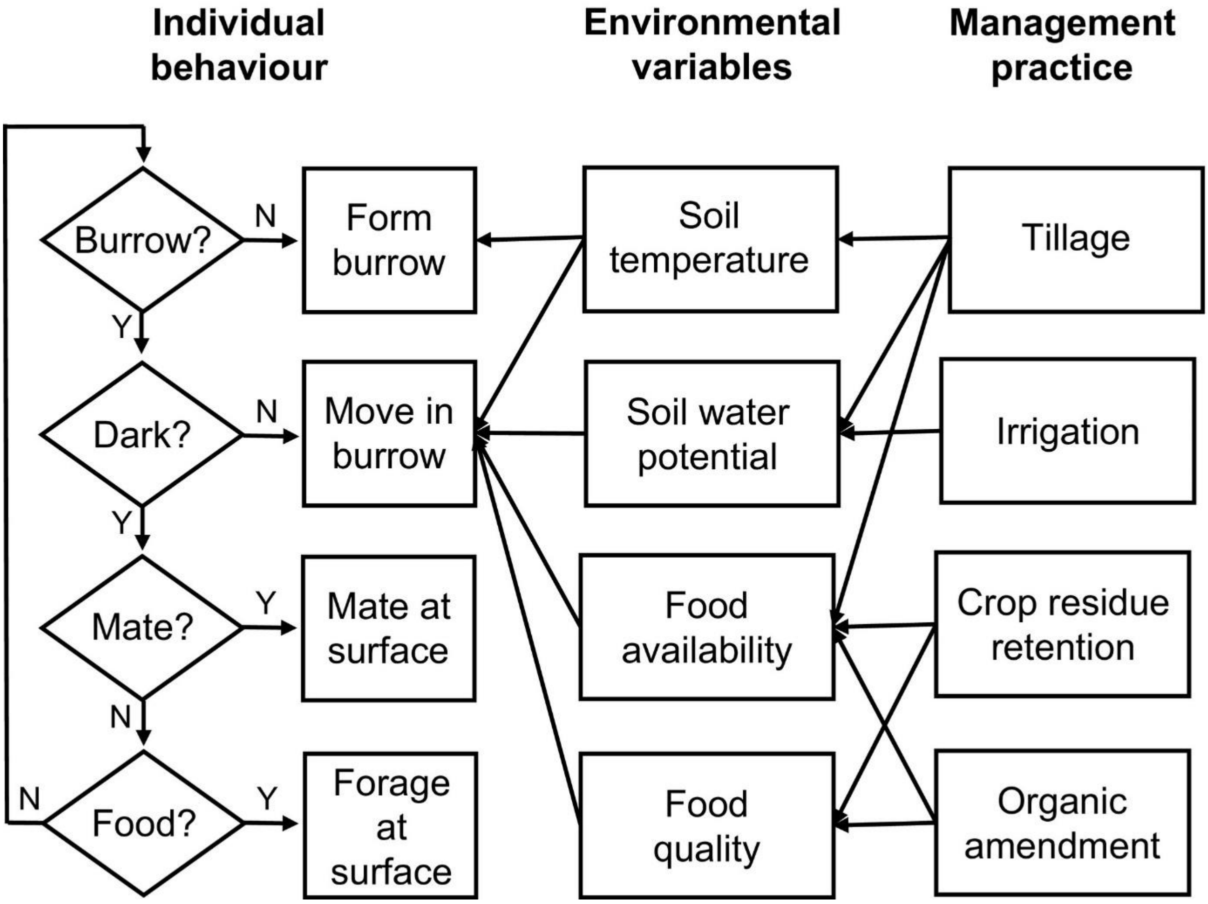


Figure 9.1. Conceptual model of the individual behaviour of *L. terrestris* per hourly time step in EEEworm model (Johnston et al, 2018).

Modelling *L. terrestris* behaviour has enabled the quantification of its behavioural responses to environmental management practices in agroecosystems (Figure 9.1). The results within this thesis could be added to this model to increase the accuracy of behavioural responses to organic amendments and crop residue retention. There are also a number of new behavioural responses determined in this thesis which could be added to this model, where the relationship between food quality (particle size) on surface foraging has been examined. Inclusion of midden formation as a variable within the "form burrow" section of the model may also require the connection of more environmental variables, where it was evident in Chapter 7 that organic matter structure had a significant impact on how *L. terrestris* utilises it within the midden-burrow complex.

9.4. Contributions to knowledge

This research has contributed the following to knowledge:

1. A smaller organic matter particle size can increase the rate of earthworm population development, increase decomposition and reduce the effects of organic matter type on earthworm growth rates.
2. Of the most abundant UK earthworm species, *L. terrestris* can incorporate the largest masses of waste organic matter into belowground ecosystems, where a significant effect of density on the rate of straw residue incorporation was recorded in the field.
3. Web camera analysis of *L. terrestris* aboveground behaviour identified that selection of organic matter aboveground is not limited by particle size, where increased activity occurred with larger particle sizes (10 cm).
4. *L. terrestris* have the potential to utilise larger particle sizes than other earthworms and in no-till agroecosystems this could promote a wider earthworm species richness, as their middens are hotspots for other juvenile earthworms.
5. Size dependant use of waste organic matter by *L. terrestris* has been recorded; smaller particles are significant for growth and larger particle sizes can influence midden development and hence earthworm community structure.

9.5. Limitations

Whilst this thesis effectively addressed the research aims defined at the outset, there were certain limitations that impacted upon the scope of the investigations that could be undertaken. For example, time limitations on laboratory and field experiments meant that long-term trends could not be identified, which would have yielded results which could have indicated e.g. the effects of harvest time on the bioturbation rate of straw by *L. terrestris* populations.

This research project utilised numerous fields to determine the effects of waste organic matter applications on earthworm populations and the effects of *L. terrestris* density on the decomposition of cereal residues. Increased availability of no-till agroecosystems for research could have provided the opportunity to analyse the effects of earthworms and waste organic matter applications on crop growth. Although resources meant that it was not possible to analyse the effects of earthworm populations directly on crop growth, findings from interactions between earthworms and waste organic matter on soil properties have provided a thorough basis for future experiments.

The soil nutrient analysis conducted in this study was chosen based on the resources available at UCLan. Other chemical analysis could have been conducted, such as soil nitrate and phosphate concentration, which would have provided more chemical data related to crop nutrient availability. Chemical exploration of the organic matter in Chapter 5 (such as metal and ammonia analysis) may have discovered why liquid anaerobic digestate was toxic and provided evidence to explain why horse manure was the most effective feed. The evidence in this thesis provided qualitative information of nutrient properties. Future experiments could analyse nutrient dispersal based on isotopic chemical analysis, which would provide a more targeted approach.

There could be some criticism of the accuracy of recording waste organic matter incorporation without the use of litter bags for the controlled experiments in Sections 6.2 and 6.4, and in the field research in Finland (Chapter 8). The use of litter bags may have made mass determination of organic matter more accurate and the methodology simpler, however following a trial, the selection behaviour of surface feeding earthworms and their midden creation was affected. Earthworm surface behaviour was an important component of this research project, so it was decided to apply straw without litter bags. Future experiments could be conducted to determine differences in mass determination between methods.

There are many waste organic amendments applied in agroecosystem settings to increase crop production. Here, there were limitations on the amendments used based on resource availability, the amount of application required and the consistency of materials. For example, following initial experiments of Chapters 4-6, anaerobic digestate was investigated no further, due to inconsistency of inputs between farms.

Spatial and temporal implications of some field and laboratory experiments within this thesis resulted in a low number of replicates. An increased number of replicates would improve the likelihood of statistically significant differences. If some of the experiments within this thesis were extended, this may provide further data to address hypotheses.

9.6. Future research

To build on the findings and limitations of the research presented in this thesis, the following suggestions are made regarding future investigations which would be of merit:

1. Investigations into the effects of temporal applications of waste organic matter on earthworm population development in no-till cereal agroecosystems.
2. In depth physical, chemical and biological analysis of earthworm casts and their erosion properties under different organic matter treatments to provide information on nutrient dispersal into soil and C sequestration.
3. Further exploration of the effects of harvest time and resulting organic matter applications on earthworm population activity to determine impacts on bioturbation and soil properties.
4. Experiments recording *L. terrestris* aboveground (infra-red) and belowground activity (X-ray tomography) *in-situ* to determine the movement of organic matter between sub-components of ecosystems.
5. Long-term laboratory and field experiments analysing the effects of earthworm and waste organic matter interactions on the macro- and micronutrient concentration in soils.

REFERENCES

Abail, Z., Sampedro, L., Whalen, J., (2017). Short-term carbon mineralization from endogeic earthworm casts as influenced by properties of the ingested soil material. *Applied Soil Ecology*, **116**, 79–86.

Abail, Z., Whalen, J. K., (2018) Corn residue inputs influence earthworm population dynamics in a no-till corn-soybean roatation. *Applied Soil Ecology*, **127**, 120-128.

Abbasi, M., Khaliq, A., (2016). Nitrogen mineralization of a loam soil supplemented with organic-inorganic amendments under laboratory incubation. *Frontiers in Plant Science*, **7**, 1038.

Adamczyk, B., Sietiö, O-M., Straková, P., Pommer, J., Wild, B., Hagner, M., Pihlatie, M., Fritze, H., Richter, A., Heinonsalo, J., (2019) Plant roots increase both decomposition and stable organic matter formation in boreal forest soil. *Nature Communications*, **10:3982**, 1-9.

Aga, D. S., Lenczewski, M., Snow, D. Muurinen, J., Sallach, B. J., Wallace, J. S., (2016). Challenges in the Measurement of Antibiotics and in Evaluating Their Impacts in Agroecosystems: A Critical Review. *Journal of Environmental Quality*, **45:2**, 407–419.

Agapit, C., Gigon, A., Puga-Freitas, R., Zeller, B., Blouin, M. (2018) Plant-earthworm interactions: influence of age and proportion of casts in soil on plant growth, morphology and nitrogen uptake. *Plant Soil*, **424**, 49-61.

Aguilera, E., Lassaletta, L., Sanz-Cobena, A., Garnier, J., Vallejo, A., (2013) The potential of organic fertilizers and water management to reduce N_2O emissions in Mediterranean climate cropping systems. A review. *Agriculture, Ecosystems and Environment*. **164**, 32–52.

Aira, M., Monroy, F., Domínguez, J., (2005) Ageing effects on nitrogen dynamics and enzyme activities in casts of *Aporrectodea caliginosa* (Lumbricidae). *Pedobiologia*, **49:5**, 467–473.

Aira, M., Sampedro, L., Monroy, F., Domínguez, J., (2008) Detritivorous earthworms directly modify the structure, thus altering the functioning of a microdecomposer food web. *Soil Biology and Biochemistry*. **40:10**, 2511–2516.

Aira, M., Mcnamara, N. P., Piearce, T. G., Domínguez, J., (2009) Microbial communities of *Lumbricus terrestris* L. middens: structure, activity and changes through time in relation to earthworm presence. *Journal of Soils and Sediments*, **9:1,** 54-61.

Aira, M., Pérez-Losada, M., Domínguez, J., (2019) Microbiome dynamics during cast ageing in the earthworm *Aporrectodea caliginosa*. *Applied Soil Ecology*, **139**, 56–63.

Aitken, M. N., Evans, B., Lewis, L. G., (1998) Effect of applying paper mill sludge to arable land on soil fertility and crop yields. *Soil Use Management*, **14**, 215-222.

Alaoui, A., Rogger, M., Peth, S., Blöschl, G., (2018) Does soil compaction increase floods? A review. *Journal of Hydrology*, **557**, 631-642.

Alakukku, L., Ristolainen, A., Salo, T., (2009) Grain and nutrient balance of spring cereals in different tillage systems. ISTRO Conf., Izmir, Turkey, Paper T6-004, 1-4.

Alburqueque, J. A., de la Fuente, C., Bernal, M. P., (2012) Chemical properties of anaerobic digestates affecting C and N dynamics in amended soils. *Agriculture, Ecosystems and Environment*, **160**, 15-22.

Allen, B. L., Mallarino, A. P., (2008) Effect of liquid swine manure rate, incorporation, and timing of rainfall on phosphorus loss with surface runoff. *Journal of Environmental Quality*, **37**, 125-137.

Allison, R., (2015) Crack soil compaction to help raise crop yields. *Farmers Weekly,* **164:13**, 60-61.

Al-Maliki, S., Scullion, J., (2013) Interactions between earthworms and residues of differing quality affecting aggregate stability and microbial dynamics. *Applied Soil Ecology*, **64**, 56-62.

Ambus, P., Jensen, E. S., (1999) Nitrogen mineralization and denitrification as influenced by crop residue particle size. *Plant and Soil*, **197**, 261-270.

Andersson, R. L., (2016) Suppressing weed growth after harvest with underseeded red clover in organic farming. *Renewable Agriculture and Food Systems*, **31:3**, 185-190.

Andrés, P., Mateos, E., Tarrasón, D., Cabrera, C., Figuerola, B., (2011) Effects of digested, composted, and thermally dried sewage sludge on soil microbiota and mesofauna. *Applied Soil Ecology*, **48:2**, 236-242.

Andriamananjara, A., Rabehariosa, L., Prud'homme, L., Morel, C., (2016) Drivers of plant-availability of phosphorus from thermally conditioned sewage sludge as assessed by isotopic labelling. *Frontiers in Nutrition,* **3:19**: doi: 10.3389/fnut.2016.00019.

Andriuzzi, W. S., Bolger, T., Schmidt, O., (2013) The drilosphere concept: fine-scale incorporation of surface residue-derived N and C around natural *Lumbricus terrestris* burrows. *Soil Biology and Biochemistry*, **64**, 136-138.

Andriuzzi, W. S., Pulleman, M. M., Schmidt, O., Faber, J. H., Brussaard, L., (2015a) Anecic earthworms (*Lumbricus terrestris*) alleviate negative effects of extreme rainfall on soil and plants in field mesocosms. *Plant and Soil*, **397**, 103-113.

Andriuzzi, W. S., Ngo, P-T., Geisen, S., Keith, A. M., Dumack, K., Bolger, T., Bonkowski, M., Brussaard, L., Faber, J. H., Chabbi, A., Rumpel, C., Schmidt, O., (2015b) Organic matter composition and the protist and nematode communities around anecic earthworm burrows. *Biology and Fertility of Soils*, **52:1**, 91–100.

Andriuzzi, W. S., Schmidt, O., Brussaard, L., Bolger, T., (2016) Earthworm functional traits and interspecific interactions affect plan nitrogen acquisition and primary production. *Applied Soil Ecology*, **104**, 148-156.

Andruschkewitch, R., Geisseler, D., Dultz, S., Joergensen, R-G., Ludwig, B., (2014) Rate of soil-aggregate formatin under different organic matter amendments – a short term incubation experiment. *Journal of Plant Nutrition and Soil Science*, **177**, 297-306.

Angers, D. A., Recous, S., (1997) Decomposition of wheat straw residues as affected by particle size. *Plant and Soil*, **189**, 197-203.

Angst, Š., Mueller, C. W., Cajthaml, T., Angst, G., Lhotáková, Z., Bartuška, M., Špaldoňová, A., Frouz, J., (2017) Stabilization of soil organic matter by earthworms is connected with physical protection rather than with chemical changes in soil organic matter. *Geoderma*, **289**, 29-35.

Annabi, M., Le Bissonnais, Y., Le Villio-Poitrenaud, M., Houot, S., (2011) Improvement of soil aggregate stability by repeated applications of organic amendments to a cultivated loamy soil. *Agriculture, Ecosystems and Environment*, **144**, 382-389.

Arai, M., Tayasu, I., Komatsuzaki, M., Uchida, M., Shibata, Y., Kaneko, N., (2013) Changes in soil aggregate carbon dynamics under no-tillage with respect to earthworm biomass revealed by radiocarbon analysis. *Soil and Tillage Research*, **126**, 42-49.

Armador, J. A., Görres, J. H., Savin, M. C., (2005) Role of soil water content in the carbon and nitrogen dynamics of *Lumbricus terrestris* L. burrow soil. *Applied Soil Ecology*, **28:1**, 15-22.

Armand, R., Bockstaller, C., Auzat, A-V., Van Dijk, P., (2009) Runoff generation related to intra-field soil surface characteristics variability. Application to conservation tillage context. *Soil and Tillage Research*, **102**, 27-37.

Arvidsson, J., Etana, A., Rydberg, T., (2014) Crop yield in Swedish experiments with shallow tillage and no-tillage 1983-2012. *European Journal of Agronomy*, **52**, 307-315.

Ashwood, F., Butt, K. R., Doick, K. J., Vanguelova, E. I., (2017a) Interactive effects of composted green waste and earthworm activity on tree growth and reclaimed soil quality: a mesocosm experiment. *Applied Soil Ecology*, **119**, 226-233.

Ashwood, F., Butt, K. R., Doick, K. J., Vanguelova, E. I., (2017b) Investigating tree foliar preference by the earthworms *Apporectodea longa* and *Allollobophora chlorotica* in reclaimed loam soil. *Applied Soil Ecology*, **110**, 109-117.

Ashworth. A. J., Allen, F. L., Tyler, D. D., Pote, D. H., Shipitalo, M. J., (2017) Earthworm populations are affected by long-term crop sequences and bio-covers under no-tillage. *Pedobiologia*, **60**, 27-33.

Athmann, M., Kautz, T., Banfield, C., Bauke, S., Hoang, D. T. T., Lüsebrink, M., Pausch, J., Amelung, W., Kuzyakov, Y., Köpke, U., (2017) Six months of *Lumbricus terrestris* L. activity in root-formed biopores increases nutrient availability, microbial biomass and enzyme activity. *Applied Soil Ecology*, **120**, 135-142.

Atwood, L. W., Mortensen, D. A., Koide, R. T., Smith, R. G., (2018) Evidence of multi-trophic effects of pesticide seed treatments on non-targeted soil fauna. *Soil Biology and Biogeochemistry*, **125**, 144-155.

Auer, A., Burgt, N. H. V., Abram, F., Barry, G., Fenton, O., Markey, B. K., Nolan, S., Richards, K., Bolton, D., de Waal, T., Gordon, S. V., O'Flaherty, V., Whyte, P., Zintl, A., (2017) Agricultural anaerobic digestion power plants in Germany and Ireland: policy and practice. *Journal of Science, Food and Agriculture*, **97**, 719-723.

Bacher, M. G., Fenton, O., Bondi, G., Creamer, R. E., Karmarkar, M., Schmidt, O., (2018). The impact of cattle dung pats on earthworm distribution in grazed pastures. *BMC Ecology*, **18:1***,* 59–59.

Bachmann, S., Gropp, M., Eichler-Löbermann, B., (2014) Phosphorus availability and soil microbial activity in a 3 year field experiment amended with digested dairy slurry. *Biomass and Bioenergy*, **70**, 429-439.

Baldivieso-Freitas, P., Blanco-Moreno, J. M., Gutiérrez-López, M., Peigné, J., Pérez-ferrer, A., Trigo-Aza, D., Sans, F. X., (2018) Earthworm abundance response to conservation agriculture practices in organic arable farming under Mediterranean climate. *Pedobiologia*, **66**, 58-64.

Ball, D. F., (1964) Loss on Ignition as an estimate of organic matter and organic carbon in non-calcareous soils. *Journal of Soil Science*, **15:1**, 84-92.

Ball, B. C., Scott, A., Parker, J. P., (1999) Field N_2O, CO_2 and CH_4 fluxes in relation to tillage, compaction and soil quality in Scotland. *Soil and Tillage Research*, **52**, 29-39.

Bardgett, R. D., (2005) The Biology of Soil: a Community and Ecosystem Approach. Oxford University Press, Oxford.

Bardgett, R. D., Campbell, C. D., Emell, B. A., Jenkins, A., Whitmore, A. P., (2011) Supporting services. UK National Ecosystem Assessment Technical Report, 499-533. UK National Ecosystem Assessment, UNEP-WCMC, Cambridge.

Barnes, A. D., Scherber, C., Brose, U., Borer, E. T., Ebeling, A., Gauzens, B., Giling, D. P., Hines, J., Isbell, F., Ristok, C., Tilman, D., Weisser, W. W., Eisenhauer, N., (2020) Biodiversity enhances the multitrophic control of arthropod herbivory. *Science Advances*. **6**, eabb6603.

Barnett, C. M., Bengough, A. G., McKenzie, B. M., (2009) Quantitative image analysis of earthworm-mediated soil displacement. *Biology and Fertility of Soils*, **45**, 821-828.

Barrera, I., Andrés, P., Alcañiz, J. M., (2001) Sewage sludge application on soil: effects on two earthworm species. *Water, Air and Soil Pollution*, **129**, 319-332.

Bart, S., Amossé, J., Lowe, C. N., Mougin, C., Péry, A. R. R., Pelosi, C., (2018) *Aporrectodea caliginosa*, a relevant earthworm species for a posteriori pesticide risk assessment: current knowledge and recommendations for culture and experimental design. *Environmental Science and Pollution Research,* **25**, 33867–33881.

Basche, A. D., Kaspar, T. C., Archontoulis, S. V., Jaynes, D. B., Sauer, T. J., Parkin, T. B., Miiguez, F. E., (2016) Soil water improvements with the long-term use of a winter rye cover crop. *Agricultural Water Management*, **172**, 40-50.

Beare, M. H., Parmelee, R. W., Hendrix, P. F., Cheng, W., Coleman, D. C., Crossley, D. A., (1992) Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. *Ecological Monographs*, **62**, 569-591.

Bending, G. D., Turner, M. K., Jones, J. E., (2002) Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. *Soil Biology and Biogeochemistry*, **34**, 1073-1082.

Bennetzen, E. H., Smith, P., Porter, J. R., (2016) Decoupling of greenhouse gas emissions from global agricultural production: 1970-2050. *Global Change Biology*, **22:2**, 763-781.

Bergström, L., Brink, N., (1986) Effects of differentiated applications of fertilizer N on leaching losses and distribution of inorganic N in the soil. *Plant and Soil*, **93**, 333-345.

Berisso, F. E., Schjønning, P., Keller, T., Lamandé, M., Etana, A., de Jonge, L. W., Iversen, B. V., Arvidsson, J., Forkman, J., (2012) Persistent effects of soil compaction on pore size distribution and gas transport in loamy soil. *Soil Tillage and Research*, **122**, 42-51.

Berman, D., Meshcheryakova, E., (2013) Ranges and cold hardiness of two earthworm subspecies (*Eisenia nordenskioldi*, Lumbricidae, Oligochaeta). *Biology Bulletin*, **40:9**, 719–727.

Bernaud, P., Anderson, K., Evans, M., Farrow, L., Glendell, M., James, M. R., Quine, T. A., Quinton, J. N., Rawlins, B., Jane Rickson, R., Brazier, R. E., (2020) National-scale geodata describe widespread accelerated soil erosion. *Geoderma*, **371**, 114378.

Berruti, A., Lumini, E., Ballestrini, R., Bianciotto, V., (2016) Arbuscular mycorrhizal fungi as natural biofertilizers: lets benefit from past successes. *Frontiers in Microbiology*, **6**, 1559.

Berry, E. C., Jordan, D., (2001). Temperature and soil moisture content effects on the growth of *Lumbricus terrestris* (Oligochaeta: Lumbricidae) under laboratory conditions. *Soil Biology & Biochemistry*, **33:1**, 133–136.

Bertrand, M., Barot, S., Blouin, M., Whalen, J., de Oliveira, T., Roger-Estrade, J. (2015a). Earthworm services for cropping systems. A review. *Agronomy for Sustainable Development*, **35:2**, 553–567.

Bertrand, M., Blouin, M., Barot, S., Charlier, A., Marchand, D., Reger-Estrade, J., (2015b). Biocontrol of eyespot disease on two winter wheat cultivars by an anecic earthworm (*Lumbricus terrestris*). *Applied Soil Ecology,* **96**, 33–41.

Beylich, A., Oberholzer, H-R., Schrader, S., Höper, H., Wilke, B-M., (2010) Evaluation of soil compaction effects on soil biota and soil biological processes in soils. *Soil and Tillage Research*, **109**, 133-143.

Bhoghal, A., Williams, J. R., Nicholson, F. A., Chadwick, D. R., Chambers, K. H., Chambers, B. J., (2016) mineralization of organic nitrogen from farm manure applications. *Soil Use and Management*, **32:S1**, 32-43.

Bicki, T. J., Guo. L., (1991) Tillage and simulated rainfall intensity effect on bromide movement in an argiudoll. *Soil Science Society of America Journal,* **55**, 794-799.

Bigorgne, E, Cossou-Liguille, C., Bonnard, M., Nahmani, J., (2010) Genotoxic effects of nickel, trivalent and hexavalent chromium on the *Eisenia fetida* earthworm. *Chemosphere*, **80:9**, 1109-1112.

Bilialis, D., Tzortzi, I., Vavoulidou, E., Karkanis, A., Emmanouel, N., Efthimiadou, A., Katsenios, N., Patsiali, S., Dellaporta, L., (2013) Effects of aluminium and moisture levels on aluminium bioaccumulation and protein content on the earthworm *Octadrilus complanatus*. *Journal of Soil Science and Plant Nutrition*, **13:4**, 845-854.

Bingeman, C. W., Varner, J. E., Martin, W. P., (1953) The effect of the addition of organic materials on the decomposition of an organic soil. *Soil Science Society of America Journal*, **17**, 34-38.

Binet, F., Le Bayon, R. C., (1999) Space-time dynamics in situ of earthworm casts under temperate cultivated soils. *Soil Biology and Biochemistry,* **31:1**, 85-93.

Biot, Y., Liu, Y. Y., (1995) Loss of yield caused by soil erosion on sandy soils in the UK. *Soil Use and Management*, **11:4**, 157-162.

Bityutskii, N.P., Kaidun, P.I., Yakkonen, K.L., (2012) The earthworm (*Aporrectodea caliginosa*) primes the release of mobile and available micronutrients in soil. *Pedobiologia*, **55**, 93-99.

Bityutskii, N., Kaidun, P., Yakkonen, K., (2016) Can earthworms alleviate nutrient disorders of plants subjected to calcium carbonate excess? *Applied Soil Ecology*, **98**, 20-29.

Blackshaw, R. E., Andersson, R. L., Lemerle, D., Chapter 3. Cultural weed management. Pages 35–48 in M. K. Upadhyaya and R. E. Blackshaw, eds. Non-Chemical Weed Management: Principles, Concepts and Technology, Wallingford, UK: C.

Blair, R. M., Savin, M. C., Chen. P., (2014) 'Composted and formulated poultry litters promote soil nutrient availability but not plant uptake or edamame quality', *Agronomy for sustainable development*, **34:4**, 849–856.

Blanchart, E., Albrecht, A., Brown, G., Decaëns, T., Duboisset, A., Lavelle, P., Mariani, L., Roose, E., (2004) Effects of tropical endogeic earthworms on soil erosion. *Agriculture, Ecosystems and Environment*, **104:2**, 303-315.

Blanco-Canqui, H., Lal, R., (2009) Crop residue removal impacts on soil productivity and environmental quality. *Critical Reviews in Plant Science*, **28:3**, 139-163.

Blanco-Canqui, H., Ruis, S. J., (2018) No-tillage and soil physical environment. *Geoderma*, **326**, 164-200.

Blevins R. L., Frye, W. W., (1993) Conservation tillage: An ecological approach to soil management. *Advances in Agronomy*, **51**, 33–78.

Blouin, M., Hodson, M. E., Delgado, E. A., Baker, G., Brussaard, L., Butt, K. R., Dai, J., Dendooven, L., Peres, G., Tondoh, J. E., Brun, J. -J., (2013) A review of earthworm impact on soil function and ecosystem services. *European Journal of Soil Science*, **64:2**, 161-182.

Bockus, W. W., Shroyer, J. P., (1998) The impact of reduced tillage on soilborne plant pathogens. *Annual Review of Phytopathology*, **36**, 485-500.

Bohan, D. A., Powers, S. J., Champion, G., Haughton, A. J., Hawes, C., Squires, G., Cussans, J., Mertens, S. K., (2011) Modelling rotations: can crop sequences explain arable weed seedbank abundance? *Weed Research*, **51**, 422-432.

Bohlen, P. J., Parmelee, R. W., McCartney, D. A., Edwards, C. A., (1997) Earthworm effects on carbon and nitrogen dynamics of surface litter in corn agroecosystems. *Ecological Applications*, **7:4**, 1341-1349.

Bojórquez-Quintal, E., Escalante-Magaña C., Echevarría-Machado I., Martínez-Estévez M., (2017) Aluminum, a Friend or Foe of Higher Plants in Acid Soils. *Frontiers in Plant Science*, **8**, 1767.

Börgesson, G., Kirchmann, H., Kätterer, T., (2014) Four Swedish long-term field experiments with sewage sludge reveal a limited effect on soil microbes and metal uptake by crops. *Journal of Soils and Sediments*, **14:1**, 164-177.

Börgesson, G., Kätterer, T., (2018) Soil fertility effects of repeated applications of sewage sludge in two 30-year-old field experiments. *Nutrient Cycling in Agroecosystems*, **12**, 369-385.

Boström, U., Löfs-Holmin, A., (1986) Growth of earthworms (*Apporectodea caliginosa*) fed shoots and roots of barley, meadow fescue and lucerne. Studies in relation to particle size, protein, crude fibre content and toxicity. *Pedobiologia*, 29, 1-12.

Botinelli, N., Hallaire, V., Menasseri-Aubry, S., Le Guillou, C., Cluzeau, D., (2010a) Abundance and stability of belowground earthworm casts influenced by tillage intensity and depth. *Soil and Tillage Research*, **103**, 263-267.

Botinelli, N., Hedde, M., Joquet, P., Capowiez, Y., (2020a) An explicit definition of earthworm ecological categories – Marcel Bouché's triangle revisited. *Geoderma*, **372**, 114361.

Bottinelli, N., Henry-des-Tureaux, T., Hallaira, V., Mathieu, J., Benard, Y., Duc Tran, T., Jouquet, P., (2010b) Earthworms accelerate soil porosity turnover under watering conditions. *Geoderma*, **156**, 43-47.

Bottinelli, N., Jouquet, P., Capowiez, Y., Podwojewski, P., Grimaldi, M., Peng, X., (2015) Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? *Soil and Tillage Research*, **146**, 118-124.

Bottinelli, N., Kaupenjohann, M., Märten, M., Jouquet, P., Soucémarianadin, L., Baudin, F., Tran, T., Rumpel, C. (2020b) Age matters: Fate of soil organic matter during ageing of earthworm casts produced by the anecic earthworm *Amynthas khami*. *Soil Biology and Biochemistry*, **148,** 107906–.

Bouché, M.B. (1977) Strategies Lombriciennes. *Ecological Bulletins*, **25**, 122-132.

Bouché, M., Al-Addan, F., (1997). Earthworms, water infiltration and soil stability: Some new assessments. *Soil Biology and Biochemistry*, **29:3**, 441–452.

Boyle, K. E., (1990) The ecology of earthworms (Lumbricidae) in grassland on reclaimed cutover peatland and their impact on soil physical properties and grass yield. Unpublished PhD. Thesis, National University of Ireland. Cited in Edwards, C.A. (ed) Earthworm Ecology. St Lucie Press, Boca Raton, 684-690.

Brankatsch, G., Finkbeiner, M., (2017) Crop rotations and crop residues are relevant parameters for agricultural carbon footprints. *Agronomy for Sustainable Development*, **37:6**, 1-14.

Brar, B. S., Singh, J., Singh, G., Kaur, G., (2015) Effects of long term application of inorganic and organic fertilizers on soil organic carbon and physical properties in maize-wheat rotation. *Agronomy*, **5**, 220-238.

Brennan, E. B., Acosta-Martinez, V., (2017) Cover cropping frequency is the main driver of soil microbial changes during six years of organic vegetable production. *Soil Biology and Biochemistry*, **109**, 188-204.

Briones, M. J., T., Schmidt, O., (2017) Conventional tillage decreases the abundance and biomass of earthworms and alters their community structure in a global meta-analysis. *Global Change Biology*, **23:10**, 4396-4419.

Brown, P. D., Morra, M. J., (1997) Control of soil-borne plant pests using glucosinolate-containing plants. *Advances in Agronomy*, **61**, 167-231.

Brown, P. D., Morra, M. J., Borek, V., (1994) Gas-chromatography of allelochemicals produced during glucosinolate degradation in soil. *Journal of Agricultural Food Chemistry*, **42**, 2029-2034.

Brown, P. H., Welch, R. M., Cary, E. E., Checkai, R. T., (1987) Beneficial effects of nickel on plant growth. *Journal of Plant Nutrition*, **10:9-16**, 2125-2135.

Bruno, J.F., Fridley, J.D., Bromberg, K.D., and Bertness, M.D. (2005) Insights into biotic interactions from studies of species invasions. In Species invasions: insights into ecology, evolution, and biogeography. Edited by D.F. Sax, J.J. Stachowicz, and S.D. Gaines. Sinauer Associates Inc., Sunderland, Mass. pp. 14–40.

Brussaard, L., de Ruiter, P. C., Brown, G. G., (2007) Soil biodiversity for agricultural sustainability. *Agriculture, Ecosystems and Environment*, **121:3**, 233-244.

Bullock, D. G., (1992) Crop Rotation. *Critical Reviews in Plant Sciences*, **11:4**, 309-326.

Burg, V., Bowman, G., Haubensak, M., Baier, U., Thees, O., (2018). Valorization of an untapped resource: Energy and greenhouse gas emissions benefits of converting manure to biogas through anaerobic digestion. *Resources, Conservation and Recycling*, **136**, 53–62.

Butt, K. R. (1991) The effects of temperature on the intensive production of *Lumbricus terrestris*. *Pedobiologia*, **35:4**, 257–264.

Butt, K. R. (1993) Reproduction and growth of three deep burrowing earthworms (Lumbricidae) in laboratory culture in order to assess production for soil restoration. *Biology and Fertility of Soils* **16**, 135-138.

Butt, K. R. (1997) Reproduction and growth of the earthworm *Allolobophora chlorotica* (Savigny, 1826) in controlled environments. *Pedobiologia* **41**, 362-367.

Butt, K. R., (1998) Interactions between selected earthworm species: A preliminary laboratory-based study. *Applied Soil Ecology*, **9:1**, 75-79.

Butt, K. R., (2000) Earthworms of the Malham Tarn Estate (Yorkshire Dales National Park). *Field Studies*, **9**, 701-710.

Butt, K. R. (2002) Depth of cocoon deposition by three earthworm species in mesocosms. *European Journal of Soil Biology*, **38:2**, 151–153.

Butt, K.R., (2011) Food quality affects production in *Lumbricus terrestris (L.)* under controlled environmental conditions. *Soil Biology and Biochemistry*, **43**, 2169-2175.

Butt, K. R., Frederickson, J., and Morris, R. M., (1992) The intensive production of *Lumbricus terrestris* L. for soil amelioration. *Soil Biology and Biochemistry*, **24:12**, 1321–1325.

Butt, K. R., Frederickson, J., and Morris, R. M., (1994) Effect of earthworm density on the growth and reproduction of *Lumbricus terrestris* L. (Oligochaeta: Lumbricidae) in culture. *Pedobiologia*, **38**, 254-261.

Butt, K. R., Frederickson, J., Morris, R. M., (1995) An earthworm cultivation and soil inoculation technique for land restoration. *Ecological Engineering*, **4**, 1-9.

Butt, K. R., Frederickson, J., Morris, R. M., (1997) The earthworm inoculation unit technique: An integrated system for cultivation and soil inoculation of earthworms. *Soil Biology and Biochemistry*, **29:3**, 251-257.

Butt, K. R., and Grigoropoulou, N., (2010) Basic research tools for earthworm ecology. *Applied and Environmental Soil Science*, 1-12.

Butt, K. R., Lowe, C. N., (2007) Presence of earthworm species within and beneath *Lumbricus terrestris* (L.) middens. *European Journal of Soil Biology*, **43:1**, S57-S60.

Butt, K. R., Meliné, C., Pérès, G., (2020) Marine macroalgae as food for earthworms: growth and selection experiments across ecotypes. *Environmental Science and Pollution Research International*, **27:27**, 33493–33499.

Butt, K. R., Nieminen, M. A., Siren, T., Ketoja, E., Nuutinen, V., (2005) Population and behavioural level responses of arable soil earthworms to boardmill sludge application. *Biology and Fertility of Soils*, 42, 163-167.

Butt, K. R., Nuutinen, V., (2005) Dawn of the dew worm. *Biologist*, **52**, 218-223.

Butt, K. R., Nuutinen, V., Sirén, T., (2003) Resource distribution and surface activity of adult *Lumbricus terrestris* L. in an experimental system. *Pedobiologia*, **47**, 548-553.

BSI, 2010, PAS, 110:2010 Specification for whole digestate, separated liquor and separated fibre derived from the anaerobic digestion of source-segregated biodegradable materials. British Standards institute, London. Available at: http://www.wrap.org.uk /sites/files/wrap/PAS110_vis_10.pdf.

Cai, F., Pang, G., Li, R., Li, R., Gu, Xiao-Long, Shen, Qi-Rong, Chen, W., (2017) Bioorganic fertiliser maintains a more stable soil microbiome than chemical fertiliser for monocropping. *Biology and Fertility of Soils*, **53:8**, 861-872.

Cai, L., Gong, X., Sun, X., Li, S., Yu., X., (2018) Comparison of chemical and microbiological changes during the aerobic composting and vermicomposting of green waste. *PloS one*, **13:11**, e0207494.

Cameron., E. K., Bayne, E., M., (2011). An experimental test of facilitation between non-native earthworms. *Canadian Journal of Zoology*, **89:12***,* 1223–1230.

Cameron, E. K., Cahill, J. F., Bayne, E. M., (2014) Root foraging influences plant growth responses to earthworm foraging. *Plos One*, **9:9**, e1088873.

Campagnolo, E. R., Johnson, K. R., Karpati, A., Rubin, C. S., Kolpin, D. W., Meyer, M. T., Esteban, J. E., Currier, R. W., Smith, K., Thu, K. M., McGheein, M., (2002) Antimicrobial residues in animal waste and water resources proximal to large scale swine and poultry feeding operations. *Science of the Total Environment*, **299**, 89-95.

Candemir, F., Gülser, C., (2010) Effects of different agricultural wastes on some soil quality indexes in clay and loamy sand fields. *Communications in Soil Science and Plant Analysis*, **42:1**, 13-28.

Cannell, R. Q., Davies, D. B., Mackney, D., Pigeon, J. D., (1978) The suitability of soils for sequential direct drilling of combine-harvested crops in Britain: a provisional classification. *Outlook on Agriculture*, **9:6**, 306-316.

Capowiez, Y., Cadoux, S., Bouchant, P., Guy, R., Hubert, B., (2009) Experimental evidence for the role of earthworms in compacted soil regeneration based on field observations and results from a semi-field experiment. *Soil Biology and Biochemistry*, **41:4**, 711-717.

Capowiez, Y., Bottinelli, N., Jouquet, P., (2014a). Quantitative estimates of burrow construction and destruction, by anecic and endogeic earthworms in repacked soil cores. *Applied Soil Ecology*, **74**, 46–50.

Capowiez, Y., Samartino, S., Cadoux, S., Bouchant, P., Guy, R., Boizard, H., (2012) Role of earthworms in regenerating soil structure after compaction in reduced tillage systems. *Soil Biology and Biochemistry*, **55**, 93-103.

Capowiez, Y., Sammartino, S., Michel, E., (2014b) Burrow systems of endogeic earthworms: Effects of earthworm abundance and consequences for soil water infiltration. *Pedobiologia*, **57**, 303-309.

Caricasole, P., Hatcher, P. G., Ohno, T., (2018) Biodegradation of crop residue-derived organic matter is influenced by its heteroatomic stoichiometry and molecular composition. *Applied Soil Ecology*, **130**, 21-25.

Caro, G., Hartmann, C., Decaëns, T., Barot, S., Mora, P., Mathieu, J., (2014) Impact of soil engineering by two contrasting species of earthworms on their dispersal rates. *Applied Soil Ecology*, **84**, 223-230.

Carter, M. R., Mele, P. M., Steed, G. R. (1994) The effects of direct drilling and stubble retention on water and bromide movement and earthworm species in a duplex soil. *Soil Science,* **157**, 224-231.

Castro-Huerta, R., Falco, L., Sandler, R., Coviella, C. (2015). Differential contribution of soil biota groups to plant litter decomposition as mediated by soil use. *PeerJ (San Francisco, CA)*, **3**, e826–.

Cesarz, S., Craven, D., Dietrich, C., Eisenhauer, N., (2016) Effects of soil and leaf litter quality on the biomass of two endogeic earthworm species. *European Journal of Soil Biology*, **77**, 9-16.

Chan, K. Y. (2001) An overview of some tillage impacts on earthworm population abundance and diversity – implications for functioning in soils. *Soil and Tillage Research*, **57**, 179-191.

Chatelain, M., Mathieu, J., (2017). How good are epigeic earthworms at dispersing? An investigation to compare epigeic to endogeic and anecic groups. *Soil Biology and Biochemistry*, **111**, 115–123.

Chen, J., Heiling, M., Resch, C., Mbaye, M., Gruber, R., Dercon, G., (2018) Does maize and legume crop residue mulch matter in soil organic carbon sequestration? *Agriculture, Ecosystems and Environment*, **265**, 123-131.

Chen, Z., Zhang, J., Lu, M., Qin, C., Chen, Y., Yang, L., Huang, Q., Wang, J., Shen, Z., Shen, Q., (2016) Microbial communities of an arable soil treated for 8 years with organic and inorganic fertilizers, *Biology and fertility of soils*, **52:4**, 455–467.

Chiyoka, W., Zvomuya, F., Hao, X., (2014) 'A Bioassay of Nitrogen Availability in Soils Amended with Solid Digestate from Anaerobically Digested Beef Cattle Feedlot Manure', *Soil Science Society of America journal*, **78:4**, 1291–1300.

Christensen, B. T., (1987) Decomposability of organic matter in particle size fractions from field soils with straw incorporation. *Soil Biology and Biogeochemistry*, **19**, 429-435.

Christian, D. G., Ball, B. C., (1994) Reduced cultivations and direct drilling for cereals in Great Britain. In: Carter, M.R., (Ed.), Conservation Tillage in Temperate Ecosystems. Lewis Publishers, Boca Raton, Florida USA, 101-110.

Clause, J., Barot, S., Richard, B., Decaëns, T., Forey, E., (2014) The interactions between soil type and earthworm species determine the properties of earthworm casts. *Applied Soil Ecology*, **83**, 149-158.

Clause, J., Forey, E., Eisenhauer, N., Seal, C. E., Soudey, A., Colville, L., Barot, S., (2017) Seed selection by earthworms: chemical seed properties matter more than morphological traits. *Plant and Soil*, **413:1-2**, 97-110.

Clement, J. S., Simpson, A. J., Simpson, M. J., (2011) Association of specific organic matter compounds in size fractions of soils under different environmental controls. *Organic Geochemistry*, **42**, 1169-1180.

Coban, H., Miltner, A., Elling, F. J., Kätsner, M., (2015) The contribution of biogas residues to soil organic matter formation and CO_2 emissions in an arable soil. *Soil Biology and Biochemistry*. **86**, 108-115.

Coelho, J. J., Prieto, M. L., Dowling, S., Hennessy, A., Casey, I., Woodcock, T., Kennedy, N., (2018) Physical-chemical traits, phytotoxicity and pathogen detection in liquid anaerobic digestates. *Waste Management*, **78**, 8-15.

Colombani, N., Boz, B., Gumiero, B., Mastrocicco, M., (2017) Monitoring nutrients fate after digestate spreading in a short rotation buffer area. *Environmental Science and Pollution Research International*, **24:29**, 22816-22826.

Commission of the European Communities (CEC), (2006) Proposal for a directive of the European Parliament and of the Council, establishing a framework for the protection of soil, and amending directive 2004/35/EC. COM 231 Final, Brussels.

Conant, R. T., Ryan, M. G., Ågren, G. I., Birge, H. E., Davidson, E. A., Eliasson, P. E., Evans, S. E., Frey, S. D., Giardina, C. P., Hopkins, F. M., Hyvönen, R., Kirschbaum, M. U. F., Lavallee, J. M., Leifeld, J., Parton, W. J., Megan Steinweg, J., Wallenstein, M. D., Martin Wetterstedt, J. Å., Bradford, M. A., (2011) Temperature and soil organic matter decomposition rates – synthesis of current knowledge and a way forward. *Global Change Biology*, **17:11**, 3392-3404.

Constanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S. J., Kubiszewski, I., Farber, S., Turner, R. K., (1997) Changes in the global value of ecosystem services. *Global Environmental Change*, **26**, 152-158.

Cook, R. T., Bailey, S. E. R., McCrohan, C. R., (1997) The potential for common weeds to reduce slug damange to winter wheat: laboratory and fields studies. *Journal of Applied Ecology*, **34:1**, 79–87.

Coq, S., Barthès, B., Oliver, R., Rabary, B., Blanchart, E. (2007). Earthworm activity affects soil aggregation and organic matter dynamics according to the quality and localization of crop residues—An experimental study (Madagascar). *Soil Biology and Biochemistry*, **39:8**, 2119–2128.

Costa, A., Gusmara, C., Gardoni, D., Zaninelli, M., Tambone, F., Sala, V., Guarino, M., (2017) 'The effect of anaerobic digestion and storage on indicator microorganisms in swine and dairy manure', *Environmental science and pollution research international*, **24:31**, 24135–24146.

Costanza, R., Fisher, B., Mulder, K., Liu, S., Christopher, T., (2007) Biodiversity and ecosystem services: A multi-scale empirical study of the relationship between species richness and net primary production. *Ecological Economics*, **61**, 478-491.

Couëdel, A., Alletto, L., Tribouillois, H., Justes, É., (2018) Cover crop crucifer-legume mixtures provide effective nitrate catch crop and nitrogen green manure ecosystem services. *Agriculture, Ecosystems and Environment*, **254**, 50-59.

Creamer, R., Hannula, S., Leeuwen, J., Stone, D., Rutgers, M., Schmelz, R., Ruiter, P., Hendriksen, N., Bolger, T., Bouffaud, M., Buee, M., Carvalho, F., Costa, D., Dirilgen, T., Francisco, R., Griffiths, B., Griffiths, R., Martin, F., Silva, P. M., Mendes, S., Morais, P. V., Pereira, C., Philippot, L., Plassart, P., Redecker, D., Römbke, J., Sousa, J. P., Wouterse, M., Lemanceau, P. (2016). Ecological network analysis reveals the inter-connection between soil biodiversity and ecosystem function as affected by land use across Europe. *Applied Soil Ecology*, **97**, 112–124.

Crittenden, S. J., Eswaramurthy, T., de Goede, R. G.M., Brussaard, L., Pulleman, M. M., (2014) Effect of tillage on earthworms over short- and medium-term in conventional and organic farming. *Applied Soil Ecology*, **83**, 140-148.

Crittenden, S. J., Poot, N., Heinen, M., van Balen, D. J. M., (2015) Soil physical quality in contrasting tillage systems in organic and conventional farming. *Soil and Tillage Research*, **154**, 136-144.

Crossley Jr, D. A., Blood, E. R., Hendrix, P. F., Seastedt, T. R., (1995) Turnover of Cobalt-60 by earthworms (*Eisenia fetida*) (Lumbricidae, Oligochaeta). *Applied Soil Ecology*, **2**, 71-75.

Curry, J. P. (2004). Factors affecting the abundance of earthworms in soils. In: Edwards, C. A. (ed.), Earthworm Ecology, Boca Raton, FL: CRC Press, p.91–113.

Curry, J. P., Bolger, T., (1983) Growth, reproduction and litter and soil consumption by *Lumbricus terrestris L.* in reclaimed peat. *Soil Biology and Biochemistry*, **16:3**, 253-257.

Curry, J. P., Byrne, D., (1992) The role of earthworms in straw decomposition and nitrogen turnover in arable land in Ireland. *Soil Biology and Biochemistry*, **24:12**, 1409-1412.

Curry, J. P., Byrne, D., (1997) Role of earthworms in straw decomposition in a winter cereal field. *Soil Biology and Biochemistry*, **29:3/4**, 555-558.

Curry, J. P., Schmidt, O., (2007) The feeding ecology of earthworms: a review. *Pedobiologia*, **50:6**, 463-477.

Dainese, M., Schneider, G., Krauss, J., Steffan-Derwenter, I., (2017) Complementarity among natural enemies enhances pest suppression. *Scientific Reports*, **7:1**, 8172-8178.

Darwin, C. R., (1881) The formation of a vegetable mould through the action of worms. Murray, London.

Daryanto, S., Wang, L., Jacinthe, P-A., (2017) Impacts of no-tillage management on nitrate loss from corn, soybean and wheat cultivation: a meta-analysis. *Scientific Reports*, **7:1**, 12117-12119.

Davies D., Finney J., (2002) Reduced cultivations for cereals: research, development and advisory needs under changing economic circumstances. Research Review No. 48. Home Grown Cereals Authority, Kenilworth, UK.

Dawson, C. J., Hilton, J., (2011) Food availability in a resource-limited world: Production and recycling of nitrogen and phosphorus. *Food Policy*, **36**, S14-S22.

De Baets, S., Poesen, J., Meersmans, J., Serlet, L., (2011) Cover crops and their erosion-reducing effects during concentrated flow erosion. *CATENA*, **85**, 237-244.

De Notaris, C., Sørensen, P., Møller, H. B., Wahid, R., Eriksen, J., (2018) 'Nitrogen fertilizer replacement value of digestates from three green manures', *Nutrient cycling in agroecosystems*, **112:3**, 355–368.

Decaëns, T., (2000) Degradation dynamics of surface earthworm casts in grasslands of the eastern plains of Colombia. *Biology and Fertility of Soils*, **32:2**, 149-156.

Delgado, M., Rodríguez, C., Martín, J. V., Miralles de Imperial, R., Alonso, F., (2012) Environmental assay on the effect of poultry manure application on soil organisms in agroecosystems. *Science of the Total Environment*, **416**, 532-535.

Derpsch, R., Friedrich, T., Kassam, A., Hongwen, L., (2009) Current status of adoption of no-till farming in the world and some of its main benefits. *International Journal of Agricultural and Biological Engineering*, **3:1**, 1-25.

Denef, K., Six, J., Paustian, K., Merckx, R. (2001) Importance of macroaggregate dynamics in controlling soil carbon stabilization: short-term effects of physical disturbance induced by dry–wet cycles. *Soil Biology and Biochemistry*. **33:15**, 2145–2153.

Devliegher, W., Verstraete, W., (1995) *Lumbricus terrestris* in a soil core experiment: Nutrient-enrichment processes (NEP) and gut-associated processes (GAP) and their effect on microbial biomass and microbial activity. *Soil Biology and Biochemistry*, **27:12**, 1573–1580.

Devliegher, W., Veerstraete, W., (1996) *Lumbricus terrestris* in a soil core experiment: effects of nutrient-enrichment processes (NEP) and gut-associated processes (GAP) on the availability of plant nutrients and Heavy Metals. *Soil Biology and Biochemistry*, **28**, 489-496.

Devliegher, W., Verstraete. W., (1997). Microorganisms and soil physico-chemical conditions in the drilosphere of *Lumbricus terrestris*. *Soil Biology and Biochemistry*, **29:11-12**, 1721–1729.

De Vries, F. T., Hoffland, E., van Eekeren, N., Brussaard, L., Bloem, J., (2006) Fungal/bacterial ratios in grasslands with contrasting nitrogen management. *Soil Biology and Biochemistry*, **38**, 2092-2103.

De Vries, J., Vinken, T., Hamelin, L., & De Boer, I. (2012). Comparing environmental consequences of anaerobic mono- and co-digestion of pig manure to produce bio-energy – A life cycle perspective. *Bioresource Technology*, **125**, 239–248.

Dinter, A., Oberwalder, C., Kabouw, P., Coulson, M., Ernst, G., Leicher, T., Miles, M., Weyman, G., Klein, O., (2013) Occurrence and distribution of earthworms in agricultural landscapes across Europe with regard to testing for responses to plant protection products. *Journal of Soils and Sediments*, **13**, 278-293.

Dittbrenner, N., Moser, I., Triebskorn, R., Capowiez, Y., (2011) Assessment of short and long-term effects of imidacloprid on the burrowing behaviour of two earthworm species (*Aporrectodea caliginosa and Lumbricus terrestris*) by using 2D and 3D post-exposure techniques. *Chemosphere*, **84**, 1349-1355.

Dobson, A. M., Blossey, B., Richardson, J. B., (2017) Invasive earthworms change nutrient availability and uptake by forest understory plants. *Plant and Soil*, **421:1-2**, 175–190.

Domínguez-Crespo, M., Sánchez-Hernández, Z., Torres-Huerta, A., Negrete-Rodríguez, M., Conde-Barajas, E., Flores-Vela, A. (2012) Effect of the heavy metals Cu, Ni, Cd and Zn on the growth and reproduction of epigeic earthworms (*E. fetida*) during the vermistabilization of municipal sewage sludge. *Water, Air, and Soil Pollution*, **223:2**, 915–931.

Doube, B. M., Schmidt, O., Killham, K., Correll, R., (1997) ‘Influence of mineral soil on the palatability of organic matter for lumbricid earthworms: A simple food preference study’, *Soil Biology and Biochemistry*, **29:3**, 569–575.

Douglas, J. T., Goss, M. J., Hill, D., (1980) Measurements of pore characteristics in a clay soil under plowing and direct drilling, including use of a radioactive tracer (144Ce) technique. *Soil and Tillage Research*. **1**, 11.

Douglas, M. R., Tooker, J. F., (2012) Slug (*Mollusca: Agriolimacidae, Arionidae*) ecology and management in no-till field crops, with an emphasis on the Mid-Atlantic region. *Journal of Integrated Pest Management*, **3:1**, C1–C9.

Downing, T. E., (1993) The effects of climate change on agriculture and food security. *Renewable Energy*, **3:4-5**, 491-497.

Duah-Yentumi S., Johnson. D. B., (1986) Changes in soil microflora in response to repeated applications of some pesticides. *Soil Biology and Biochemistry*, **18:6**, 629-635.

Duczek, L. J., Sutherland, K. A., Reed, S. L., Bailey, K. L., Lafond, G. P., (1999) Survival of leaf spot pathogens on crop residues of wheat and barley in Saskatchewan. *Canadian Journal of Plant Pathology*, **21:2**, 165-173.

Düring, R-A., Hummel, H. E., (1997) Glyphosphate: importance in no-till agriculture with regard to environmental side effects. In: Tebrüge, F., Böhrnsen A., (Eds.), Experience with the applicability of no-tillage crop production in the west European countries. Final report, Fachverlag Köhler, 35396, Glessen, Germany, 45-61.

Duval, M. E., Galantini, J. A., Capurro, J. E., Martinez, J. M., (2016) Winter cover crops in soybean monoculture: effects on soil organic carbon and its fractions. *Soil and Tillage Research*, **161**, 95-105.

Edwards, C. A. (2004) Earthworm ecology. Boca Raton, FL: CRC Press

Edwards, C.A., Bohlen, P. J., (1996) Biology and Ecology of Earthworms. 3rd Edition, Chapman and Hall.

Edwards, C. A., Lofty, J. R. (1972) Biology of Earthworms. Chapman and Hall, London.

Edwards, C. A., Lofty, J. R. (1982) The effect of direct drilling and minimal cultivation on earthworm populations. *Journal of Applied Ecology,* **19***,* 723–734.

Edwards W. M., Shipitalo, M. J., Owens, L. B., Norton, L. D., (1990) Effect of *Lumbricus terrestris* burrows on hydrology of continuous no-till corn fields. *Geoderma*. **46**, 73-- 84.

Eden, M., Gerke, H., Houot, S., (2017) Organic waste recycling in agriculture and related effects on soil water retention and plant available water: a review. *Agronomy for Sustainable Development*, **37:2**, 1-21.

Eggerton-Warbuton, L. M., Johnson, N. C., Allen, E. B., (2007) Mycorrhizal community dynamics following nitrogen fertilization: A cross site test in five grasslands. *Ecological Monographs*, **77**, 527-544.

Ehlers, W., (1975) Observations on earthworm channels and infiltration on tilled and untilled loess soil. *Soil Science,* **119**, 242-247.

Eichinger, E., Bruckner, A., Stemmer, M., (2007) Earthworm expulsion by formalin has severe and lasting side effects on soil biota and plants. *Ecotoxicology and Environmental Safety*, **67:2**, 260-266.

Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., Partsch, S., Sabais, A. C. W., Scherber, C., Steinbeiss, S., Weigelt, A., Weisser, W. W., Scheu, S., (2010) Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology*, **91**, 485-496.

Ekeburg, E., Riley, H. C. F., (1997) Tillage intensity effects on soil properties and crop yields on a long term trial on morainic loam soil in southeast Norway. *Soil and Tillage Research*, **112**, 122-132.

Ellis, S. R., Hodson, M. E., Wege, P., (2010) Determining the influence of rainfall patterns and carbendazim on the surface activity of the earthworm *Lumbricus terrestris*. *Environmental Toxicology and Chemistry*, **29:8**. 1821-1827.

Elliston, T., Oliver, I. W., (2019) Ecotoxicological assessments of biochar additions to soil employing earthworm species *Eisenia fetida* and *Lumbricus terrestris*. *Environmental Science and Pollution Research*, **27**, 33410–33418.

Eriksen-Hamel, N. S., Whalen, J. K., (2006) Growth rates of *Aporrectodea caliginosa* (Oligochaetae: Lumbricidae) as influenced by soil temperature and moisture in disturbed and undisturbed soil columns. *Pedobiologia*, **50:3***,* 207–215.

Eriksen-Hamel N. S., Whalen, J. K., (2007a) Competitive interactions affect the interactions between *Lumbricus terrestris* and *Aporrectodea caliginosa* (Lumbricidae, Oligochaeta) in single and mixed species laboratory cultures. *European Journal of Soil Biology*, **43:3**, 142-150.

Eriksen-Hamel, N. S., Whalen, J. K., (2007b) Impacts of earthworms on soil nutrients and plant growth in soybean and maize agroecosystems. *Agriculture, Ecosystems and Environment*, **120:2**, 442–448.

Eriksen-Hamel, N. S., Speratti, A. B., Whalen, J. K., Légère, A., Madramootoo, C. A., (2009) Earthworm populations and growth rates related to long-term crop residue and tillage management. *Soil and Tillage Research*, **104**, 311-316.

Ernst, G., Emmerling, C., (2009) Impact of five different tillage systems on soil organic carbon content and the density, biomass and community composition of earthworms after a ten-year period. *European Journal of Soil Biology*, **45:3**, 247-251.

Ernst, G., Felten, D., Vohland, F., Emmerling, C., (2009) Impact of ecologically different earthworm species on soil water characteristics. *European Journal of Soil Biology*, **45**, 207-213.

Ernst, G., Frey, B., (2007) The effect of feeding behaviour on Hg accumulation in the ecophysically different earthworm species *Lumbricus terrestris* and *Octolasion cyaneum*: A microcosm experiment. *Soil Biology and Biochemistry*, **39:1**, 386-390.

Ernst, G., Henseler, I., Felten, D., Emmerling, C., (2009) Decomposition and mineralization of energy crop residues governed by earthworms. *Soil Biology and Biochemistry*, **41**, 1548-1554.

European Union (1986) Council Directive of 12 June 1986 on the protection of the environment, and in particular the soil, when sewage sludge is used in agriculture (86/278/EEC). Official Journal of the European Communities, L181-6-12. Accessed online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31986L0278&from=EN

European Union, (2013) COMMISSION IMPLEMENTING REGULATION (EU) No 485/2013 of 24 May 2013 amending Implementing Regulation (EU) No 540/2011, as regards the conditions of approval of the active substances clothianidin, thiamethoxam and imidacloprid, and prohibiting the use and sale of seeds treated with plant protection products containing those active substances. Official Journal of the European Union, L139-12-26. Accessed online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013R0485&from=EN

Euteneuer, P., Wagentristl, H., Steinkellner, S., Scheibreithner, C., Zaller, J. G., (2019) Earthworms affect decomposition of soil-borne plant pathogen *Sclerotinia sclerotiorum* in a cover crop field experiment. *Applied Soil Ecology*, **138**, 88-93.

Euteneuer, P., Wagentristl, H., Steinkellner, S., Fuchs, M., Zaller, J. G., Piepho, H-P, Butt, K. R., (2020) Contrasting effects of cover crops on earthworms: results from field monitoring and laboratory experiments on growth, reproduction and food choice. *European Journal of Soil Biology*, **100**, 103225.

Evans, D.L., Quinton, J. N., Davies, J. A. C., Zhao, J., Govers, G., (2020) Soil lifespans and how they can be extended by land use and management change. *Environmental Research Letters*, **15:9**, 1-10.

Evers, A. K., Demers, T. A., Gordon, A. M., Thevathasan, N. V., (2010) The Effect of Earthworm (*Lumbricus terrestris* L.) Population Density and Soil Water Content Interactions on Nitrous Oxide Emissions from Agricultural Soils. *Applied and Environmental Soil Science*, **2010**, 1–9.

Fahey, T., Yavitt, J., Sherman, R., Maerz, J., Groffman, P., Fisk, M., Bohlen, P., (2013) Earthworm effects on the incorporation of litter C and N into soil organic matter in a sugar maple forest. *Ecological Applications*, **23:5**, 1185–1201.

Fan, J., Xiao, J., Liu, D., Ye, G., Luo, J., Houlbrooke, D., Laurenson, S., Yan, J., Chen, L., Tian, J., Ding, W., (2017) Effect of application of dairy manure, effluent and inorganic fertilizer on nitrogen leaching in clayey fluvo-aquic soil: A lysimeter study. *Science of the Total Environment*, **592**, 206-214.

FAO, (2015) *Soil Functions: Soils deliver ecosystem services that enable life on earth*. Accessed [Online] on 20.10.20, URL: http://www.fao.org/3/a-ax374e.pdf

FAO, (2017) World Fertliser Trends and Outlook to 2020. Accessed [Online] on 05.05.20, URL: World fertilizer trends and outlook to 2020 (fao.org)

FAO, (2019a) *World Agriculture: Towards 2015/2030 – An FAO perspective.* Accessed [Online] on 02.09.19, URL: fao.org/3/y4252e/y4252e06.htm.

FAO, (2019b) *The State of the World's Biodiversity for Food and Agriculture*, J. Bélanger & D. Pilling (eds.). FAO Commission on Genetic Resources for Food and Agriculture Assessments. Rome. 572 pp. (http://www.fao.org/3/CA3129EN/CA3129EN.pdf).

Fernandez, M. C., Rubio, G., (2015) Root morphological traits related to phosphorus uptake efficiency of soybean, sunflower and maize. *Journal of Plant Nutrition and Soil Science*, **178:5**, 807-815.

Figuerola, E. L. M., Guerrero, L. D., Türkowsky, D., Wall, L. G., Erijman, L., (2015) Crop monoculture rather than agriculture reduces the spatial turnover of soil bacterial communities at a regional scale. *Environmental Microbiology*, **17:3**, 678-688.

Finzi, A. C., Abramoff, R., Spiller, K., Brzostek, E., Darby, B., Kramer, M., Phillips, R., (2015) Rhizosphere processes are quantitatively important components of carbon and nutrient cycles. *Global Change Biology*, **21:5**, 2082-2094.

Foot, K. J., Hislop, M., McNeilly, S., (2003) The effect of composted green waste on tree establishment in landfill. Moore, H. M., Fox, H. R., Elliot, S., (Eds.) Land Reclaimation – Extending the Boundaties. AA Balkema, Lisse, 213-221.

Fortuna, A., Harwood, R., Kizilkaya, K., Paul, E. A., (2003) Optimizing nutrient availability and potential carbon sequestration in an agroecosystem. *Soil Biology and Biochemistry*, **35**, 1005-1013.

Fox, R. H., Hoffman, L. D., (1981) The effect of N fertilizer source on grain yield, N uptake, soil pH and lime requirement in no-till corn. *Agronomy Journal*, **73**, 891-895.

Foy, C. D., Chaney, R. L., and White, M. C., (1978). The physiology of metal toxicity in plants. *Annual Reviews in Plant Physiology*, **29**, 511–566.

Fraser, P., Piercy, J. (1998). The effects of cereal straw management practices on lumbricid earthworm populations. *Applied Soil Ecology*, ***9*:1**, 369–373.

Fraser, P. M., Beare, M. H., Butler, R. C., Harrison-Kirk, T., and Piercy, J. E., (2003) Interactions between earthworms (*Apporectodea caliginosa*), plants and crop residues for restoring properties of a degraded arable soil. *Pedobiologia*, **47**, 870-876.

Frazão J., de Goede, R. G. M., Brussaard, L., Faber, J. H., Groot, J. C. J., Pulleman, M. M., (2017) Earthworm communities in arable fields and restored field margins, as related to management practices and surrounding landscape diversity. *Agriculture, Ecosystems and Environment*, **248**, 1-8.

Frazão, J., de Goede, R. G. M., Capowiez, Y., Pulleman, M. M., (2019) Soil structure formation and organic matter distribution as affected by earthworm species interactions and crop residue placement. *Geoderma*, **338**, 453-463.

Frederickson, B., Butt, K. R., Morris, R. M., Daniel, C., (1997) Combining vermiculture with traditional green waste composting systems, *Soil Biology and Biochemistry*, **29:3-4**, 725–730.

Frøseth, R. B., Bakken, A. K., Bleken, M. A., Riley, H., Pommeresche, R., Thorup-Kristensen, K., Hansen, S., (2014) Effects of green manure herbage management and its digestate from biogas production on barley yield, N recovery, soil structure and earthworm populations. *European Journal of Agronomy*, **52**, 90-102.

Frouz, J., Špaldoňová, A., Fričová, K., Bartuška, M., (2014) The effect of earthworms (*Lumbricus rubellus*) and simulated tillage on soil organic carbon in a long-term microcosm experiment. *Soil Biology and Biochemistry*, **78**, 58-64.

Fründ, H. C., Butt, K. R., Capowiez, Y., Eisenhauer, N., Emmerling C., Ernst, G., Potthoff, M., Schädler, M., Schrader, S., (2010) Using earthworms as model organisms in the laboratory: recommendations for experimental interpretations. *Pedobiologia*, **53:2**, 119-125.

Fu, F., Wang, Q., (2011) Removal of heavy metal ions from wastewaters: a review. *Journal of Environmental Management*, **92**, 407-418.

Gao, H., Chen, X., Wei, J., Zhang, Y., Zhang, L., Chang, J., Thompson, M. (2016) Decomposition Dynamics and Changes in Chemical Composition of Wheat Straw Residue under Anaerobic and Aerobic Conditions. *PloS One*, **11:7**, e0158172–.

Gaupp-Berghausen, M., Hofer, M., Rewald, B., Zaller, J. G., (2015) Glyphosphate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations. *Scientific Reports*, **5**, 12886.

Gell, K., van Groenigen, J., Cayuela, M. L., (2011) Residues of bioenergy production chains as soil amendments: immediate and temporal phytotoxicity. *Journal of Hazardous Materials*, **186**, 2017-2025.

Gerard, B. M., Hay, R. K. M., (1979) The effect on earthworms of ploughing, tined cultivation, direct drilling and nitrogen in a barley monoculture system. *The Journal of Agricultural Science*, **93:1**, 147-155.

Giannopoulous, G., Pulleman, M. M., van Groenigen, J. W., (2010) Interactions between residue placement and earthworm ecological strategy affect aggregate turnover and N2O dynamics in agricultural soil. *Soil Biology and Biochemistry*, **42:4**, 618-625.

Giannitsopoulos, M. L., Burgess, P.J., Rickson, J. R., (2019). Effects of conservation tillage drills on soil quality indicators in a wheat–oilseed rape rotation: organic carbon, earthworms and water-stable aggregates. *Soil Use and Management*, **36:1**, 139–152.

Gill, M., (2011) 'Effect of Organic Mulches on Soil Surface Insects and Other Arthropods', *The Florida Entomologist*, **94:2**, 226–232.

Gish, C. D., Christensen, R. E., (1973) Cadmium, Nickel, Zinc and Lead in earthworms from roadside soil. *Environmental Science and Technology*, **7:11**, 1060-1062.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M., Toulmin, C., (2010) Food security: the challenge of feeding 9 billion people. *Science*, **327:5967**, 812-818.

Godfray, H. C. J., Blacquiere, T., Field, L. M., Hails, R. S., Petrokofsky, G., Potts, S. G., Raine, N. E., Vanbergen, A. J., McClean, A. R., (2014) A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proceedings of the Royal Society B., 2151821.

Goh, K. M., Tutuna, S. S., (2004) Effects of organic and plant residue quality and orchard management practices on decomposition rates of residues. *Communications in Soil Science and Plant Analysis*, **35**, 441-460.

Gopinath, K. A., Saha, S., Mina, B. L., Pande, H, Kundu, S., Gupta, H. S., (2008) ‘Influence of organic amendments on growth, yield and quality of wheat and on soil properties during transition to organic production’, *Nutrient cycling in agroecosystems*, **82:1**, 51–60.

Gómez-Brandón, M., Aira, M., Lores, M., Domínguez, J., (2011) Epigeic earthworms exert a bottleneck effect on microbial communities through gut associated processes. *PloS One*, **6:9,** e24786–e24786.

Gómez-Brandón, M., Lores, M., Domínguez, J., (2012) Species-Specific Effects of Epigeic Earthworms on Microbial Community Structure during First Stages of Decomposition of Organic Matter. *PloS One*, **7:2,** e31895–e31895.

Goncharov, A. A., Glebova, A. A., Tiunov, A. V., (2020) Trophic interactions between fusarium species and soil fauna: a meta-analysis of experimental studies. *Applied Soil Ecology*, **145**, 103302.

Görres, J. H., Savin, M. C., Amador, J. A., (2001) Soil micropore structure and carbon mineralization in burrows and casts of an anecic earthworm (*Lumbricus terrestris*). *Soil Biology and Biochemistry*, **33:14**, 1881-1887.

Götze, P., Rücknagel, J., Jacobs, A., Märländer, B., Koch, H-J., Christen, O., (2016) Environmental impacts of different crop rotations in terms of soil compaction. *Journal of Environmental Management*, **181**, 54-63.

Gramig, B. M., Massey, R., Yun, S. D., (2017) Nitrogen application decision-making under climate risk in the U.S. Corn Belt. *Climate Risk Management*, **15**, 82-89.

Grant, J. D., (1983) The activities of earthworms and the fates of seeds. In: Satchell, J. E., (Ed.), Earthworm Ecology, Chapman and Hall, London, 107-122.

Grande, J. D., Karthikeyan, K. G., Miller, P. S., Powell, M. J., (2005) Corn residue level and manure application timing effects on phosphorus losses in runoff. *Journal of Environmental Quality*, **34:5**, 1620-1631.

Griffiths, B. S., Ball, B. C., Daniell, T. J., Hallet, P. D., Neilson, R., Wheatley, R. E., Osler, G., Bohanec, M., (2010) Integrating soil quality changes to arable agricultural systems following organic matter addition, or adoption of a ley-arable rotation. *Applied Soil Ecology*, **46**, 43-53.

Griffiths, B. S., Römbke, J., Schmelz, R., Scheffczyk, A., Faber, J., Bloem, J., Pérès, G., Cluzeau, D., Chabbi, A., Suhadolc, M., Sousa, J., Martins da Silva, P., Carvalho, F., Mendes, S., Morais, P., Francisco, R., Pereira, C., Bonkowski, M., Geisen, S., Bardgett, R. D., de Vries, F. T., Bolger, D. T., Dirilgen, t., Schmidt, O., Widning, A., Hendriksen, N. B., Johansen, A., Philippot, L., Plassart, P., Bru, D., Thomson, B., Griffiths, R. I., Bailey, M. J., Keith, A., Rutgers, M., Mulder, C., Hannula, S. E., Creamer, R., Stone, D. (2016). Selecting cost effective and policy-relevant biological indicators for European monitoring of soil biodiversity and ecosystem function. *Ecological Indicators*, **69,** 213–223.

Grigoropoulou, N., Butt, K. R., (2010) Field investigations of *Lumbricus terrestris* spatial distribution and dispersal through monitoring of manipulated, enclosed plots. *Soil Biology and Biochemistry* **42**, 40-47.

Grigoropoulou, N., Butt, K. R., (2012) Assessment of burrow re-use by *Lumbricus terrestris* L. through field experimentation. *Zeszyty Naukowe*, **15**, 43-51.

Grigoropoulou, N., Butt, K. R., Lowe, C. N., (2009) Interactions of juvenile *Lumbricus terrestris* with adults and their burrow systems in a two dimensional microcosm. *Pesquisa Agropecuária Brasileira*, **44:8**, Online: https://doi.org/10.1590/S0100-204X2009000800025

Grüter, R., Costerousse, B., Bertoni, A., Mayer, J., Thonar, C., Frossard, E., Schulin, R., Tandy, S., (2017) Green manure and long-term fertilization effects on soil zinc and cadmium availability ad uptake by wheat (*Triticum aestivum* L.) at different growth stages. *Science of the Total Environment*, **599-600**, 1330-1343.

Gutser, R., Ebertseder, T, H., Werber, A., Schraml, M., Schmidhalter, U., (2005) Short term and residual availability of nitrogen after long term application of organic fertilizers in arable land. *Journal of Plant Nutrition and Soil Science*, **168**, 439-446.

Guy, C., Shura, O., Murray, R. L., Sandhu, P., (2018) Worms make risky choices too: the effect of starvation on foraging in the common earthworm (*Lumbricus terrestris*). *Canadian Journal of Zoology*, **96:11***,* 1278–1283.

Hallam, J., Hodson, M., (2020) Impact of different earthworm ecotypes on water stable aggregates and soil water holding capacity. *Biology and Fertility of Soils*, **56:5**, 607–617.

Hamilton, W. E., Sillman, D. Y., (1989) Influence of earthworm middens on the distribution of soil microarthropods. *Biology and Fertility of Soils*, **8**, 279-284.

Hannan, J., (2017) Ultrafast Agricultural soil analysis using the ThermoScientific iCAP 7600 ICP-OES Radial. *ThermoFisher Scientific*, Application Note 43156, 1-3.

Hansen, E. M., Munkholm, L. J., Melander, B., Olesen, J. E., (2010) Can non-inversion tillage and straw retainment reduce N leaching in cereal based crop rotations? *Soil and Tillage Research*, **109**, 1-8.

Harrison, M. J., (1999) Biotrophic interfaces and nutrient transport in plant/fungal symbioses. *Journal of Experimental Botany*, **50**, 1013-1022.

Harvey, R. J., Chadwick, D. R., Sánchez-Rodríguez, A. R., Jones, D. L., (2019) Agroecosystem resilience in response to extreme winter flooding. Agriculture, *Ecosystems and Environment*, **279**, 1-13.

Haynes, R. J., Fraser, P. M., Piercy, J. E., Tregurtha, R. J., (2003). Casts of *Aporrectodea caliginosa* (Savigny) and *Lumbricus rubellus* (Hoffmeister) differ in microbial activity, nutrient availability and aggregate stability. *Pedobiologia*, **47:5**, 882–887.

Heiner, B., Drapela, T., Frank, T., Zaller, J. (2011) Stable isotope ^{15}N and ^{13}C labelling of different functional groups of earthworms and their casts: A tool for studying trophic links. *Pedobiologia*, **54:3**, 169–175.

Hernandez-Soriano, M. C., Jemenez-Lopez, J. C., (2012) Effects of total soil water content and organic matter addition on the speciation and bioavailability of heavy metals. *Science of the Total Environment*, **423**, 55-61.

Hirano, T., Tamae, K., (2010) Heavy metal induced oxidative DNA damage in earthworms: a review. *Applied and Environmental Soil Science*, **2010**, 1-7.

Hoang, D. T. T., Pausch, J., Razavi, B. S. Kuzyakova, I., Banfield, C. C., Kuzyakov, Y., (2016) Hotspots of microbial activity induced by earthworm burrows, old root channels, and their combination in subsoil. *Biology and Fertily of Soils* **52,** 1105–1119.

Hoeffner, M., Monard, C., Cluzeau, D., Santonja, M., (2019) Response of temperate anecic earthworm individual biomass to species interactions. *Applied Soil Ecology*, **144**, 8–11.

Hoeffner, K., Monard, C., Santonja, M., Cluzeau, D., (2018) Feeding of epi-anecic earthworm species and their impacts on soil microbial communities. *Soil Biology and Biochemistry*, **125**, 1-9.

Hoeffner, K., Santonja, M., Cluzeau, D., Monard, C., (2019) Epi-anecic rather than strict anecic earthworms enhance soil enzyme activities. *Soil Biology and Biogeochemistry*, **132**, 93-100.

Holland, E., Coleman, D. (1987). Litter Placement Effects on Microbial and Organic Matter Dynamics in an Agroecosystem. *Ecology, **68***:**2**, 425-433.

Hollis, J. M., Jones, R. J. A., Palmer, R. C., (1977) The effects of organic matter and particle size on the water retention properties of some soils in the West Midland of England. *Geoderma*, **17**, 225-238.

Holmstrup, M., (1992) Cold hardiness strategy in cocoons of the Lumbricid earthworm *Dendrobaena octaedra* Savigny. *Comparative Biochemistry and Physiology*, **102**, 49-54.

Holmstrup, M., (1994) Physiology of cold hardiness in cocoons of five earthworm taxa Lumbricidae: Oligochaeta. *Journal of Comparative Physiology*, **164**, 222-228.

Holmstrup, M., Hansen, B.T., Nielsen, A., Ostergaard, I.K., (1990) Frost tolerance of lumbricid earthworm cocoons. *Pedobiologia*, **34**, 361-366.

Holmstrup, M., Ostergaard, I.K., Nielsen, A. Hansen, B.T., (1991) The relationship between temperature and cocoon incubation time for some lumbricid earthworm species. *Pedobiologia*, **35**, 179-184.

Holmstrup, M., Ostergaard, I.K., Nielsen, A., Hansen, B.T., (1996) Note on the incubation of earthworm cocoons at three constant temperatures. *Pedobiologia*, **40**, 477-478.

Holmstrup, M., Overgaard, J. (2007) Freeze tolerance in *Aporrectodea caliginosa* and other earthworms from Finland. *Cryobiology*, **55:1**, 80–86.

Holmstrup, M., Westh, P., (1994) Dehydration of earthworm cocoons exposed to cold: a novel cold hardiness mechanism. *Journal of Comparative Physiology*, 164, 312-315.

Horn, M. A., Schramm, A., Drake, H. L., (2003) The earthworm gut: an ideal habitat for ingested N_2O-producing microorganisms. *Applied and Environmental Microbiology*, **69**, 1662-1669.

Howard, P. J. A., Howard, D. M., (1990) Use of organic carbon and loss-on-ignition to estimate soil organic matter in different soil types and horizons. *Biology and Fertility of Soils*, **9**, 306-310.

Houben, D., Michel, E., Nobile, C., Lambers, H., Kandeler, E., Faucon, M-P., (2019) Response of phosphorus dynamics to sewage sludge application in an agroecosystem in France. *Applied Soil Ecology*, **137**, 178-186.

Hoyle, F. C., Murphy. D. V., (2011) 'Influence of organic residues and soil incorporation on temporal measures of microbial biomass and plant available nitrogen', *Plant and soil*, **347:1-2**, 53–64.

Hu., G., Liu, X., He, H., Zhang, W., Xie, H., Wu, Y., Cui, J., Sun, C., Zhang, X., (2015) Multi-seasonal nitrongen recoveries from crop residue in soil and crop in a temperate agro-ecosystem. *Plos One*, **10:7**, 1-12.

International Standards Organisation (2006) *ISO 23611-1:2006: Soil Quality – Sampling of soil invertebrates – Part 1: Hand-sorting and formalin extraction of earthworms.*

Iritani, W. M., Arnold, C. Y., (1960) Nitrogen release of vegetable crop residues during incubation as related to their chemical composition. *Journal of Soil Science*, **89**, 74-72.

Irmler, U., (2010) Changes in earthworm populations during conversion from conventional to organic farming. *Agriculture, Ecosystems and Environment*, **135**, 194-198.

Isbell, F., Adler, P. R., Eisenhauer, N., Fornara, D., Kimmel, K., Kremen, C., Letourneau, D. K., Liebman, M., Polley, H. W., Quijas, S., Scherer-Lorenzen, M., Bardgett, R., (2017) 'Benefits of increasing plant diversity in sustainable agroecosystems', *Journal of Ecology*, **105:4**, 871–879.

Jarvis, N. J., (2007) A review of non-equillibrium water flow and solute transport in soil macropores: principles, controlling factors and consequences for water quality. *European Journal of Soil Science*, **58**, 523-546.

Janssen, B.H., (1996) Nitrogen mineralization in relation to C:N ratio and decomposability of organic materials. *Plant and Soil*, **181**, 39-45.

Jegou, D., Cluzeau, D., Hallaire, V., Balesdent, J., Tréhen, P., (2000) Burrowing activity of the earthworms *Lumbricus terrestris* and *Apporectodea giardia* and consequences on C transfers in soil. *European Journal of Soil Biology*, **36**, 27-34.

Jensen, E. S., (1994) Mineralization-immobilization of nitrogen in soil amended with low C:N ratio plant residues with different particle sizes. *Soil Biology and Biochemistry*, **31**, 1135-1149.

Johnson, J. M. F., Reicosky, D., Sharratt, B., Lindstrom, M., Voorhees, W., Carpenter-Boggs, L., (2004) Characterization of soil amended with the by-product of corn stover fermentation. *Soil Science Society of America Journal*, **68**, 139-147.

Johnson, K. N., Kleinman, P. J. A., Beegle, D. B., Elliott, H. A., Saporito, L. S., (2011) Effect of dairy manure application in a no till system on phosphorus runoff. *Nutrient Cycling in Agroecosystems*, **90**, 201-212.

Johnston, A. S. A., Sobly, R. M., Thorbek, P., (2017) Forecasting tillage and soil warming effects on earthworm populations. *Journal of Applied Ecology*, **55**, 1498-1509.

Jones, C. G., Lawton, J. H., Shachak, M., (1994). Organisms as ecosystem engineers. *Oikos*, p.373– 386.

Jongmans, A. G., Pulleman, M. M., Marinissen, J. C. Y., (2001) Soil structure and earthworm activity in a marine silt loam under pasture verses arable land. *Biology and Fertility of Soils*, **33:4**, 279-285.

Joschko M., Diestel H., Larink O., (1989) Assessment of earthworm burrowing efficiency in compacted soil with a combination of morphological and soil physical measurements. *Biology and Fertility of Soils*, **8**, 191–196.

Jouquet, P., Podwojewski, P., Bottinelli, N., Mathieu, J., Ricoy, M., Orange, D., Tran, T., Valentin, C. (2008). Above-ground earthworm casts affect water runoff and soil erosion in Northern Vietnam. *Catena*, **74:1**, 13–21.

Karami, A., Homaee, M., Afzalinia, S., Ruhipour, H., Basirat, S., (2012) Organic resource management: Impacts on soil aggregate stability and other soil physio-chemical properties. *Agriculture, Ecosystems and Environment*, **148**, 22-28.

Kardol, P., Throop, H. L., Adkins, J., de Graaf, M-A., (2016) A hierarchial framework for studying the role of biodiversity in soil food web processes and ecosystem services. *Soil Biology and Biochemistry*, **102**, 33-36.

Kätterer, T., Bolinder, M. A., Andrén, O., Kirchmann, H., Menichetti, L., (2011) Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. *Agriculture, Ecosystems and Environment*, **141**, 184-192.

Kavamura, V. N., Hayat, R., Clark, I. M., Rossmann, M., Mendes, R., Hirsch, P. R., Mauchline, T. H., (2018) Inorganic nitrogen application affects both taxonomical and predicted functional structure of wheat rhizosphere bacterial communities. *Frontiers in Microbiology*, **9**, 1-15.

Kchaou, R., Baccar, R., Bouzid, J., Rejeb, S., (2018) The impact of sewage sludge and compost on winter triticale. *Environmental science and pollution research international*, **25:19**, 18314–18319.

Kennedy, T. F., Connery, J., Fortune, T., Forristal, D., Grant, J., (2013) A comparison of the effects of mimimum-till and conventional-till methods, with and without straw incorporation, on slugs, slug damage, earthworms and carabid beetles in autumn-sown cereals. *The Journal of Agricultural Science*, **151:5**, 609-629.

Kennette, D., Hendershot, W., Tomlin, A., Sauvé, S., (2002) Uptake of trace metals by the earthworm *Lumbricus terrestris* L. in urban contaminated soils. *Applied Soil Ecology*, **19:2**, 191-198.

Kibblewhite, M. G., Ritz, K., Swift, M. J., (2008). Soil health in agricultural systems. Philosophical Transactions of the Royal Society of London. Series B, *Biological sciences*, **363**, p.685–701.

Kidd, J., Manning, P., Simkin, J., Peacock, S., Stockdale, E., (2017) Impacts of 120 years of fertilizer addition on a temperate grassland ecosystem. *PLoS One,* **12:3**, 1-26.

Kilowasid, L. M. H., Herlina, H., Syaf, H., Safuan, L. O., Tufaila, M., Leomo, S., Widiawan, B., (2015) Engineering of soil biological quality from nickel mining stockpile using two earthworm ecological groups. *Journal of Degraded and Mining Land Management*, **2:3**, 361-367.

Kirchberger, J., Eisenhaur, N., Weisser, W. W., Türke, M., (2015) Experimental evaluation of herbivory on live plant seedlings by the earthworm *Lumbricus terrestris* L. in the presence and absence of soil surface litter. *PloS One*, **10:4***,* e0123465.

Kiss, T. B. W., Chen, X., Ponting, J., Sizmur, T., Hodson, M. E., (2021) Dual stresses of flooding and agricultural land use reduce earthworm populations more than the individual stressors. *The Science of the Total Environment*, **754**, 142102–142102.

Kizilkaya, R., (2004) Cu and Zn accumulation by *Lumbricus terrestris* L. in sewage sludge amendments. *Ecological Engineering*, **22:2**, 141-151.

Kizilkaya, R., (2005) The role of different organic wastes on zinc bioaccumulation by earthworm *Lumbricus terrestris* L. (Oligochaeta) in successive Zn enhanced soil. *Ecological Engineering*, **25:4**, 322-331.

Kladivko, E. J., Akhouri, N. M., Weesies, G., (1997) Earthworm populations and species distributions under no-till and conventional tillage in Indiana and Illinois. *Soil Biology and Biochemistry*, **29**, 613-615.

Kladivko, E. J. *(*2001*).* Tillage systems and soil ecology*. Soil and Tillage Research,* **61***,* 61–76*.*

Koch, H. J., Heuer, H., Tomanová, O., Märländer, B., (2008) Cumulative effect of annually repeated passes of heavy agricultural machinery on soil structural properties and sugar beet yield under two tillage systems. *Soil and Tillage Research*, **101**, 69-77.

Kochian, L. V., Piñeros, M. A., and Hoekenga, O. A., (2005) The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. *Plant Soil*, **274**, 175–195.

Kochian, L. V., Piñeros, M. A., Liu, J., and Magalhaes, J. V., (2015). Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. *Annual Review of Plant Biology*, **66**, 571–598.

Koeller, K., (1989) Machinery requirements and possible energy savings by reduced tillage. In: Bäumer, K., Ehlers, W., (Eds.) Agriculture. Energy saving by reduced soil tillage. Commission of the European Communities, Report EUR 11258, 7-16.

Korucu, T., Shipitalo, M.J., Kaspar, T.C., (2018) Rye cover crop increases earthworm populations and reduces losses of broadcast, fall applied, fertilizers in surface run-off. *Soil Tillage and Research*, **180**, 99-106.

Kozlowski, J., JasKulska, M., Kozlowska, M., (2014) Evaluation of the effectiveness of iron phosphate and the parasitic nematode *Phasmarhabditis hermaphrodita* in reducing plant damage caused by the slug *Arion vulgaris Moquin-Tandon*, 1885. *Folia Malacologica,* **22:4,** 293–300.

Kraska, P., (2012) The effect of soil extracts from a monoculture of spring wheat (*Triticum aestivum* L.) grown under different tillage systems on the germination of its seeds. *Acta Agrobotanica*, **64:1**, 79–90.

Księżak, K., Bojarszczuk, J., Staniak, M., (2018) Comparisons of maize yield and soil chemical properties under maize (*Zea Mays L.*) grown in monoculture and crop rotation. *Journal of Elementology*, **23:2**, 531-543.

Kuntz, M., Berner, A., Gattinger, A., Scholberg, J. M. S., Mäder, P., Pfiffner, L., (2013) Influence of reduced tillage on earthworm and microbial communities under organic arable farming. *Pedobiologia*, **56:4-6**, 251–260.

Kuzyakov, Y., Friedel, J. K., Stahr, K., (2000) Review of the mechanisms and quantification of priming effects. *Soil Biology and Biogeochemistry*, **32**, 1485-1498.

Lagerlof, J., Palsson, O., Arvidsson, J., (2012) Earthworms influenced by reduced tillage, conventional tillage and energy forest in Swedish agricultural field environments. Acta Agriculturae Scandinavia: Section B, *Soil and Plant Science*, **62:3**, 235-244.

Lal, R., (2007) Farming Carbon. *Soil Tillage and Research*, **96**, 1-5.

Lal, R., (2008) Crop residues as soil amendments and feedstock for bioethanol production. *Waste Management*, **28**, 747-758.

Laossi, K-R., Ginot, A., Noguera, D. C., Blouin, M., Barot, S., (2010) Earthworm effects on plant growth do not necessarily decrease with soil fertility. *Plant and Soil*, **328:1/2**, 109–118.

Latifi, F., Musa, F., Musa, A. (2020) Heavy metal content in soil and their bioaccumulation in earthworms (*Lumbricus terrestris* L.). *Agriculture and Forestry*, **66:1**, 57-67.

Lavalle, C., Micale, F., Houston, T. D., Camia, A., Hiederer, R., Lazar, C., Conte, C., Amatulli, G., Genovese, G., (2009) Climate change in Europe. 3. Impact on agriculture and forestry. A review. *Agronomy for Sustainable Development*, **29:3**, 433-446.

Lavelle, P., (1988) Earthworm activities and the soil system. *Biology and Fertility of Soils,* **6**, 237-251.

Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P., Heal, O. W. and Dhillion, S. (1997) Soil function in a changing world: The role of invertebrate ecosystem engineers. *European Journal of Soil Biology*, **33:4**, p.159–193.

Lavelle, P., Brussaard, L., Hendrix, P., (1999). Earthworm management in tropical agroecosystems. Wallingford: CABI Publishing.

Lavelle, P., Spain, A. V., (2001) Soil Ecology. Kluwer Academic Publishers, Dordrecht, Netherlands.

Lazcarno, C. Gómez-Brandón, M., Revilla, P., Domínguez, J., (2013) Short term effects of organic and inorganic fertilisers on soil microbial community structure and function. *Biology and Fertility of Soils*, **49**, 723-733.

Le Bayon, R., Binet, F., (1999) Rainfall effects on erosion of earthworm casts and phosphorus transfers by water runoff. *Biology and Fertility of Soils*, **30:1**, 7–13.

Le Bayon, R., Binet, F., (2001) Earthworm surface casts affect soil erosion by runoff water and phosphorus transfer in a temperate maize crop. *Pedobiologia*, **45:5**, 430–442.

Le Bayon, R. C., Moreau, S., Gascuel-Odoux, C., Binet, J., (2002) Annual variations in earthworm surface casting activity and soil transport by water runoff in a temperate maize agroecosystem. *Geoderma*, **106:1**, 121-135.

Le Couteulx, A., Wolf, C., Hallaire, V., Pérès, G. (2015) Burrowing and casting activities of three endogeic earthworm species affected by organic matter location. *Pedobiologia,* **58:2-3**, 97–103.

Lee, K. E., (1985) Earthworms: their ecology and relationships with soil and land use. Academic Press, Sydney.

Lees, K. J., McKenzie, A. J., Newell Price, J. P., Critchley, C. N., Rhymer, C. M., Chambers, B. J., Whittingham, M. J., (2016) The effects of soil compaction mitigation on below-ground fauna: How earthworms respond to mechanical loosening and power harrow cultivation. *Agriculture, Ecosystems and Environment*, **232**, 273-282.

Lehman, R. M., Taheri, W. I., Osborne, S. L., Buyer, J. S., Douds Jr, D. D., (2012) Fall cover cropping can increase arbuscular mycorrhizae in soils supporting intensive agricultural production. *Applied Soil Ecology*, **61**, 300-304.

Lehmann, J., Kleber, M., (2015) The contentious nature of soil organic matter. *Nature,* **528:7580**, pp. 60-68.

Leroy, B. L. M., Van Den Bossche, E., De Neve, S., Reheul, D., Moens, M., (2007) The quality of exogenous organic matter: short term influence on earthworm abundance. *European Journal of Soil Biology*, **43**, S196-S200.

Leroy, B. L. M., Schmidt, O., den Bossche, A. V., Reheul, D., Moens, M., (2008) Earthworm population dynamics as influence the quality of exogenous organic matter. *Pedobiologia*, **52**, 139-150.

Lévêque, T., Capowiez, Y., Schreck, E., Mazzia, C., Auffan, M., Foucault, Y., Austruy, A., Dumat, C. (2013). Assessing ecotoxicity and uptake of metals and metalloids in relation to two different earthworm species (*Eiseina hortensis* and *Lumbricus terrestris*). *Environmental Pollution*, **179**, 232–241.

Lévêque, T., Dumat, C., Lagier, L., Schreck, E., Ruales, J., Capowiez, Y., (2019). Influence of earthworm bioturbation on metals phytoavailability and human gastric bioaccessibility. *Environmental Science and Pollution Research International*, **26:20**, 20052–20063.

Li, H., Shen, J., Zhang, F., Clairotte, M., Drevon, J. J., Le Cadre, E., Hinsinger, P., (2007) Dynamics of Phosphorus fractions in the rhizosphere of common bean (*Phaseolus vulgaris* L.) and durum wheat (*Triticum turgidum durum*, L.) grown in monocropping and intercropping systems. *Plant and Soil*, **312:1-2**, 139-150.

Li, Y., Liu, Q., Liu, F., Zhu, P., Zhang, L., Zhou, X., Sun, C., Cheng, Y., (2016) Effects of Different Ratios of Sewage Sludge and Cattle Manure on Growth and Propagation of *Eisenia Fetida*. *PloS One*, **11:6**, e0156492.

Liang, Q., Chen, H., Gong, Y., Fan, M., Yang, H., Lal, R., Kuzyakov, Y., (2011) Effects of 15 years of manure and inorganic fertilizers on soil organic carbon fractions in a wheat-maize system in the North China Plain. *Nutrient cycling in agroecosystems*, **92:1**, 21–33.

Liebman, M., Graef, R. L., Nettleton, D., Cambardella, C. A., (2011) Use of legume green manures as nitrogen sources for corn production. *Renewable Agriulture and Food Systems*, **27:3**, 180-191.

Lin, Y., King, J. Y., (2014) Effects of UV exposure and litter position on decomposition in a California grassland. *Ecosystems,* **17:1**, 158-168.

Lindstrom, M. J., (1986) Effects of residue harvesting on water runoff, soil-erosion and nutrient loss. *Agriculture, Ecosystems and Environment*, **16**, 103-112.

Little, N. G., Mohler, C. L., Quirine, K. M., DiTommaso, A., (2015) Effects of Organic Nutrient Amendments on Weed and Crop Growth. *Weed science*, **63:3**, 710–722.

Liu, L. H., (2011) 'Greenhouse gases emissions from agricultural residues burning' A case study of Jiangsu province, China. MS thesis, Graduate School, Nanjing Agricultural University. http://www.biochar-international.org/sites/default/files/Straw_burning_revised0708.pdf

Lofs-Holmin A., (1983) Reproduction and growth of common arable land and pasture species of earthworms (Lumbricidae) in laboratory cultures. *Swedish Journal of Agricultural Research*, **13**, 31–37.

Logan, M., Visvanathan, C., (2019) Management strategies for anaerobic digestate of organic fraction of municipal solid waste: Current status and future prospects. *Waste Management and Research*. **37**, 27–39.

Lorenz, K., Lal, R., Preston, C. M., Nierop, K. G. J., (2007) Strengthening the soil organic carbon pool by increasing contributions from recalcitrant aliphatic bio(macro)molecules. *Geoderma,* **142**, 1-10.

Loria, E. R., Sawyer, J. E., (2005) Extractable Soil Phosphorus and Inorganic Nitrogen following Application of Raw and Anaerobically Digested Swine Manure. *Agronomy journal*, **97:3** 879–885.

Lowe C. N., Butt K. R., (1999) Interspecific interactions between earthworms: a laboratory-based investigation. *Pedobiologia*, **43**, 808–817.

Lowe, C. N., Butt, K. R., (2002) Influence of organic matter on earthworm production and behaviour: a laboratory-based approach with applications for soil restoration. *European Journal of Soil Biology*, **38**, 173-176.

Lowe, C. N., Butt, K. R., (2003) Influence of food particle size on inter and intra-specific interactions of *Allolobophora chlorotica* (Savigny) and *Lumbricus terrestris*. *Pedobiologia*, **27**, 574-577.

Lowe, C. N., Butt, K. R., (2005) Culture techniques for soil dwelling earthworms: a review. *Pedobiologica*, **49**, 401-413.

Lowe, C. N., Butt, K. R., (2008) *Allolobophora chlorotica* (Savigny, 1826): Evidence for classification as two separate species. *Pedobiologia*, **52**, 81-84.

Lubbers, I., Van Groenigen, K., Fonte, S., Six, J., Brussaard, L., Van Groenigen, J., (2013) Greenhouse gas emissions from soils increased by earthworms. *Nature Climate Change*, **3:3**, 187-194.

Lubbers, I. M., van Groenigen, K. J., Brussaard, L., van Groenigen, J. W., (2015) Reduced greenhouse gas mitigation potential of no-tillage soils through earthworm activity. *Scientific Reports*, **5:1**, 13787–13787.

Lubbers, I. M., Pulleman, M. M., Van Groenigen, J. W., (2017) Can earthworms simultaneously enhance decompositions and stabilization of plant residue carbon? *Soil Biology and Biochemistry*, **105**, 12-24.

Macholdt, J., Honermeier, B., (2017) Importance of variety choice: Adapting to climate change in organic and conventional farming systems in Germany. *Outlook on Agriculture*, **46:3**, 178-184.

Maillard, É., Angers, D. A., (2014) Animal manure application and soil organic carbon stocks: a meta-analysis. *Global Change Biology*, **20**, 666-679.

Majeed, M., Miambi, E., Barois, L., Blanchart, E., Brauman, A., (2013) Emissions of nitrous oxide from casts of tropical earthworms belonging to different ecological categories. *Pedobiologia*, **56**, 49-58.

Manlay, R. J., Feller, C., Swift, M. J., (2007) Historical evolution of soil organic matter concepts and their relationships with the fertility and sustainability of cropping systems. *Agriculture, Ecosystems and Environment*, **119**, 217-233.

Maraun, M., Alphei, J., Bonknowski, M., Buryn, R., Migge, S., Peter, M., Schaefer, M., Scheu, S., (1999) Middens of the earthworm *Lumbricus terrestris* (Lumbricidae): microhabitats for micro- and mesofauna in forest soil. *Pedobiologia*, **43**, 276-287.

Marinari, S., Masciandaro, B., Ceccanti, B., Grego, S., (2000) Influence of organic and mineral fertilizers on soil biological and physical properties. *Bioresource Technology*, **72**, 9-17.

Martínez-García, L. B., Korthals, G., Brussaard, L., Jørgensen, H. B., De Deyn, G. B., (2018) Organic management and cover crop species steer soil microbial community structure and functionality along with soil organic matter properties. *Agriculture, Ecosystems and Environment*, **263**, 7-17.

Mathew, R. P., Feng, Y., Githinji, L., Ankumah, R., Balkcom, K. S., (2012) Impact of No-Tillage and Conventional Tillage Systems on Soil Microbial Communities. *Applied and Environmental Soil Science*, **2012**, 1–10.

Massey, P. A., Creamer., R. E., Schulte, R. P. O., Whelan, M. J., Ritz, K., (2013) The effects of earthworms, botanical diversity and fertiliser type on the vertical distribution of soil nutrients and plant nutrient acquisition: A mesocosm study. *Biology and Fertility of Soils*, **49:8***,* 1189–1201.

Maute, K., French, K., Story, P., Bull, C. M., Hose, G. C., (2017) Short and long-term impacts of ultra-low-volume pesticide and biopesticide applications for locust control on non-target arid zone arthropods. *Agriculture, Ecosystems and Environment*, **240**, 233-243.

McBratney, A., Field, D. J., Koch, A., (2014) The dimensions of soil security. *Geoderma*, **213**, 203-213.

McDaniel, M. D., Grandy, A.S., Tiemann, L. K., Weintraub, M. N., (2014) Crop rotation complexity regulates the decomposition of high and low quality residues. *Soil Biology and Biogeochemistry*, **78**, 243-254.

McGrath, S. P., Chambers, B. J., Taylor, M. J., Carlton-Smith, C. H., (2012) Biofortification of zinc in wheat grain by the application of sewage sludge. *Plant and Soil*, **361**, 97-108.

McTavish, M. J., Basiliko, N., Sackett, T. E., (2013) Environmental factors influencing immigration behaviour of the invasive earthworm *Lumbricus terrestris*. *Canadian Journal of Zoology*, **91:12***,* 859–865.

Médiène, S., Valantin-Morison, M., Sarthou, J., de Tourdonnet, S., Gosme, M., Bertrand, M., Roger-Estrade, J., Aubertot, J-N., Rusch, A., Motisi, N., Pelosi, C., Dorè, T., (2011) Agroecosystem management and biotic interactions: a review. *Agronomy and Sustainable Development* **31,** 491–514.

Medina-Sauza, R. M., Álvarez-Jiménez, M., Delhal, A., Reverchon, F., Blouin, M., Guerro-Analco, J., Cerdán, C., Guevara, R., Villain, L., Barois, I., (2019) Earthworms building up soil biota: a review. *Frontiers in Environmental Science*, **7:81**, 1-20.

Medková, H., Vačkář, D., Weinzettel, J., (2017) Appropriation of potential net primary production by cropland in terrestrial ecoregions. *Journal of Cleaner Production*, **150**, 294–300.

Menšík, L., Hlisnikovský, L., Pospíšilová, L., Kunzová, E., (2018) The effect of application of organic manures and mineral fertilizers on the state of soil organic matter and nutrients in the long-term field experiment. *Journal of Soils and Sediments*, **18:8**, 2813–2822.

Menta, C., Conti, F. D., Lozano Fondón, C., Staffilani, F., Remelli, S., (2020) Soil Arthropod Responses in Agroecosystem: Implications of Different Management and Cropping Systems. *Agronomy (Basel)*, **10:7**, 982.

Met Office (2019) Record breaking February, mild winter. [Online]. [1 June 2020] Available from: https://www.metoffice.gov.uk/about-us/press-office/news/weather-and-climate/2019/february-and-winter-statistics

Meyer-Wolfarth, F., Schrader, S., Oldenburg, E., Weinert, J., Brunotte, J., (2017). Biocontrol of the toxigenic plant pathogen *Fusarium culmorum* by soil fauna in an agroecosystem. *Mycotoxin Research*, **33:3**, 237–244.

Mickelson, S. K., Boyd, P., Baker, J. L., Ahmed, S. I., (2001) Tillage and herbicide incorporation effects on residue cover, runoff, erosion and herbicide loss. *Soil and Tillage Research*, **60**, 55-66.

Mikutta, R., Kleber, M., Torn, M. S., Jahn, R., (2006) Stabilization of soil organic matter: associations with minerals or chemical recalcitrance? *Biogeochemistry*, **77**, 25-56.

Milcu, A., Schumacher. J., Scheu, S., (2006). Earthworms (*Lumbricus terrestris*) Affect Plant Seedling Recruitment and Microhabitat Heterogeneity. *Functional Ecology*, **20:2**, 261–268.

Milinković, M., Lalević, B., Jovičić-Petrović, J., Golubović-Ćurguz, V., Kljujev, I., Raičević, V., (2019). Biopotential of compost and compost products derived from horticultural waste—Effect on plant growth and plant pathogens' suppression. *Process Safety and Environmental Protection*, **121**, 299–306.

Millennium Ecosystem Assessment, (2005) Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.

Milleret, R., Le Bayon, R-C., Gobat, M-J., (2009) Root, mycorrhiza and earthworm interactions: their effects on soil structuring processes, plant and soil nutrient concentration and plant biomass. *Plant and Soil,* **316:1-2**, 1–12.

Mirsky, S. B., Curran, W. S., Mortenseny, D. M., Ryany, M. R., Shumway, D. L., (2011) Timing of cover crop management effects on weed suppression in no-till planted soybean using a roller-crimper. *Weed Science*, **59:3,** 380-389.

Moffatt A.S., (1998) Global nitrogen overload problem grows critical. *Science,* **279,** 988–989.

Moinard, V., Redondi, C., Etiéviant, V., Savoie, A., Duchene, D., Pelosi, C., Houot, S., Capowiez, Y., (2021) Short- and long-term impacts of anaerobic digestate spreading on earthworms in cropped soils. *Applied Soil Ecology*, **168**, 1041-49.

Möller, K., Müller, T., (2012) Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Engineering in Life Sciences*, **12:3**, 242–257.

Möller, K., Stinner, W., Deuker, A., Leithgold, G., (2008) Effects of different manuring systems with and without biogas digestion on the nitrogen cycle and crop yields in mixed organic dairy systems. *Nutrient Cycling in Agroecosystems*, **82**, 209-232.

Möller, K., Stinner, W., (2009) Effects of different manuring systems with and without biogas digestion on soil mineral nitrogen content and on gaseous nitrogen losses (ammonia, nitrous oxides). *European Journal of Agronomy*. **30:1**, 1–16.

Mombo, S., Laplanche, C., Besson, P., Sammartino, S., Schreck, E., Dumak, C., Capowiez, Y., (2018) Metal soil pollution differentially affects both the behaviour and exposure of *A. caliginosa* and *L. terrestris*: a mesocosm study. *Biology and Fertility of Soils*, **54:3**, 319–328.

Monteiro, F. P., Pacheco, L. P., Lorenzetti, E. R., Armesto, C., De Souza, P. E., De Abreu, M. S., (2012) Substrates and cover crop residues on the suppression of cover crop *Sclerotinia sclerotiorum*. *Science Communications*, **3**, 199-205.

Montusiewicz, S., Szaja, A., Musielewicz, I., Cydzik-Kwiatkowska, A., Lebiocka, M., (2020) ‘Effect of bioaugmentation on digestate metal concentrations in anaerobic digestion of sewage sludge’, *PloS one*, **15:7**, e0235508–e0235508.

Monroy, F., Aira, M., Gago, J.A., Domínguez, J., (2007) Lifecycle of the earthworm *Octadrilus complanatus* (Oligochaeta, Lumbricidae). *Animal Biology and Pathology*, **330**, 389-391.

Monroy, F., Aira, M., Domínguez, J., (2011) Epigeic earthworms increase soil arthropod populations during first steps of decomposition of organic matter. *Pedobiologia*, **54**, 93-99.

Moody, S. A., Briones, M. J. I., Piearce, T. G., Dighton, J., (1995) Selective consumption of decomposing wheat straw by earthworms. *Soil Biology and Biochemistry*, **27:9**, 1209-1213.

Moos, J. H., Schrader, S., Paulsen, H. M., Rahmann, G., (2016) Occasional reduced tillage in organic farming can promote earthworm performance and resource efficiency. *Applied Soil Ecology*, **103**, 22-30

Moran, K. K., Six, J., Horwarth, W. R., van Kessel, C., (2005) Role of mineral-nitrogen in residue decomposition and stable soil organic matter formation. *Soil Science Society of America Journal*, **69**, 1730-1736.

Morris, N. L., Miller, P. C. H., Orson, J. H., Froud-Williams, R. J., (2010) The adoption of non-inversion tillage systems in the United Kingdom and the agronomic impact on soil, crops and the environment – a review. *Soil and Tillage Research*, **108**, 1-15.

Mulumba, L., N., Lal, R., (2008) Mulching effects on selected soil physical properties. *Soil and Tillage Research*, **98**, 106-111.

Murtaza, G., Usman, Y., Niazi, N., Usman, M., Hussain, T., (2017) Bioaccumulation of Potentially Toxic Elements in Cereal and Legume Crops: A Review. *CLEAN – Soil, Air, Water*, *45*:12, 1700548.

Naderi, R., Edalat, M., Egan, T. P., (2018) 'Effects of urea nitrogen and residual composted municipal waste and sheep manure on the growth and chemical composition of two triticale genotypes', *Journal of plant nutrition*, **41:2**, 178–185.

Nason, M., Williamson, J., Tandy, S., Christou, M., Jones, D., Healey, J., (2007) Using organic wastes and composts to remediate and restore land: Best practice manual. Bangor, School of Natural Resources, Bangor University.

Nayal, F. S., Mammadov, A., Ciliz, N., (2016) Environmental assessment of energy generation from agricultural and farm waste through anaerobic digestion. *Journal of Environmental Management*, **184**, 389-399.

Neuhauser, E. F., Meyer, J. A., Malenki, M. R., Thomas, J. M., (1984) Dietary cobalt supplements and the growth and reproduction of the earthworm *Eisenia fetida*. *Soil Biology and Biochemistry*, **16**, 521-523.

Nevins, C. J., Nakatsu C., Armstrong, S., (2018) Characterization of microbial community response to cover crop residue decomposition. *Soil Biology and Biochemistry*, **127**, 39-49.

Nicholson, F., Bhogal, A., Rollett, A., Taylor, M., Williams, J., (2018) Precision application techniques reduce ammonia emissions following food-based digestate applications to grassland. *Nutrient Cycling in Agroecosystems*, **110:1**, 151–159.

Nieminen, M., Hurme, T., Mikola, J., Regina, K., Nuutinen, V., (2015) Impact of earthworm *Lumbricus terrestris* living sites on the greenhouse gas balance of no-till arable soil. *Biogeosciences*, **12:18**, 5481-5493.

Nkoa, R., (2013) Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. *Agronomy and Sustainable Development*, **34:2**, 473-492.

Nordström, S., Rundgren, S., (1974) Environmental factors and lumbricid associations in southern Sweden. *Pedobiologia,* **14**, 1–27

Nuutinen, V., (2011) The meek shall inherit the burrow: Feedback in earthworm soil modification. In: Karaca, A. (ed.) The Biology of Earthworms. Springer, Heidelberg, 123-140.

Nuutinen V., Butt, K. R., (1997) The mating behaviour of *Lumbricus terrestris* (Oligochaeta; Lumbricidae). *Journal of Zoology*, **242:4**, 783-798.

Nuutinen, V., Butt, K. R., (2003) Interactions of *Lumbricus terrestris* burrows with field subdrains. *Pedobiologia*, **47**, 578-581.

Nuutinen, V., Butt, K. R., (2005) Homing ability widens the sphere of the earthworm *Lumbricus terrestris* L. *Soil Biology and Biochemistry*, **37:4**, 805-807.

Nuutinen, V., Butt, K. R., (2009). Worms from the cold: Lumbricid life stages in boreal clay during frost. *Soil Biology and Biochemistry*, **41:7**, 1580–1582.

Nuutinen, V., Butt, K. R., (2019) Earthworm dispersal of plant litter across the surface of agricultural soils. *Ecology* **100**: e02669.

Nuutinen, V., Butt, K., Jauhiainen, L., (2011) Field margins and management affect settlement and spread of an introduced dew-worm (*Lumbricus terrestris* L.) population. *Pedobiologia - International Journal of Soil Biology*, **54**, S167–S172.

Nuutinen, V., Butt, K. R., Hyväluoma, J., Ketoja, E., Mikola, J., (2017) Soil faunal and structural responses to the settlement of a semi-sedentary earthworm *Lumbricus terrestris* in an arable clay field. *Soil Biology and Biochemistry*, **115**, 285-296.

Nuutinen, V., Butt, K. R., Jauhiainen, L., Shipitalo, M. J., Sirén, T., (2014) Dew worms in white lights: High latitude light constrains earthworm (*Lumbricus terrestris*) behaviour at the soil surface. *Soil Biology and Biochemistry*, **72**, 66-74.

Omara, P., Macnack, N., Aula, L., Raun, B., (2017) Effect of long-term beef manure application on soil test phosphorus, organic carbon, and winter wheat yield. *Journal of Plant Nutrition*, **40:8**, 1143-1151.

Ondreičková, K., Piliarová, M., Klčová, L., Žofajová, A., Gubiš, J., Horník, M., Gubišová, M., Hudcovicová, M., Kraic, J., (2021). The impact of sewage sludge on the fungal communities in the rhizosphere and roots of barley and on barley yield. *Open Life Sciences*, **16:1**, 210–221.

Onrust, J., Piersma, T., (2019) How dairy farmers manage the interactions between organic fertilizers and earthworm ecotypes and their predators. *Agriculture, Ecosystems and Environment*, **273**, 80-85.

Orazova, M. K., Semenova, T. A., Tiunov, A. V., (2003) The microfungal community of *Lumbricus terrestris* middens in a linden (*Tilia cordata*) forest. *Pedobiologia*, **47**, 27-32.

Orzi, V., Riva, C., Scaglia, B., D'Imporzano, G., Tambone, F., Adani, F., (2018) Anaerobic digestion coupled with digestate injection reduced odour emissions from soil during manure distribution. *Science of the Total Environment*, **621**, 168–176.

Øvsthus, I., Seljåsen, R., Stockdale, E., Uhlig, C., Torp, T., Breland, T. A., (2017) Yield, nitrogen recovery efficiency and quality of vegetables grown with organic waste-derived fertilisers. *Nutrient Cycling in Agroecosystems,* **109:3**, 233-248.

Pagliai, M., Guidi, G., La Marca, M., Giachetti, M., Lucamante, G., (1981) Effects of sewage sludges and composts on soil porosity and aggregation. *Journal of Environmental Quality*, **10**, 556-61.

Pagliai, M., Vittori Antisari, L., (1993) Influence of waste organic matter on soil micro- and mactostructure. *Bioresource Technology*, **43**, 205-213.

Palm, C.*,* Blanco-Canqui, H.*,* DeClerck, F.*,* Gatere, L.*,* Grace, P., (2014) Conservation agriculture and ecosystem services: An overview*. Agriculture, Ecosystems & Environment,* **187***,* 87–105*.*

Pang, J., Qiao, Y., Sun, Z., Zhang, S., Li, Y., Zhang, R., (2012) Effects of Epigeic Earthworms on Decomposition of Wheat Straw and Nutrient Cycling in Agricultural Soils in a Reclaimed Salinity Area: A Microcosm Study. *Pedosphere*, **22:5**, 726-735.

Pang, X. P., Letey, J., (1998) Organic farming challenge of timing nitrogen availability to crop nitrogen requirements. *Soil Science Society of America Journal*, **64:1**, 247-253.

Parkin T. B., Berry, E. C., (1999) Microbial nitrogen transformations in earthworm burrows. *Soil Biology and Biochemistry* **31:13**, 1765–1771.

Parmar, K. R., Ross, A. B., (2019) ‘Integration of Hydrothermal Carbonisation with Anaerobic Digestion; Opportunities for Valorisation of Digestate’, *Energies (Basel)*, **12:9**, 1586.

Parry, M. A. J., Hawesford, M. J., (2010) Food security: increasing yield and improving resource use efficiency. *Proceedings of the Nutrition Society*, **69**, 592-600.

Parker, C. F., Sommers, L. E., (1983) Mineralization of nitrogen in sewage sludges. *Journal of Environmental Quality*, **12:1**, 150-156.

Paschold, J. S., Wienhold, B. J., Ferguson, R. B., McCallister, D. L., (2008) Soil nitrogen and phosphorus availability for field applied slurry from swine fed traditional and low-phytate corn. *Soil Science Society of America Journal*, **72:4**, 1096-1101.

Paul, B. K., Lubbers. I. M., van Groenigen, J. W., (2011) Residue incorporation depth is a controlling factor of earthworm-induced nitrous oxide emissions. *Global Change Biology*, **18:3**, 1141–1151.

Peigné, J., Ball, B. C., Roger-Estrade, J., David, C., (2007) Is conservation tillage suitable for organic farming. *Soil Use Management*, **23**, 129-144.

Peigné, J., Cannavaciuolo, M., Gautronneau, Y., Aveline, A., Giteau, J. L., Cluzeau, D., (2009) Earthworm populations under different tillage systems in organic farming. *Soil Tillage Research*, **104**, 207-214.

Peigné, J., Vian, J-F., Payet, V., Saby, N. P. A., (2018) Soil fertility after 10 years of conservation tillage in organic farming. *Soil and Tillage Research*, **175**, 194-204.

Pelosi, C., Bertrand, M., Roger-Estrade, J., (2009) Earthworm community in conventional, organic and direct seeding with living mulch cropping systems. *Agronomy and Sustainable Development*, **29**, 287-295.

Pelosi, C., Grandeau, G., Capowiez, Y., (2017) Temporal dynamics of earthworm-related macroporosity in tilled and non-tilled cropping systems. *Geoderma*, **289**, 169-177.

Pelosi, C., Pey, B., Caro, G., Cluzeau, D., Peigne, J., Bertrand, M., Hedde, M., (2016) Dynamics of earthworm taxonomic and functional diversity in ploughed and no-tilled cropping systems. *Soil and Tillage Research*, **156**, 25-32.

Pelosi, C., Pey, B., Hedde, M., Caro, G., Capowiez, Y., Guernion, M., Peigne, J., (2014) Reducing tillage in cultivated fields increases earthworm functional diversity. *Applied Soil Ecology*, **83**, 79-87.

Peltre, C., Gregorich, E., Bruun, S., Jensen, L., Magid, J. (2017) Repeated application of organic waste affects soil organic matter composition: Evidence from thermal analysis, FTIR-PAS, amino sugars and lignin biomarkers. *Soil Biology and Biochemistry*, **104**, 117–127.

Peng, W., Lü, F., Hao, L., Zhang, H., Shao, L., He, P., (2020) Digestate management for high-solid anaerobic digestion of organic wastes: a review. *Bioresource technology*, **297**, 122485.

Perrault, J. M., Whalen, J. P., (2006) Earthworm burrowing in laboratory microcosms as influenced by soil moisture and temperature. *Pedobiologia*, **50:5**, 397-403.

Persson J., Kirchmann, H., (1994) Carbon and nitrogen in arable soils as affected by a supply of N fertilizers and organic manures. *Agriculture, Ecosystems and Environment*, **51:1-2**, 249-255.

Peterson, C. A., Eviner, V. T., Gaudin, A. C. M., (2019) Ways forward for resilience research in agroecosystems. *Agricultural Systems*, **162**, 19-27.

Petrik, I., Mustafić, N., (2015) Dynamic modelling of the composting process of the mixture of poultry manure and wheat straw. *Journal of Environmental Management*, **161**, 392-401.

Pfiffner, L., Mäder, P., (1997) Effects of biodynamic, organic and conventional production systems on earthworm populations. *Biological Agriculture and Horticulture*, **15:1-4**, 2-10.

Phillips, H., R., P., Guerra, C., A., Bartz, M.L.C., Briones, M. J. I., Brown, G., Crowther, T. W., Ferlian, O., Gongalsky, K.B., van den Hoogen, J., Krebs, J., Orgiazi, A., Routh, D., Schwarz, B., Bach, E. M., Bennet, J., Brose, U., Decaëns, B., König-Ries, B., Loreau, M., Mathieu, J., Mulder, C., van der Puttern, W. H., Ramirez, K. S., Rillig, M. C., Russell, D., Rutgers, M., Thakur, M. P., de Vries, F. T., Wall, D. H., Wardle, D. A., Arai, M., Ayuke, F. O., Baker, G. H., Beauséjour, R., Bedano, J. C., Birkhofer, K., Blanchart, E., Blossey, B., Bolger, T., Bradley, R. L., Callaham, M. A., Capowiez, Y., Caulfield, M. E., Choi, A., Crotty, F. V., Dávalos, A., Cosin, D. J. D., Domínguez, A., Durhour, A. E., van Eekeren, N., Emmerling, C., Falco, L. B., Fernández, R., Fonte, S. J., Fragoso, C., Franco, A. L. C., Fugère, M., Fusilero, A. T., Gholami, S., Gundale, M. J., López, M. G., Hackenberger, D. K., Hernández, L. M., Hishi, T., Holdsworth, A. R., Holmstrup, M., Hopfensperger, K. N., Lwange, E. H., Huhta, V., Hurisso, T. T., Iannone, B. V., Lordache, M., Joshko, M., Kaneko, N., Kanianska, R., Keith, A. M., Kelly, C. A., Hernecker, M. L., Klaminder, J., Koné, A. W., Kooch, Y., Kukkonen, S. T., Lalthanzara, H., Lammel, D. R., Lebedev, I. M., Li, Y., Lidon, J. B. J., Lincoln, N. K., Loss, S. R., Marichal, R., Matula, R., Moos, J. H., Moreno, G., Morón-Ríos, A., Muys, B., Neirynck, J., Norgrove, L., Novo, M., Nuutinen, V., Nuzzo, V., Rahman, P. M., Pansu, J., Paudel, S., Pérèz, G., Pérez-Camacho, L., Pineiro, R., Ponge, J-F., Rashid, M. I., Rebollo, S., Rodeiro-Iglesias, J., Rodríguez, M. Á., Roth, A. M., Rousseau, G. X., Rozen, A., Sayad, E., van Schaik, L., Scharenbroch, B.C., Schirrmann, M., Schmidt, O., Schröder, B., Seeber, J., Shashkov, M. P., Singh, J., Smith, S. M., Steinwandter, M., Talavera, J. A., Trigo, D., Tsukamoto, J., de Valenca, A. W., Vanek, S. J., Virto, I., Wackett, A. A., Warren, M. W., Wehr, N. H., Whalen, J. K., Wironen, M. B., Wolters, V., Zenkova, I. V., Zhang, W., Cameron, E. K., Eisenhauer, N., (2019) Global distribution of earthworm diversity. *Science*, **366**, 480–485. https://doi.org/10.1126/science.aax4851

Piccolo, A., Mbagwu, J. S. C., (1990) Effects of different organic waste amendments on soil microaggregates stability and molecular sizes of humic substances. *Plant and Soil*, **123**, 27-37.

Piearce, T. G., (1978) Gut contents of some lumbricid earthworms. *Pedobiologia*, **18**, 153-157.

Piearce, T. G., Boone, G. C., (1998) Responses of invertebrates to paper sludge application to soil. *Applied Soil Ecology*, **9**, 393-397.

Pierie, F., Dsouza, A., van Someren, C. E. J., Benders, R. M. J., van Gemert, W. J. T., Moll, H. C., (2017) Improving the sustainability of farming practices through the use of a symbiotic approach for anaerobic digestion and digestate processing. *Resources*, **6**, **50**, 1-23.

Pinto, R., Brito, L. M., Coutinho, J., (2017) Organic production of horticultural crops with green manure, composted farmyard manure and organic fertiliser. *Biological Agriculture and Horticulture*, **33:4**, 269–284.

Piron, D., Boizard, H., Heddadj, D., Pérèz, G., Hallaire, V., Cluzeau, D., (2017) Indicators of earthworm bioturbation to improve visual assessment of soil structure. *Soil Tillage and Research*, **173**, 53-63.

Pitkänen, J., Nuutinen, V. (1997) Distribution and abundance of burrows formed by *Lumbricus terrestris* L. and *Aporrectodea caliginosa* Sav. in the soil profile. *Soil Biology and Biochemistry*, ***29*:3**, 463–467.

Postma-Blaauw, M., Bloem, J., Faber, J., van Groenigen, J., de Goede, R., Brussaard, L. (2006) Earthworm species composition affects the soil bacterial community and net nitrogen mineralization. *Pedobiologia*, **50:3**, 243–256.

Potvin, L. R., Lilleskov, E. A., (2017) Introduced earthworm species exhibited unique patterns of seasonal activity and vertical distribution, and *Lumbricus terrestris* burrows remained usable for at least 7 years in hardwood and pine stands. *Biology and Fertility of Soils*, **53:2**, 187-198.

Poudel, D. D., Horwarth, W. R., Mitchell, J. P., Temple, S. R., (2001) Impacts of cropping systems on soil nitrogen storage and loss. *Agricultural Systems*, **68**, 253-268.

Powlson, D. S., Brookes, P. C., Christensen, B. T., (1987) Measurement of soil microbial biomass provide an early of changes in total soil organic matter due to straw incorporation. *Journal of Soil Biology and Biochemistry*, **19**, 159-64.

Puget, P., Chenu, C., Balesdent, J., (2000) Dynamics of soil organic matter associated with particle-size fractions of water-stable aggregates. *European Journal of Soil Science*, **51**, 595-605.

Pulleman, M. M., Bourma, J., van Essen, E. A., Meijles, E. W., (2000) Soil organic matter content as a function of different land use history. *Soil Science Society of America Journal*, **64**, 689-693.

Pulleman, M. M., Six., J., van Breemen, N., Jongmans, A. G., (2005) Soil organic matter distribution and microaggregate characteristics as affected by agricultural management and earthworm activity. *European Journal of Soil Science*, **56**, 453-467.

Pulleman, M., Creamer, R., Hamer, U., Helder, J., Pelosi, C., Pérès, G., Rutgers, M., (2012) Soil biodiversity, biological indicators and soil ecosystem services-an overview of European approaches. *Current Opinion in Environmental Sustainability*, **4:5**, p.529–538.

Råberg, T., Carlsson, G., Jensen, E. S., (2018) Nitrogen balance in a stockless organic cropping system with different strategies for internal N cycling via residual biomass. *Nutrient Cycling in Agroecosystems*, **112**, 165-178.

Radford, B. J., Cowie, B. A., (2011) Surface stratification of soil nutrients in no-till limits nutrient availability and reduces grain yield. *Environmental Research Journal*, **5:5**, 577-591.

Rajapaksha, N. S. S., Butt, K. R., Vanguelova, E. I., Moffat, A. J., (2013) Earthworm selection of Short Rotation Forestry leaf litter assessed through preference testing and direct observation. *Soil Biology and Biochemistry*, **67**, 12-19.

Ratnadass, A., Fernandes, P., Avelino, J., Habib, R., (2012) Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. *Agronomy for Sustainable Development*, **32**, 273-303.

Reddell, P., Spain, A., (1991) Earthworms as vectors of viable propagules of mycorrhizal fungi. *Soil Biology and Biochemistry*, **23:8**, 767–774.

Redin, M., Recous, S., Aita, C., Dietrich, G., Skolaude, A., Ludke, W., Schmatz, R., Giacomini, S. (2014) How the chemical composition and heterogeneity of crop residue mixtures decomposing at the soil surface affects C and N mineralization. *Soil Biology and Biochemistry*, **78**, 65–75.

Reeleeder, R. D., Miller, J. J., Ball Coelho, B. R., Roy, R. C., (2006) Impacts of tillage, cover crop, and nitrogen on populations of eartwhorms, microarthropods, and soil fungi in a cultivated fragile soil. *Applied Soil Ecology*, **33**, 243-257.

Regina, K., Alakukku, L., (2010) Greenhouse gas fluxes in varying soil types under conventional and no-tillage practices. *Soil Tillage and Research*, **109**, 144-152.

Reijnders, L., (2014) Phosphorus sources, their depletion and conservation: a review. *Resources, Conservation and Recycling*, **93**, 32-49.

Ren, A., Abbott, L., Chen, Y., Xiong, Y., Mickan, B., (2020) Nutrient recovery from anaerobic digestion of food waste: impacts of digestate on plant growth and rhizosphere bacterial community composition and potential function in ryegrass. *Biology and Fertility of Soils*. [online] https://doi.org/10.1007/s00374-020-01477-6

Resner, K., Yoo, K, Sebestyen, S. D., Aufdenkampe, A., Hale, C., Lyttle, A., Blum, A., (2015) Invasive Earthworms Deplete Key Soil Inorganic Nutrients (Ca, Mg, K, and P) in a Northern Hardwood Forest. *Ecosystems (New York)*, **18:1**, 89–102.

Rickson, R. J., Deeks, L. K., Graves, A., Harris, J. A. H., Kibblewhite, M. G., Sakrabani, R., (2015) Input constraints to food production: the impact of soil degradation. *Food Security*, **7**, 351-364.

Riley, H., Pommeresche, R., Eltun, R., Hansen, S., Korsaeth, A., (2008) Soil structure, organic matter and earthworm activity in a comparison of cropping systems with contrasting tillage, rotations, fertilizer levels and manure use. *Agriculture, Ecosystems and Environment*, **124**, 275-284.

Rinkes, Z. L., DeForest, J. L., Grandy, S. A., Moorhead, D. L., Weintraub, M. N., (2014) Interactions between leaf litter quality, particle size and microbial activity during the earliest stages of decay. *Biogeochemistry*, **117:1**, 153-168.

Riva, C., Orzi, V., Carozzi, M., Acutis, M., Boccasile, G., Lonati, S., Tabomne, F., D'Imporzano, G., Adani, F. (2016) Short-term experiments in using digestate products as substitutes for mineral (N) fertilizer: Agronomic performance, odours, and ammonia emission impacts. *Science of the Total Environment*, **547**, 206–214.

Riverford Organic Farmers (no date) https://www.riverford.co.uk/ (last accessed 01 November 2020).

Roarty, S., Hackett, R. A., Schmidt, O., (2017) Earthworm populations in twelve cover crop and weed management combinations. *Applied Soil Ecology*, **114**, 142-151.

Rolette, A. J., Bhogal, A., Scullion, J., Nicholson, F. A., Taylor, M. J., Williams, J. R., (2020) The effect of field application of food-based anaerobic digestate on earthworm populations. *Soil Use and Management*, 1-10.

Ros, M. B. H., Hiemstra, T., van Groenigen, J. W., Chareesri A., Koopmans, G. F., (2017) Exploring the pathways of earthworm-induced phosphorus availability. *Geoderma*, **303**, 99-109.

Roubíčková, A., Frouz, J., (2014). Performance of the earthworm *Aporrectodea caliginosa* on unreclaimed spoil heaps at different successional stages. *European Journal of Soil Biology*, **65**, 57–61.

Rousk, J., Brookes, P. C., Bååth, E., (2009) Contrasting soil pH effects on fungal and bacterial growth suggests functional redundancy in carbon mineralization. *Applied and Environmental Microbiology*, **75**, 1589-1596.

Rowcliffe, J. M., Kays, R., Kranstauber, B., Carbone, C., Jansen, P. A., (2014) Quantifying levels of animal activity using camera trap data. *Methods in Ecology and Evolution*, **5**, 1170-1179.

Ruiz Diaz, D. A., Sawyer, J. E., Barker, D. W., Mallarino, A. P., (2010) Runoff nitrogen loss with simulated rainfall immediately following poultry manure application for corn production. *Soil Science Society Journal of America*, **74:1** 221-230.

Rusinamhodzi, L., Corbeels, M., van Wijk, M. T., Rufino, M. C., Nyamangara, J., Giller, K. E., (2011) 'A meta-analysis of long-term effects of conservation agriculture on maize grain yield under rain-fed conditions', *Agronomy for sustainable development*, **31:4**, 657–673.

Ryals, R., Hartman, M. D., Parton, W. J., Delonge, M. C., Silver, W. L., (2015) Long-term climate change mitigation potential with organic matter management on grasslands. *Ecological Applications*. **25:2**, 531–545.

Ryals, R., Silver, W. L., (2013) Effects of organic matter amendments on net primary productivity and greenhouse gas emissions in annual grasslands. *Ecological Applications*, **23:1**, 46-59.

Sainju, U. M., Allen, B. L., Caesar-TonThat, T., Lenssen, A. W., (2015) Dryland soil chemical properties and crop yields affected by long-term tillage and cropping sequence. *SpringerPlus*, **4:320**.

Samson, M-E., Menasseri-Aubry, S., Chantigny, M. H., Angers, D. A., Royer, I., Vanasse, A., (2019) Crop response to soil management practices is driven by interactions among practices, crop species and soil type. *Field crops research*, **243**, 107623.

Sanchez, J. E., Willson, T. C., Kizilkaya, K., Parker, E., Harwood, R. R., (2001) Enhancing the mineralizable nitrogen pool through substrate diversity in long-term cropping systems. *Soil Science Society of America Journal*, **65**, 1442-1447.

Satchell, J. E., (1967) Earthworms. In: Burges A, Raw F (eds) Soil biology. Academic Press, London, New York, 259–322.

Scheu, S., (1987) The role of substrate feeding earthworms (Lumbricadae) for bioturbation in a beechwood soil. *Oecologia*, **72**, 192-196.

Schipanski, M. E., Barbercheck, M., Douglas, M. R., Finney, D. M., Haider, K., Kaye, J. P., Kemanian, A. R., Mortensen, D. A., Ryan, M. R., Tooker, J., (2014) A framework for evaluating ecosystem services provided by cover crops in agroecosystems. *Agricultural Systems,* **125,** 12–22.

Schlatter, D. C., Kahl, K., Carlson, B., Huggins, D. R., Paulitz, T., (2018) Fungal community composition and diversity vary with soil depth and landscape position in a no-till based wheat cropping system. *FEMS Microbiology Ecology*, **94:7**, FIY098.

Schlatter, D. C., Reardon, C. L., Johnson-Maynard, J., Brooks, E., Khal, K., Norby, J., Huggins, D., Paulitz, T. C., (2019) Mining the Drilosphere: Bacterial Communities and Denitrifier Abundance in a No-Till Wheat Cropping System. *Frontiers in Microbiology*, **10**, 1339–1339.

Schlegel, A. J., Assefa, Y., Haag, L. A., Thompson, C. R., Stone, L. R., (2018) Long-term tillage on yield and water use of grain sorghum and winter wheat. *Agronomy Journal*, **110:1**, 269-280.

Schlerbak, L., Millar, N, Robertson, G.P., (2014) Global meta-analysis of the nonlinear response of soil nitrous oxide (N_2O) emissions to fertilizer nitrogen. *PNAS*, **111**, 9199.

Schlüter S., Großmann, C., Diel, J., Wu, G-M., Tischer, S., Deubel, A., Rücknagel, J., (2018) Long-term effects of conventional and reduced tillage on soil structure, soil ecological and hydraulic properties. *Geoderma*, **332**, 10-19.

Schmidt, O., Curry, J. P., Hackett, R. A., Purvis, G, Clements, R. O., (2001) Earthworm communities in conventional wheat monocropping and low-input wheat-clover intercropping systems. *Annals of Applied Biology*, **138**, 377-388.

Schmidt, O., Scrimgeour, C., Handley, L., (1997) Natural abundance of ^{15}N and ^{13}C in earthworms from a wheat and a wheat-clover field. *Soil Biology & Biochemistry*, **29:9**, 1301–1308.

Schmidt, M. W., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kögel-Knabner, L., Lehmann, J., Manning, D. A., Nannipieri, P., Rasse, D. P., Weiner, S., Trumbore, S. E., (2011) Persistence of soil organic matter as an ecosystem property. *Nature*, **478**, 49-56.

Schrader, S., (1992) Energy dispersive X-ray microanalysis of the calciferous glands of *Lumbricus terrestris* L. (Oligochaeta) contaminated with heavy metals. *Soil Biology and Biochemistry*, 24:12, 1755-1761.

Schrader, S., Seibel, C., (2001) Impact of cultivation management in an agroecosystem on hotspot effects of earthworm middens. *European Journal of Soil Science*, **37**, 309-313.

Schrader, S., Zhang, H., (1997) Earthworm casting: Stabilization or destabilization of soil structure? *Soil Biology and Biochemistry*. **29:3-4**, 469–475.

Scullion, J., Neale, S., Philipps, L., (2002) Comparisons of earthworm populations and cast properties in conventional and organic arable rotations. *Soil Use and Management*, **18**, 293-300.

Searle, S., Malins, C., (2013) 'Availability of cellulosic residues and wastes in the EU' *International Council on Clean Transportation*, White Paper, 1-29. https://www.theicct.org/sites/default/files/publications/ICCT_EUcellulosic-waste-residues_20131022.pdf

Shah, A., Tanveer, M., Shahzad, B., Yang, G., Fahad, S., Ali, S., Bukhari, M. A., Tung, S. A., Hafeez, A., Souliyanonh, B., (2017) Soil compaction effects on soil health and crop productivity: an overview. *Environmental Science and Pollution Research*, **24:11**, 10056-10067.

Shaver, T., Peterson, G., Ahuja, L., Westfall, D., (2002) Surface soil physical properties after twelve years of dryland no-till management. *Soil Science Society of America Journal*, **66:4**, 1296-1303.

Sheehy, J., Nuutinen, V., Six, J., Palojärvi, A., Knuutila, O., Kaseva, J., Regina, K., (2019) Earthworm *Lumbricus terrestris* mediated redistribution of C and N into large macroaggregate-occluded soil fractions in fine textured no-till soils. *Applied Soil Ecology*, **140**, 26-34.

Shipitalo, M. J., Le Bayon, R. C., (2004) Quantifying the effects of earthworms on soil aggregation and porosity. In: Edwards, C. A. (Ed.), Earthworm Ecology, 2nd edition, 183-200.

Shipitalo, M. J., Protz, R., (1988) Factors influencing the dispersibility of clay in worm casts. *Soil Science Society of America Journal*, **52:3**, 764-769.

Shipitalo, M. J., Protz, R., Tomlin, A. D., (1988) Effect of diet on the feeding and casting activity of *Lumbricus terrestris* and *Lumbricus rubellus* in laboratory culture. *Soil Biology and Biochemistry*, **20**, 233-237.

Shiwakoti, S., Zheljazkov, V. D., Gollany, H. T., Kleber, M., Xing, B., Astatkie, T., (2019) Micronutrients in the soil and wheat: Impact of 84 years of organic or synthetic fertilization and crop residue management. *Agronomy*, **9**, 464, 1-13.

Simberloff, D., (2006) Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? *Ecology Letters*, **9:8**, 912– 919.

Simonsen, J., Posner, J., Rosemeyer, M., Baldock, J., (2010) Endogeic and anecic earthworm abundance in six Midwestern cropping systems. *Applied Soil Ecology*, **44:2**, 147–155.

Sims, J. T., Simard, R. R., Joern, B. C., (1998) Phosphorus loss in agricultural drainage: historical perspective and current research. *Journal of Environmental Quality*, **27**, 277-293.

Sims, R., Gerard, B., (1985) *Earthworms: Keys and Notes For The Identification And Study Of The Species*, n.p.: London: E.J. Brill/W. Backhuys for the Linnean Society of London and the Estuarine and Brackish-water Sciences Association.

Sims R. W., and Gerard B. M., (1999) *Earthworms: Notes for the identification of British species*. No. 31, Linnean Society of London and the Estuarine and Coastal Sciences Association.

Singh, A., (2015) Aerobic and Anaerobic Transformations in Estrogens and Nutrients in Swine Manure: Environmental Consequences. *Agriculture (Basel)*, **5:3**, pp. 697–712.

Singh, H., (1995) Nitrogen mineralization, microbial biomass and crop yield as affected by wheat residue placement and fertilizer in a semi arid tropical soil with minimal tillage. *Journal of Applied Ecology*, **32**, 588-595.

Singh, H., Singh, K. P., (1994) Nitrogen and phosphorus availability and mineralization in dryland reduced tillage cultivation: effects of residue placement and chemical fertilizer. *Soil Biology and Biochemistry*, **26:6**, 695-702.

Singh, J., Schädler, M., Demetrio, W., Brown, G. G., Eisenhauer, N., (2020) Climate change effects on earthworms - a review. *Soil Organisms*, **91:3**, 113–137.

Singh, K., Bhartiya, D. K., Chauhan, H. K., Rai, R., Singh, R. N., (2014) Bioaccumulation of Cobalt and Lead by earthworm *Eisenia fetida* from sewage sludge with different cattle dung during vermicomposting. *Research Journal of Science and Technology*, **6:4**, 174-179.

Singh, P., Heikkinen, J., Ketoja, E., Nuutinen, V., Palojärvi, A., Sheehy, J., Esala, M., Mitra, S., Alakukku, L., Regina, K., (2015) Tillage and crop residue management methods had minor effects on stock and stabilization of topsoil carbon in a 30-year field experiment. *Science of the Total Environment*, **518-519**, 337-344.

Six, J., Conant, R. T., Paul, E. A., Paustian, K., (2002) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant and Soil*, **241**, 155-176.

Six, J., Elliott, E. T., Paustian, K., (2000) Soil structure and soil organic matter: II A normalized stability index and the effect of mineralogy. *Soil Science Society of America Journal*, **64**, 1042-1049

Sizmur, T., Martin, E., Wagner, K., Parmentier, E., Watts, C., Whitmore, A., (2017) Milled cereal straw accelerates earthworm (*Lumbricus terrestris*) growth more than selected organic amendments. *Applied Soil Ecology*, **113**, 166–177.

Sizmur, T., Palumbo-Roe, B., Watts, M., Hodson, M., (2011) Impact of the earthworm *Lumbricus terrestris* (L.) on As, Cu, Pb and Zn mobility and speciation in contaminated soils. *Environmental Pollution*, **159:3**, 742–748.

Skidmore, E. L., Layton, J. B., Armbrust, D. V., Hooker, M. L., (1986) Soil physical properties as influenced by cropping and residue management. *Soil Science Society of America Journal*, **50**, 415-419.

Smith, J., Nayak, D., Albanito, F., Balana, B., Black, H. I. J., Boke, S., Brand, A., Byg, A., Dinato, M., Habte, M., Hallett, P., Lemma Argaw, T., Mekuria, W., Moges, A., Muluneh, A., Novo, P., Rivington, M., Tefera, T., Vanni, M., Yakob, G., Phimister, E., (2019) Treatment of organic resources before soil incorporation in semi-arid regions improves resilience to El Nino, and increases crop production and economic returns. *Environmental Research Letters*, **14**, Article No. 085004.

Smith, P., Ashmore, M. R., Black, H. I. J., Burgess, P. J., Evans, C. D., Quine, T. A., Thomson, A.M., Hicks, K., Orr, H. G., (2013) The role of ecosystems and their management in regulating climate, and soil, water and air quality. *Journal of Applied Ecology*, **50**, 812-829.

Smith P., Bustamante, M., Ahammad, H., (2014) Agriculture, forestry and other land use (AFOLU). In: Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (eds Edenhofer, O. Madruga, R., Sokona, Y., Farahani, E., Kadner, S., Seyboth, K., Adler, A., Baum, I., Brunner, S., Eickemeier, P., Kriemann, B., Savolainen, J., Schlömer, S., von Stechow, C., Zwickel, T., Minx, J. C.) Cambridge University Press, Cambridge, and New York, NY.

Smith, R. G., Gross, K. L., Januchowski, S., (2005) Earthworms and weed distribution in annual crops. *Agriculture, Ecosystems and Environment*, **108**, 363-367.

Soane, B. D., Ball, B.C., Arvidsson, J., Basch, G., Moreno, F., Roger-Estrade, J., (2012) No-till in northern, western, and south western Europe: A review of problems and opportunities for crop production and the environment. *Soil and Tillage Research*, **118**, 66-87.

Speiser, B., Kistler, C., (2002) Field tests with a molluscicide containing iron phosphate. *Crop Protection*, **21:5,** 389–394.

Strickland, M. S., Rousk, J., (2010) Considering fungal:bacterial dominance in soils: Methods, controls and ecosystems implications. *Soil Biology and Biogeochemistry*, **42**, 1385-1395.

Stromberger, M. E., Keith, A. M., Schmidt, O., (2012) Distinct microbial and faunal communities and translocated carbon in *Lumbricus terrestris* drilospheres. *Soil Biology and Biochemistry*, **46**, 155–162.

Stroud, J. L., Irons, D. E., Carter, J. E., Watts, C. W., Murray, P. J., Norris, S. L., Whitmore, A. P., (2016a) *Lumbricus terrestris* middens are biological and chemical hotspots in a minimum tillage arable ecosystem. *Applied Soil Ecology*, **105**, 31-35.

Stroud, J., Irons, D., Watts, C., Storkey, J., Morris, N., Stobart, R., Fielding, H., Whitmore, A., (2017) Cover cropping with oilseed radish (*Raphanus sativus*) alone does not enhance deep burrowing earthworm (*Lumbricus terrestris*) midden counts. *Soil & Tillage Research*, **165**, 11–15.

Stroud, J. L., Irons, D. E., Watts, C. W., White, R. P., Mcgrath, S. P., Whitmore, A. P., (2016b) Population collapse of *Lumbricus terrestris* in conventional arable cultivations and response to straw applications. *Applied Soil Ecology*, **108**, 72-75.

Stroud, J. L., Irons, D., Watts, C. W., Whitmore, A. P., (2016c) *Lumbricus terrestris* abundance is not enhanced after three years of compost amendments on a reduced tillage wheat cultivation conversion. *Applied Soil Ecology*, **98**, 282-284.

Stroud J. L., (2020) Earthworms in No-Till: The Key to Soil Biological Farming. In: Dang Y., Dalal R., Menzies N. (eds) No-till Farming Systems for Sustainable Agriculture. Springer, Cham. https://doi.org/10.1007/978-3-030-46409-7_16.

Stoessel, F., Sondedregger, T., Bayer, P., Hellweg, S., (2018) Assessing environmental impacts of soil compaction in life cycle assessment. *Science of the Total Environment*, **630**, 913-921.

Styles, D., Gibbons, J., Williams, A., Dauber, J., Stichnothe, H., Urban, B., Chadwick, D., Jones, D., (2015) Consequential life cycle assessment of biogas, biofuel and biomass energy options within an arable crop rotation. *GCB Bioenergy*, **7:6**, 1305–1320.

Subler, S., Kirsch, A. S., (1998) Spring dynamics of soil carbon, nitrogen, and microbial activity in earthworm middens in a no-till cornfield. *Biology and Fertility of Soils*, **26**, 243-249.

Suthar, S., Singh, S., Dhawan, S. (2008) Earthworms as bioindicator of metals (Zn, Fe, Mn, Cu, Pb and Cd) in soils: Is metal bioaccumulation affected by their ecological category? *Ecological Engineering*, ***32*****:2**, 99–107.

Svoboda, N., Taube, F., Wienforth, B., Kluß, C., Kage, H., Herrmann, A., (2013) Nitrogen leaching losses after biogas residue application to maize. *Soil and Tillage Research*, **130**, 69-80.

Szymczak, L. S., de Faccio Carvalho, P. C., Lurette, A., de Moraes, A., de Albuquerque Nunes, P. A., Martins, A. P., Moulin, C-H., (2020) System diversification and grazing management as resilience-enhancing agricultural practices: The case of crop-livestock integration. *Agricultural Systems*, **184**, 102904.

Tahir, M., Anwar-ul-Hassan, Zahir, A. Z., Khalil-ur-Rehamn, (2012) Modelling water retention capacity and hydraulic properties of a manure-amended loam soil and its effect on wheat and maize yield. *International Journal of Agriculture and Biology*, **14:4**, 492-498.

Tambone, F., Scaglia, B., D'imporzano, G., Schievano, A., Orzi, V., Salati, S., Adani, F., (2010) Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. *Chemosphere*, **81**, 577-583.

Tampio, E., Salo, T., Rintala, J., (2016) Agronomic characteristics of five different urban waste digestates. *Journal of Environmental Management*, **169**, 293-302.

Tate, R. L., (1979) Microbial activity in organic soils as affected by soil depth and crop. *Applied and Environmental Microbiology*, **37:6**, 1085-1090.

Taylor, A., Lenoir, L., Vegerfors, B., Persson, T., (2018) Ant and Earthworm Bioturbation in Cold-Temperate Ecosystems. *Ecosystems (New York)*, ***22*****:5**, 981–994.

Tebrügge F., (2003) No-Tillage Visions- Protection of Soil, Water and Climate and Influence on Management and Farm Income. In: García-Torres L., Benites J., Martínez-Vilela A., Holgado-Cabrera A. (eds) Conservation Agriculture. Springer, Dordrecht.

Thiour-Mauprivez, C., Martin-Laurent, F., Calveyrac, C., Barthelmebs, L., (2019) Effect of herbicide on non-target organisms: towards a new class of biomarkers? *Science of the Total Environment*, **684**, 314-325.

Tiessen, H., Cuevas, E., Chacon, P., (1994) The role of organic matter in sustaining soil fertility. *Nature*, **371**, 783-785.

Townsend, R., Ramsden, S. J., Wilson, P., (2016) How do we cultivate in England? Tillage practices in crop production systems. *Soil use and management*, **32:1**, 106–117.

Tsiafouli, M. A., Thébault, E., Sgardelis, S. P., de Ruiter, P. C., van der Putten, W. H., Birkoffer, K., Hemerik, L., de Vries, F. T., Bardgett, R. D., Brady, M. V., Bjornlund, L., Jørgensen, H. B., Christensen, S., D'Hertefelt, T., Hotes, S., Gera Hol, W. H., Frouz, J., Liiri, M., Mortimer, S. R., Setälä, H., Tzanpoulos, J. H., Uteseny, K., Pizl, V., Stary, J., Wolters, V., Hedlund, K., (2015) Intensive agriculture reduces soil biodiversity across Europe. *Global Change Biology*, **21**, 973-985.

Tubiello, F. N., Amthor, J. S., Boote, K. J., Donatelli, M., Easterling, Fischer, G., Gifford, R. M., Howden, M., Reilley, J., Rosenzweig, C., (2007) Crop response to elevated CO_2 and world food supply. *Journal of Agronomy*, **26:3**, 215-223.

Turtola, E., Alakukku, L., Uusitalo, R., Kaseva, A., (2007) Surface runoff, subsurface drainflow and soil erosion as affected by tillage in a clayey Finnish soil. *Agriculture and Food Science*, **16**, 332-351.

Triberti, L., Nastri, A., Baldoni, G., (2016) Long term effects of crop rotation, manure and mineral fertilisation on carbon sequestration and soil fertility. *European Journal of Agronomy*, **74**, 47-55.

Trnka, M., Olesen, J. E., Kersebaum, K. C., Skjelvåg, A. O., Eitzinger, J., Seguin, B., Peltonen-Sainio, P., Rötter, R., Iglesias, A., Orlandini, S., Dubrovskỳ, M., Hlavinka, P., Balek, J., Eckersten, H., Cloppet, E., Calanca, P., Gobin, A., Vučetić, V., Nejedlik, P., Kumar, S., Lalic, B., Mestre, A., Rossi, F., Kozyra, J. , Alexandrov, V. , Semerádová, D., Žalud, Z., (2011) Agroclimatic conditions in Europe under climate change. *Global Change Biology*, **17**, 2298-2318.

Turner, S., Mikutta, R., Meyer-Stüve, S., Guggenberger, G., Schaarschmidt, F., Lazar, C. S., Dohrmann, R., Schippers, A., (2017) Microbial community dynamics in soil depth profiles over 120,000 years of ecosystem development. *Frontiers in Microbiology*, **8:874**.

Umiker, K. J., Johnson-Maynard, J. L., Hatten, T. D., Eigenbrode, S. D., Bosque-Pérez, N. A., (2009) Soil carbon, nitrogen, pH, and earthworm density as influenced by cropping practices in the Inland Pacific Northwest. *Soil and Tillage Research*, **105:2**, 184-191.

UN (United Nations), (2015) Sustainable Development Goals. Goal 2: Zero Hunger. Accessed [online] at: https://www.un.org/sustainabledevelopment/hunger/

UN (United Nations), (2017) World population projected to reach 9.8 billion in 2050, and 11.2 billion in 2100. Available at: https://www.un.org/development/desa/en/news/population/world-population-prospects-2017.html

Urra, J., Alkorta, I., Lanzén, A., Mijangos, I., Garbisu, C., (2019) The application of fresh and composted horse and chicken manure affects soil quality, microbial composition and antibiotic resistance. *Applied Soil Ecology*, **135**, 73–84.

Uvarov, A., (2009) Inter- and intraspecific interactions in lumbricid earthworms: Their role for earthworm performance and ecosystem functioning. *Pedobiologia*, **53**, 1-27.

Uvarov, A., Ilieva-Makulec, K., Karaban, K., Yakovenko, N., Uchmański, J. (2019) Effects of Intra- and Interspecific Interactions in Earthworm Assemblages: A Comparative Study. *Biology Bulletin*, **46:5**, 475–482.

Uvarov, A., Tiunov, A. V., Scheu., S., (2011) Effects of seasonal and diurnal temperature fluctuations on population dynamics of two epigeic earthworm species in forest soil. *Soil Biology and Biochemistry*, **43:3**, 559–570.

Valckx, J., Pennings, A., Leroy, T., Berckmans, D., Govers, G., Hermy, M., Muys, B., (2010) Automated observation and analysis of earthworm surface behaviour under experimental habitat quality and availability conditions. *Pedobiologia*, **53:4**, 259-263.

Valckx, J., Pina, A. C., Govers, G., Hermy, M., Muys, B., (2011) Food and habitat preferences of the earthworm *Lumbricus terrestris* L. for cover crops. *Pedobiologia*, **54S**, S139-S144.

van Apeldoorn, D. F., Sonneveld. M. P. W., Kok, K., (2011) Landscape asymmetry of soil organic matter as a source of agro-ecosystem resilience. *Agriculture, Ecosystems and Environment*, **140:3-4**, 401-410.

van den Bossche, A., De Bolle, S., De Neve, S., Hofman, G., (2009) Effect of tillage intensity on N mineralization of different crop residues in a temperate climate. *Soil and Tillage Research*, **103**, 316-324.

van Eekeren, N., de Boer, H., Hanegraaf, M., Bokhorst, J., Nierop, D., Bloem, J., Schouten, T., de Goede, R., Brussaard, L., (2010) Ecosystem services in grassland associated with abiotic and biotic soil parameters. *Soil Biology and Biochemistry*, **42**, 1491-1504.

van Kessell, J. S., Reeves, J. B., Meisinger, J. J., (2000) Nitrogen and carbon mineralization of potential manure compounds. *Journal of Environmental Quality*, **29**, 1669-1677.

van Groenigen, J. W., Lubbers, I. M., Vos, H. M., Brown, G. G., De Deyn, G. B., van Groenigen, K. J., (2014) Earthworms increase plant production: a meta-analysis. *Scientific Reports*, **4**, 6365.

van Groenigen, J. W., van Groenigen, K. J., Koopmans, G. F., Stokkermans, L., Vos, H. M. J., Lubbers, I. M., (2019) How fertile are earthworm casts? A meta-analysis. *Geoderma*, **338**, 525-535.

Vaneeckhaute, C., Lebuf, V., Michels, E., Belia, E., Vanrolleghem, P. A., Tack, F. M. G., Meers, E., (2016) Nutrient Recovery from Digestate: Systematic Technology Review and Product Classification. *Waste and biomass valorization*, **8:1**, 21–40.

Vernavà, M. N., Phillips-Aalten, P. M., Hughes, L. A., Rowcliffe, H., Wiltshire, C. W., Glen, D. M., (2004) Influences of preceding cover crops on slug damage and biological control using *Phasmarhabditis hermaphrodita*. *Annals of Applied Biology*, **145:3**, 279–284.

Vidal, A., Watteau, F., Remusat, L., Mueller, C.W., Tu, T-T.N., Buegger, F., Derenne, S., Quenea, K., (2019) Earthworm cast formation and development: A shift from plant litter to mineral associated organic matter. *Frontiers in Environmental Science*, **7:55**.

Vig, A. P., Vampal, C., Thind, T. S., Arora, S., (2009) Bio-protective effects of glucosinolates – a review. *LWT- Food Science and Technology*, **42**, 1561-1572.

Vogeler, I., Rogasik, J., Funder, U., Panten, K., Schnug, E., (2009) Effect of tillage systems and P fertilisation on soil physical and chemical properties, crop yield and nutrient uptake. *Soil and Tillage Research*, **103**, 137-143.

Vos, H. M. J., Koopmans, G. F., Beezemer, L., de Goede, R. G. M., Hiemstra, T., van Groenigen, J. W., (2019) Large variations in readily-available phosphorus in casts of eight earthworm species are linked to cast properties. *Soil Biology and Biochemistry*, **138**, 107583.

Wachendorf, C., Potthoff, M., Ludwig, B., Joergensen, R. G., (2014) Effects of addition of maize litter and earthworms on C mineralization and aggregate formation in single and mixed soils differing in soil organic carbon and clay content. *Pedobiologia*, **57**, 161-169.

Wall, D. H., Bardgett, R. D., Behan-Pelletier, V., Herrick, J. E., Jones, T. H., Ritz, K., Six, J., (2012) Soil ecology and ecosystem services. Oxford: Oxford University Press.

Wallace, A., Romney, E. M., Cha, J. W., Soufi, S. M., Chaudhry, F. M., (1977) Nickel phytotoxicity in relation to soil pH manipulation and chelating agents. *Communications in Soil Science and Plant Analysis*, **8**, 757-764.

Walmsley, D. C., Siemens, J., Kindler, R., Kaiser, K., Saunders, M., Fichtner, A., Kaupenjohann, M., Osborne, B. A., (2018) Reduced nitrate leaching from an Irish cropland soil under non-inversion tillage with cover cropping greatly outweighs increased dissolved organic nitrogen leaching. *Agriculture, Ecosystems and Environment*, **265**, 340-349.

Walsh, J. J., Jones, D. L., Edwards-Jones, G., Williams, A. P., (2012a) Replacing inorganic fertilizer with anaerobic digestate may maintain agricultural productivity at less environmental cost. *Journal of plant nutrition and soil science*, **175:6**, 840–845.

Walsh, J. J., Rousk, J., Edwards-Jones, G., Jones, D. L., Prysor Williams, A., (2012b) Fungal and bacterial growth following the application of slurry and anaerobic digestate of livestock manure to temperate pasture soils. *Biology and Fertility of Soils*, **48**, 889-897.

Wang, H., Wang, L., Zhang, Y., Hu, Y., Wu, J., Fu, X., Le, L., (2017a) The variability and causes of organic carbon retention ability of different agricultural straw types returned to soil. *Environmental Technology*, **38:5**, 538-548.

Wang, J., Sun, N., Xu, M., Wang, S., Zhang, J., Cai, Z., Cheng, Y. (2019) The influence of long-term animal manure and crop residue application on abiotic and biotic N immobilization in an acidified agricultural soil. *Geoderma*, ***337,*** 710–717.

Wang, L., Wang, S., Chen, W., Li, H., Deng, X., (2017b) Physiological mechanisms contributing to increased water-use efficiency in winter wheat under organic fertilization. *PLoS One*, **12:6**, [Online] e0180205.

Wang, M., Pendall, E., Fang, C., Li, B., Nie, M., (2018) A global perspective on agroecosystem nitrogen cycles after returning crop residue. *Agriculture, Ecosystems and Environment*, **266**, 49-54.

Wang, Z., Bao, X., Li, X., Jin, X., Zhao, J., Sun, J., Christie, P., Li, L., (2015) Intercropping maintains soil fertility in terms of chemical properties and enzyme activities on a timescale of one decade. *Plant and Soil*, **391:1-2**, 265-282.

Wang, Z., Jin, X., Bao, X., Li, X., Zhao, J., Sun, J., Christie, P., Li, L., (2014) Intercropping enhances productivity and maintains the most soil fertility properties relative to sole cropping. *PloS One*, **9:12**, e113984-e113984.

Wardle, D. A., (1995) Impacts of disturbance on detritus food webs in agro-ecosystems of contrasting tillage and weed management practices. *Advances in Ecological Research*, **26**, 105-185.

Warren Raffa, D., Bogdanski, D., Tittonell, P., (2015) How does crop residue removal affect soil organic carbon and yield? A hierarchical analysis of management and environmental factors, *Biomass and Bioenergy*, **81**, 345–355.

Webb, J., Menzi, H., Pain, B. F., Misselbrook, T. H., Dämmgen, U., Hendriks, G., Döhler, H., (2004) Managing ammonia emissions from livestock production in Europe. *Environmental Pollution*, **135**, 399-406.

Webb, J., Thorman, R. E., Fernanda-Aller, M., Jackson, D. R., (2014) Emission factors for ammonia and nitrous oxide emissions following immediate manure incorporation on two contrasting soil types. *Atmospheric Environment*, **82**, 280-287.

Wei, T., Zhang, P., Wang, K., Ding, R., Yang, B., Nie, J., Jia, Z., Han, Q. (2015) Effects of wheat straw incorporation on the availability of soil nutrients and enzyme activities in semiarid areas. *PloS One*, **10:4**, e0120994–.

Welsh, R. M., Shuman, L., (1995) Micronutrient nutrition of plants. *Critical Reviews in Plant Sciences*, **14:1**, 49-82.

Whalen J. K., Sampedro L., Waheed T., (2004) Quantifying surface and subsurface cast production by earthworms under controlled laboratory conditions. *Biology and Fertility of Soils*, **39**, 287–291.

Whalley, W. R., Dumitru, E., Dexter, A. R., (1995) Biological effects of soil compaction. *Soil and Tillage Research*, **35**, 53-68.

Wierzbowska, J., Sienkiewicz, S., Krzebietke, S., Bowszys, T., (2016) Heavy Metals in Water Percolating Through Soil Fertilized with Biodegradable Waste Materials. *Water, air, and soil pollution*, **227:12**, 1–10.

Wilcox, C. S., Domínguez, J., Parmelee, R. W., McCartney, D. A., (2002) Soil carbon and nitrogen dynamics in *Lumbricus terrestris* L. middens in four arable, a pasture and a forest ecosystems. *Biology and Fertility of Soils*, **36**, 26-34.

Wilhelm, W. W., Doran, J. W., Power, J. F., (1986) Corn and soybean yield response to crop residue management under no-tillage production systems. *Journal of Agronomy*, **96**, 1-17.

Wilhelm, W. W., Johnson, J. M. F., Karlen, D., Lightle, D., (2007) Corn stover to sustain soil organic cerbon further constrains biomass supply. *Journal of Agronomy*, **99**, 1665-1667.

Williams, A., Börjesson, G., Hedlund, K., (2013) The effects of 55 years of different inorganic fertiliser regimes on soil properties and microbial community composition. *Soil Biology and Biochemistry*, **67**, 41-46.

Willoughby, G. L., Kladiviko, E. J., Reza, S. M., (1997) Seasonal variations in infiltration rate under no-till and conventional (disk) tillage systems as affected by *Lumbricus terrestris* activity. *Soil Biology and Biochemistry*, **29:3**, 481–484.

Willoughby, G. L., Kladiviko, E. J., (2002) Water infiltration rates following reintroduction of *Lumbricus terrestris* into no-till fields. *Journal of Soil and Water Conservation*, **57:2**, 82-88.

Wolfarth, F., Schrader, S., Oldenburg, E., Weinert, J., Bruonette, J., (2011) Earthworms promote the reduction of fusarium biomass and deoxynivalenol content in wheat straw under controlled conditions. *Soil Biology and Biochemistry*, **43**, 1858-1865.

Wolfarth, F., Schrader, S., Oldenburg, E., Brunotte, J., (2016) Mycotoxin contamination and its regulation by the earthworm species *Lumbricus terrestris* in presence of other soil fauna in an agroecosystem. *Plant and Soil*, **402:1-2**, 331-342.

Wood, S. A., Tirfessa, D., Baudron, F., (2018) Soil organic matter underlies crop holder nutritional quality and productivity in smallholder agriculture. *Agriculture, Ecosystems and Environment*, **266**, 100-108.

Wortmann, C. S., Shapiro, C. A., (2007) The effects of manure application on soil aggregation. *Nutrient Cycling in Agroecosystems*, **80:2**, 173–180.

WRAP (2016) Digestate and compost use in agriculture: good practice guidance. Accessed online at: http://www.wrap.org.uk/sites/files/wrap/Digestate_compost_good_practice_guide_reference_version.pdf

Wright, A. L., Hons, F. M., (2005) Soil carbon and nitrogen storage in aggregates from different tillage and crop regimes. *Soil Science Society of America Journal*, **69**, 141-147.

Wright, A. L., Hons, F. M., Lemon, R. G., McFarland, M. L., Nichols, R. L., (2007) Stratification of nutrients in soil for different tillage regimes and rotations. *Soil and Tillage Research*, **96**, 19-27.

Wu, L., Liu, M., Song, X., Jiao, J., Li, H., Hu, F., (2015) Earthworm ecosystem service and dis-service in an N-enriched agroecosystem: Increase of plant production leads to no effects on yield-scaled N_2O emissions. *Soil Biology and Biochemistry*, **82**, 1-8.

Wyss, E., Glasstetter, M., (1992) Tillage treatments and earthworm distribution in a Swiss experimental corn field. *Soil Biology and Biochemistry*, **24:12**, 1635-1639.

Xia, L., Szlavecz, K., Swan, C. M., Burgess, J. L., (2011) Inter- and intra-specific interactions of *Lumbricus rubellus* (Hoffmeister, 1843) and *Octolasion lacteum* (Örley, 1881) (Lumbricidae) and the implication for C cycling. *Soil Biology and Biochemistry*, **43**, 1584-1590.

Xing, X., Li, Y., Ma, X., (2017) Effects on infiltration and evaporation when adding rapeseed-oil residue or wheat straw to a loam soil. *Water*, **9**, 1-12.

Yang, X. M., Drury, C. F., Reynolds, W. D., Tan, C. S., (2008) Impacts of long-term and recently imposed tillage practices on the vertical distribution of soil organic carbon. *Soil and Tillage Research*, **100**, 120-124.

Zaller, J., Heigl, F., Grabmaier, A., Lichtenegger, C., Piller, K., Allabashi, R., Frank, T., Drapela, T., (2011) Earthworm-mycorrhiza interactions can affect the diversity, structure and functioning of establishing model grassland communities. *PloS One*, **6:12**, e29293–.

Zaller, J., Wechselberger, K., Gorfer, M., Hann, P., Frank, T., Wanek, W., Drapela, T., (2013) Subsurface earthworm casts can be important soil microsites specifically influencing the growth of grassland plants. *Biology and Fertility of Soils*, **49:8**, 1097–1107.

Zarea M. J., Ghalavand A., Goltapeh E. M., Rejali F., Zamaniyan M., (2009) Effects of mixed cropping, earthworms (*Pheretima* sp.), and arbuscular mycorrhizal fungi (*Glomus mosseae*) on plant yield, mycorrhizal colonization rate, soil microbial biomass, and nitrogenase activity of free-living rhizosphere bacteria. *Pedobiologia*, **52**, 223–235.

Zasada, L. A., Ferris, H., (2004) Nematode suppression with brassicaceous amendments: application based on glucosinolate profiles. *Soil Biology and Biochemistry*, **36**, 1017-1024.

Zatón, K. K., Skwiercz, A. T., Adamiak, E., Szelągowska, P., Hury, G., (2018) Plant parasitic nematodes in the soil and roots of winter wheat grown in crop rotation and long-term monoculture. *Journal of Plant Protection Research*, **58:2**, 184-191.

Zhang, W., Hendrix, L. E., Dame, R. A., Burke, R. A., Wu, J., Neher, D. A., Li, J., Shao, Y., Fu, S., (2013) Earthworms facilitate carbon sequestration through unequal amplification of carbon stabilization compared with mineralization. *Nature Communications*, **4**.

Zhang, X., Ferris, H., Mitchell, J., Liang, W., (2017) Ecosystem services of the soil food web after long-term application of agricultural management practices. *Soil Biology and Biogeochemistry*, **111**, 36-43.

Zhao, B., Zhang, J., Yu, Y., Karlen, D. L., Hao, X., (2016) Crop residue management and fertilisation effects on soil organic matter and associated biological properties. *Environmental Science and Pollution Research*, **23**, 17581-17591.

Zhao, H., Shar, A. G., Li, S., Chen, Y., Shi, J., Zhang, X., Tian, X., (2018) Effect of straw return mode on soil aggregation and aggregate carbon content in an annual maize-wheat double cropping system. *Soil and Tillage Research*, **175**, 178-186.

Zhen, L., Kai, S., Bin, Z., Qingling, D., Geng, L., Huifang, H., Zengjia, L., Tangyuan, N., (2019) Impacts of straw, biogas slurry, manure and mineral fertilizer applications on several biochemical properties and crop yield in a wheat-maize cropping system. *Plant, Soil and Environment*, **65:1**, 1–8.

Zhou, C. Q., Zhang, Y. Q., Rashid, A., Ram, H., Savasli, E., Arisoy, R. Z., Ortiz-Monasterio, I., Simunji, S., Wang, Z. H., Sohu, V., Hassan, M., Kaya, Y., Onder, O., Lungu, O., Yaqub Mujahid, M., Joshi, A. K., Zelenskiy, Y., Zhang, F. S., Cakmak, I., (2012) Biofortification of wheat with zinc through fertilization in seven countries. *Plant and Soil*, **361**, 119-130.

Zikeli, S., Deil, L., Möller, K., (2017) The challenge of imbalanced nutrient flows in organic farming systems: A study of organic greenhouses in Southern Germany. *Agriculture, Ecosystems and Environment*, **244**, 1-13.

Zorn, M., Van Gestel, C., Eijsackers, H. (2005) The effect of two endogeic earthworm species on zinc distribution and availability in artificial soil columns. *Soil Biology and Biochemistry*, **37:5**, 917–925.

APPENDICES

Appendix I: Results from preliminary midden survey at Lilac Field, Light Ash farm, Myerscough College (November 2018).

Prior to the midden survey being conducted at Lilac farm, Myerscough College, midden samples (N=10) were collected for identification in November 2018. The results from this survey are presented below (Table I-1; Figure I-1).

Table I.1. The mean earthworm density, biomass and number of species per treatment (±SE) of midden samples collected at Lilac Field, Myerscough College, 2018. N=10.

Earthworm data	Result
Mean density (ind treatment^{-1} ± S.E.)	12.5 ± 1.6
Mean number of species (n treatment^{-1})	2.8 ± 0.3
Mean biomass (g treatment $^{-1}$)	4.0 ± 0.5
% adult	26.2

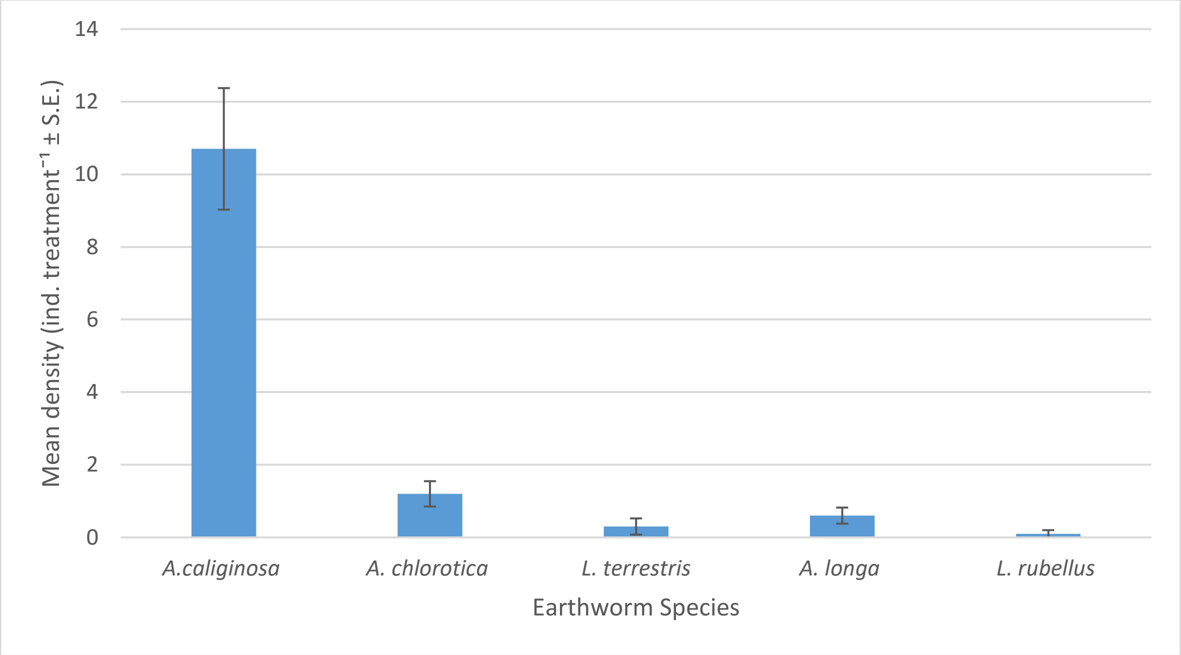


Figure I.1. The mean earthworm species density (g treatment^{-1} ± S.E.) within midden samples collected at Lilac field, Myerscough College, 2018. N = 10.

Appendix II. Supplementary analyisis from Section 4.4

1. The effect of earthworm variables on total midden straw biomass.

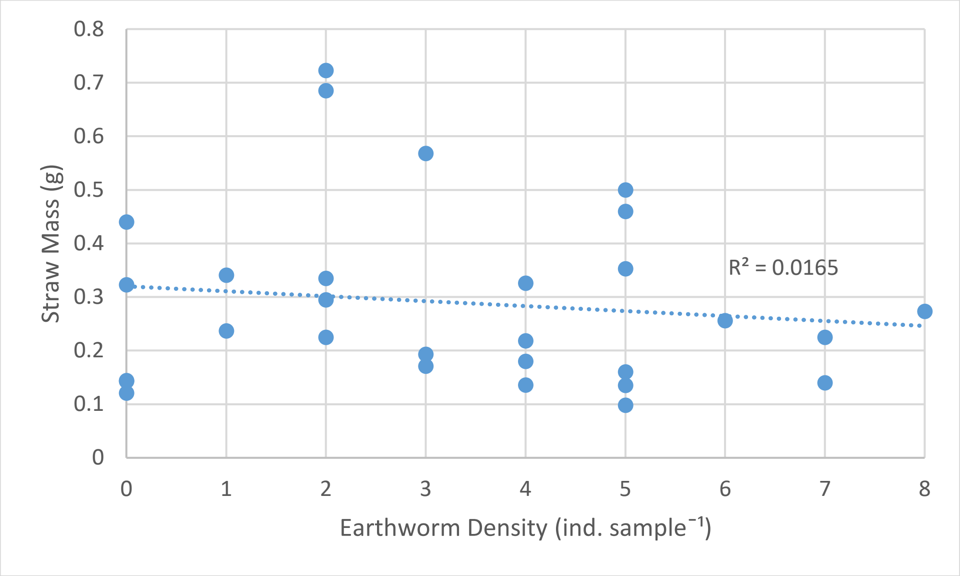


Figure II.1. The relationship between earthworm density (n) and straw biomass (g)

There was no correlation between earthworm density on midden straw mass ($r^2 = 0.0165$). There were also no effects of total earthworm biomass (g sample $^{-1}$) on the midden straw mass. As the biomass of juveniles (which feed on midden straw) increased, the midden biomass decreased (Figure II-2.), however this was not a significant effect.

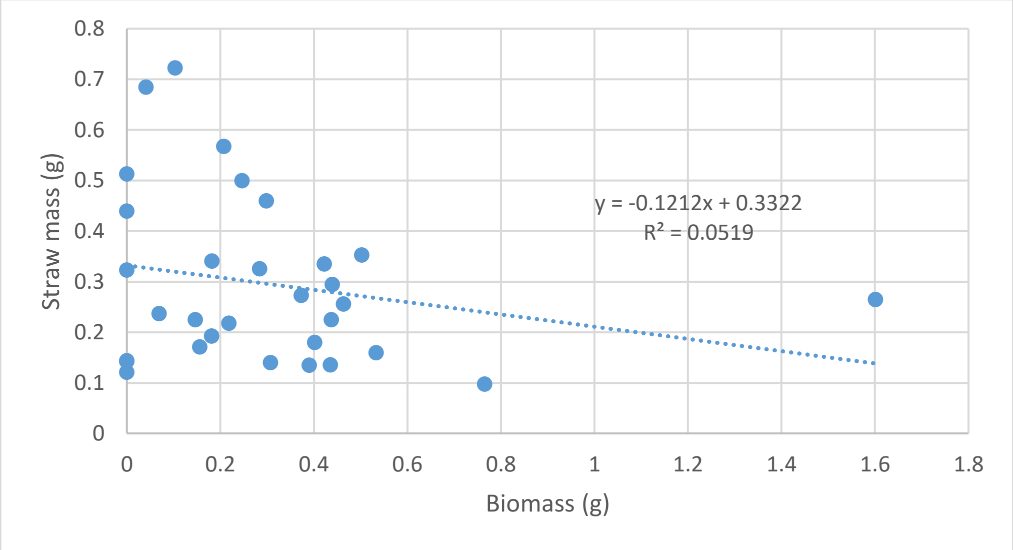


Figure II.2. The relationship between total earthworm biomass (g) of juveniles within midden cores and midden straw mass (g).

2. The effect of midden straw mass (g) on the organic nutrient concentration.

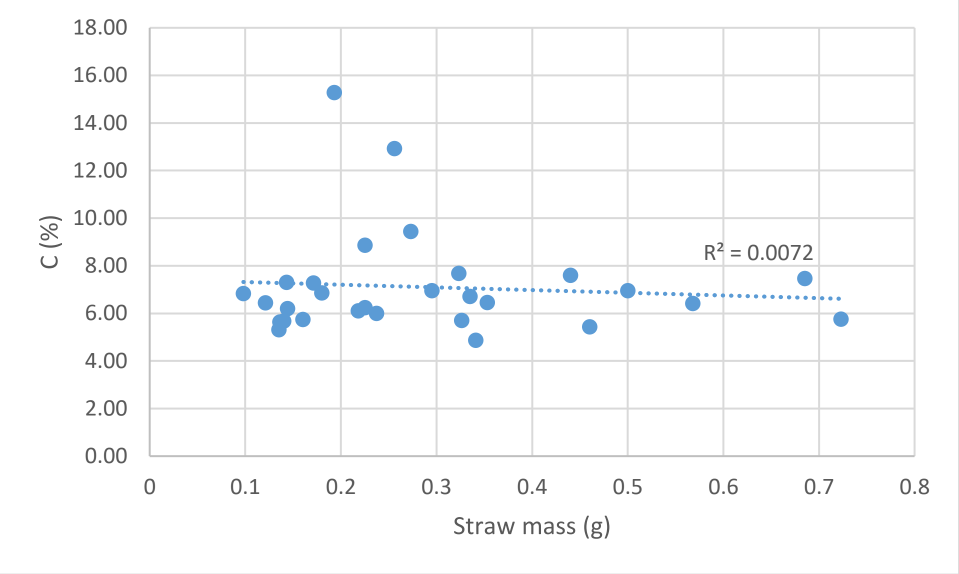


Figure II.3. The relationship between midden straw mass and midden carbon concentration (%) at each sample point.

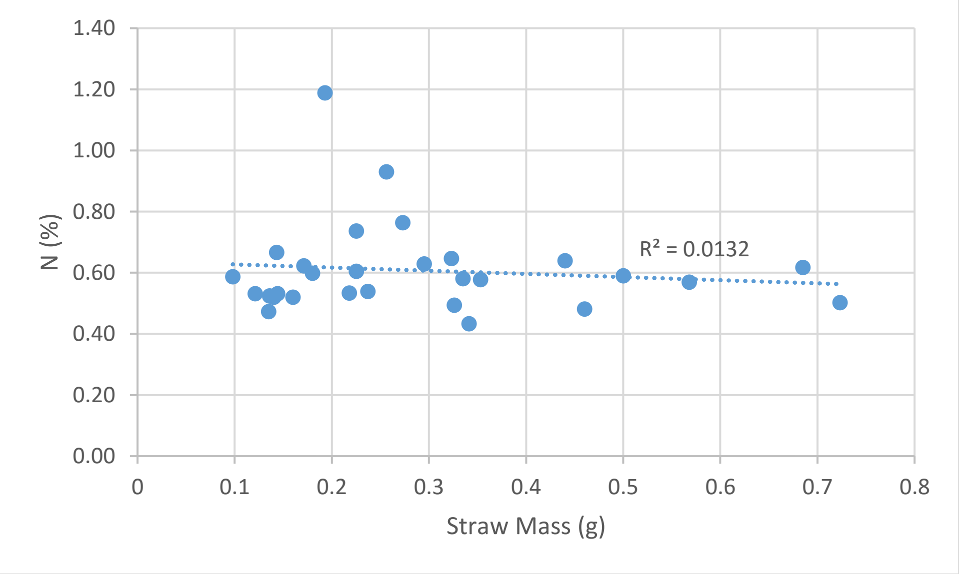


Figure II.4. The relationship between midden straw mass (g) and midden nitrogen concentration (%) at each sample point.

Appendix III. Supplementary chemical analysis data from Section 6.4.

Table III.1. Mean total nutrient concentration at increasing depth from soil surface (1 = 0-6 cm; 2 = 6-12 cm; 3 = 12-18 cm) for *L. terrestris* monocultures (N=2).

Treatment	Depth	Phosphorus		Calcium		Magnesium		Potassium		Manganese		Iron		Nickel		Zinc		Boron	
		mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE
Control	1	1502	69	1229	330	3859	161	599	20	988	106	8305	601	39	2	237	45	113	8
Control	2	1443	47	1032	221	3608	119	570	20	1197	290	8181	416	39	2	231	45	110	5
Control	3	1438	53	889	51	3650	87	577	22	801	32	7848	449	38	2	244	49	106	5
1 cm	1	1574	96	836	36	3604	123	538	29	928	143	7942	577	40	2	151	6	106	10
1 cm	2	1553	176	791	71	3544	93	576	22	763	38	7469	422	39	2	141	3	97	5
1 cm	3	1475	83	917	121	3680	260	521	37	870	157	8054	601	35	6	148	9	108	8
20 cm	1	1698	174	991	74	4989	92	733	38	1266	36	9645	484	50	2	164	3	137	6
20 cm	2	2152	379	1249	123	5061	746	694	100	1610	477	14126	3028	50	7	206	32	181	38
20 cm	3	1722	178	942	116	4477	480	660	86	999	99	10106	1273	40	5	164	12	140	17
40 cm	1	1928	87	1298	158	5624	468	844	67	1218	105	12753	859	70	5	181	9	299	19
40 cm	2	1885	201	1465	268	5172	242	804	43	1842	560	14059	1550	72	8	187	15	336	36
40 cm	3	2237	237	1817	513	5590	217	827	36	1178	97	15844	1601	71	4	213	12	373	29

Table III.2. Mean total nutrient concentration at increasing depth from soil surface (1 = 0-6 cm; 2 = 6-12 cm) for *A. caliginosa* monocultures (N=3).

Treatment	Depth (cm)	**Phosphorus**		**Calcium**		**Magnesium**		**Potassium**		**Manganese**		**Iron**		**Nickel**		**Zinc**		**Boron**	
		mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE
Control	1	1639	141	1867	266	5242	910	741	61	1143	252	12150	1704	53	6	253	50	292	25
Control	2	1633	206	2173	710	5377	856	801	75	1121	195	13004	1936	49	6	208	15	305	30
1 cm	1	1627	87	1288	58	3919	285	682	31	872	209	9067	1031	48	6	173	11	249	27
1 cm	2	1686	50	1520	183	3555	118	681	30	723	36	8370	192	39	2	167	3	228	5
20 cm	1	1722	78	1385	215	5014	107	818	29	1064	70	11414	934	64	3	187	7	319	22
20 cm	2	1851	256	1268	89	4102	230	674	19	926	177	11268	1557	54	8	205	27	314	35
40 cm	1	1542	50	1357	123	4461	274	726	40	933	76	9996	1034	55	6	189	6	300	24
40 cm	2	1462	162	2016	604	3980	400	693	54	907	147	9177	1005	53	6	173	18	277	25

Table III.3. Total nutrient concentration at increasing depth from soil surface (1 = 0-6 cm; 2 = 6-12 cm; 3 = 12-18 cm) for mixed species treatment (*L. terrestris* N=1*; A. caliginosa* N=3)

Treatment	Depth (cm)	**Phosphorus**		**Calcium**		**Magnesium**		**Potassium**		**Manganese**		**Iron**		**Nickel**		**Zinc**		**Boron**	
		mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE	mg kg^{-1}	SE
Control	1	2619	349	1565	117	6378	579	959	92	1348	78	16349	1859	78	7	213	11	365	37
Control	2	2403	416	1475	92	6003	250	886	38	1964	788	16674	3964	70	8	213	31	368	77
Control	3	2629	262	1439	144	6355	416	944	46	1923	383	17660	2005	80	6	226	13	386	43
1 cm	1	2583	406	1485	78	6815	212	931	32	1381	50	19324	2855	79	14	262	22	421	56
1 cm	2	2807	411	1776	143	6880	402	904	81	2424	836	17574	1127	80	8	244	10	370	25
1 cm	3	2183	171	1477	98	6956	468	912	92	1515	113	19477	3314	65	8	254	19	397	48
20 cm	1	2083	282	1947	515	5865	540	794	43	1365	150	18233	4499	57	5	211	21	389	88
20 cm	2	2464	640	1747	298	6925	554	961	104	1390	181	14564	1257	63	8	225	21	324	28
20 cm	3	2348	312	1772	91	6704	601	913	87	2992	1232	19805	2078	60	11	239	16	408	35
40 cm	1	2057	151	1315	83	5204	248	786	35	1704	440	16524	2466	68	7	201	23	351	52
40 cm	2	2144	195	1319	175	5384	531	775	18	2862	1760	16172	2690	71	16	215	32	346	48
40 cm	3	2066	346	1748	429	5941	651	818	51	1506	254	14510	2637	69	8	212	33	311	47

Appendix IV. Supplementary information from Chapter 7.

1: Images of deconstructed L. terrestris middens.

Figure IV.1. Deconstructed midden from Container 1.

Figure IV.2. Deconstructed midden from Container 2.

Figure IV.3. Deconstructed midden from Container 3.

Figure IV.4. Deconstructed midden from Container 4.

2. L. terrestris surface activity of each experimental unit.

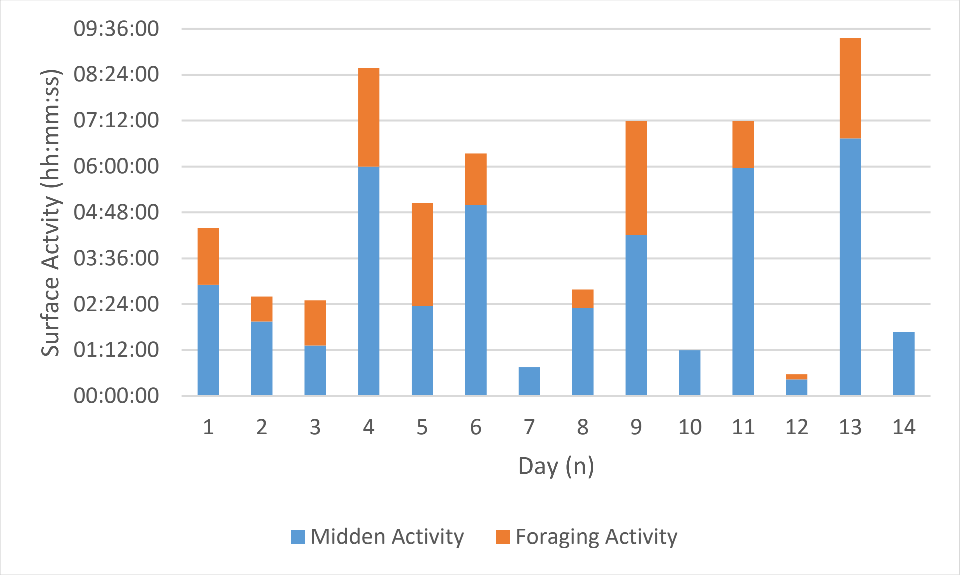


Figure IV.5. The total duration of surface activity by *L. terrestris* in Container 1, where total time is separated by midden activity and foraging activity.

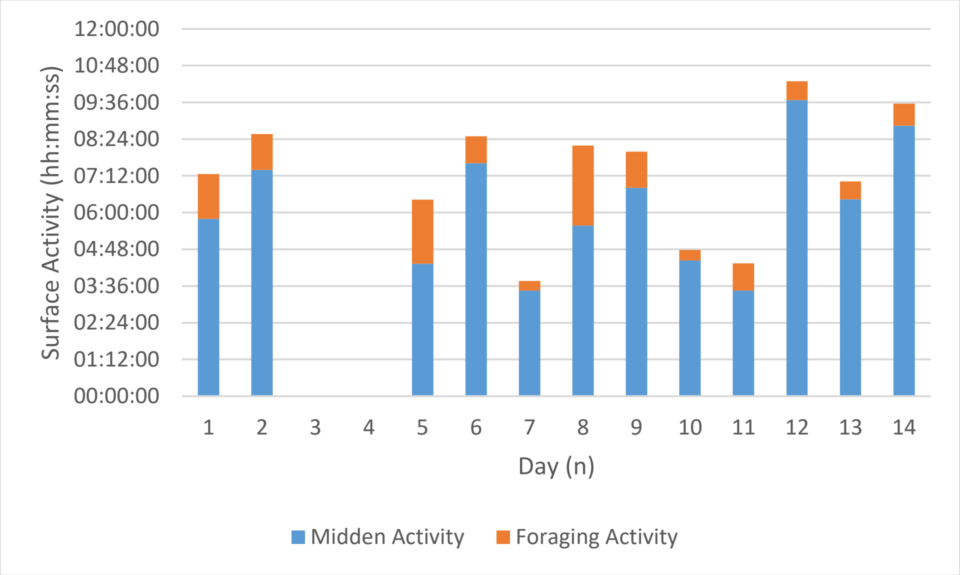


Figure IV.6. The total duration of surface activity by *L. terrestris* in Container 2, where total time is separated by midden activity and foraging activity.

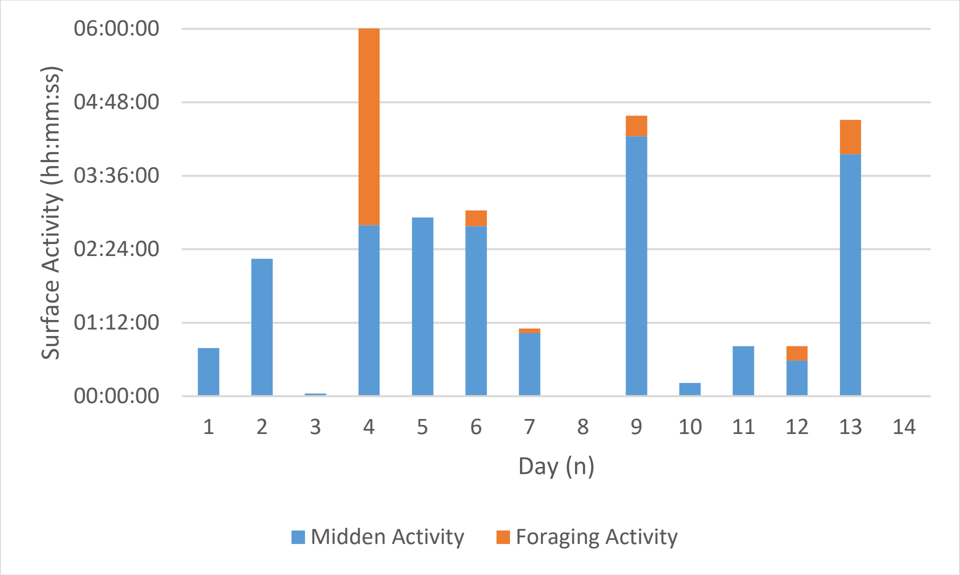


Figure IV.7. The total duration of surface activity by *L. terrestris* in Container 3, where total time is separated by midden activity and foraging activity.

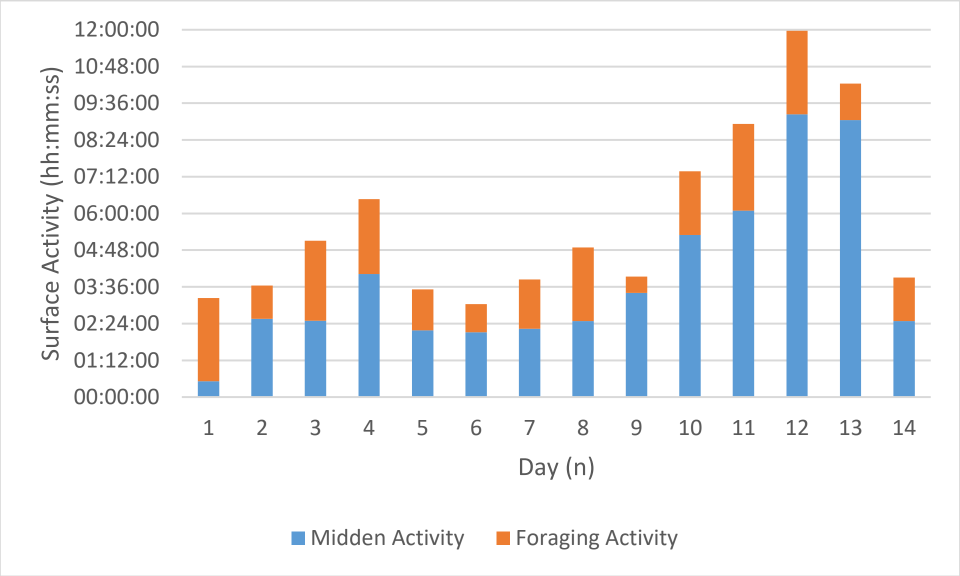


Figure IV.8. The total duration of surface activity by *L. terrestris* in Container 4, where total time is separated by midden activity and foraging activity.

3. Total straw particle selection by L. terrestris per experimental unit.

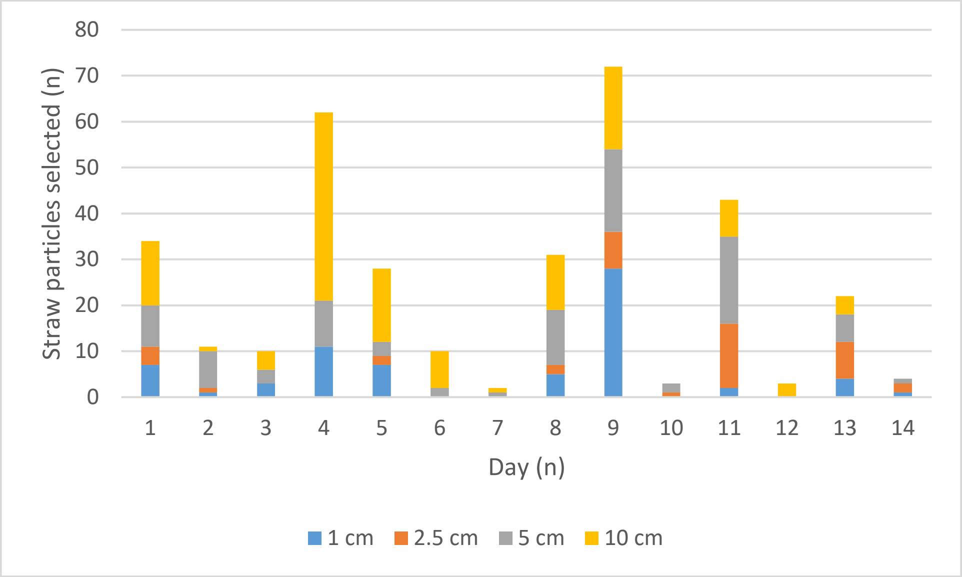


Figure IV.9. The number of straw particles selected each day in Container 1, separated by the particle size.

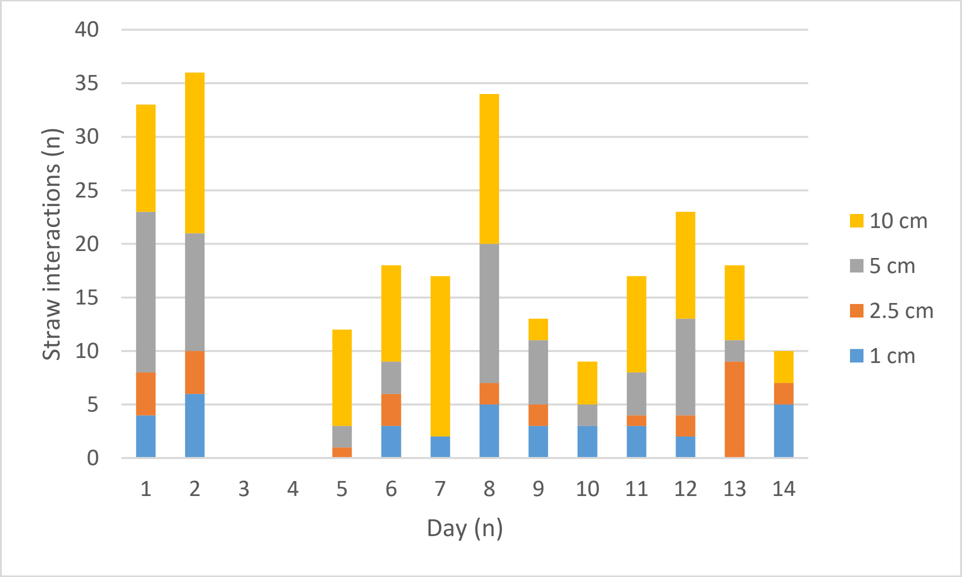


Figure IV.10. The number of straw particles selected each day in Container 2, separated by the particle size.

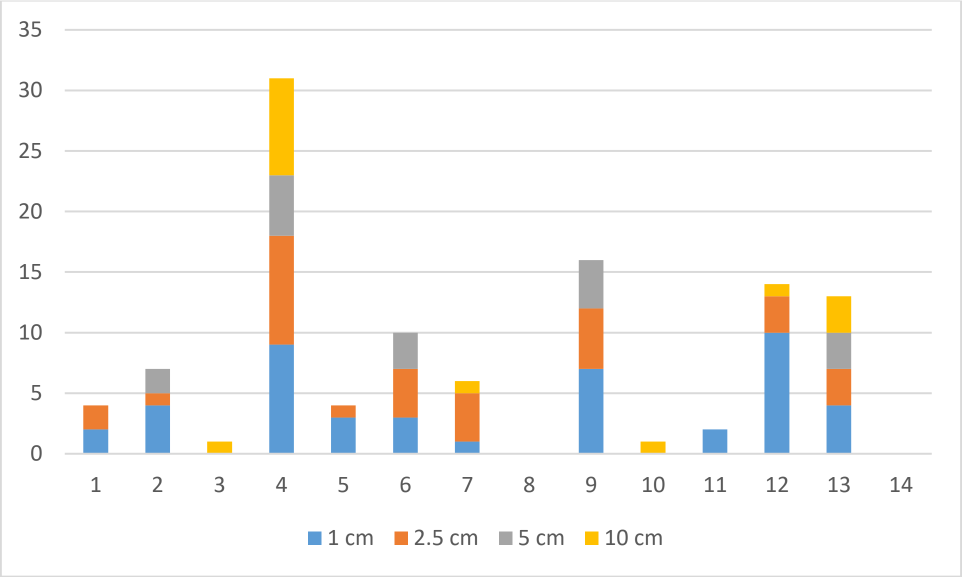


Figure IV.11. The number of straw particles selected each day in Container 3, separated by the particle size.

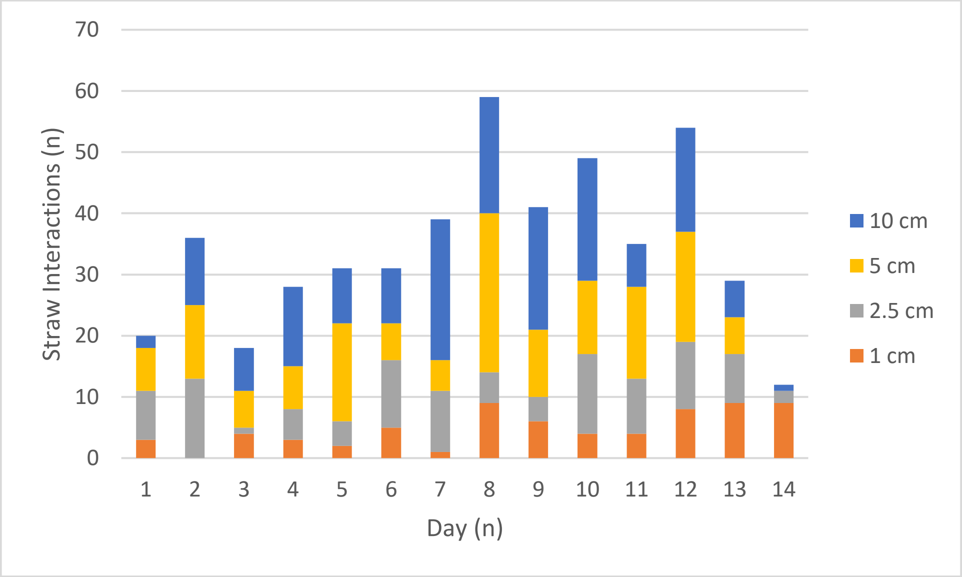


Figure IV.12. The number of straw particles selected each day in Container 4, separated by the particle size.

7.4. Macro-and micronutrient concentration (mg kg^{-1}) of midden and non midden soils at increasing depth (mm).

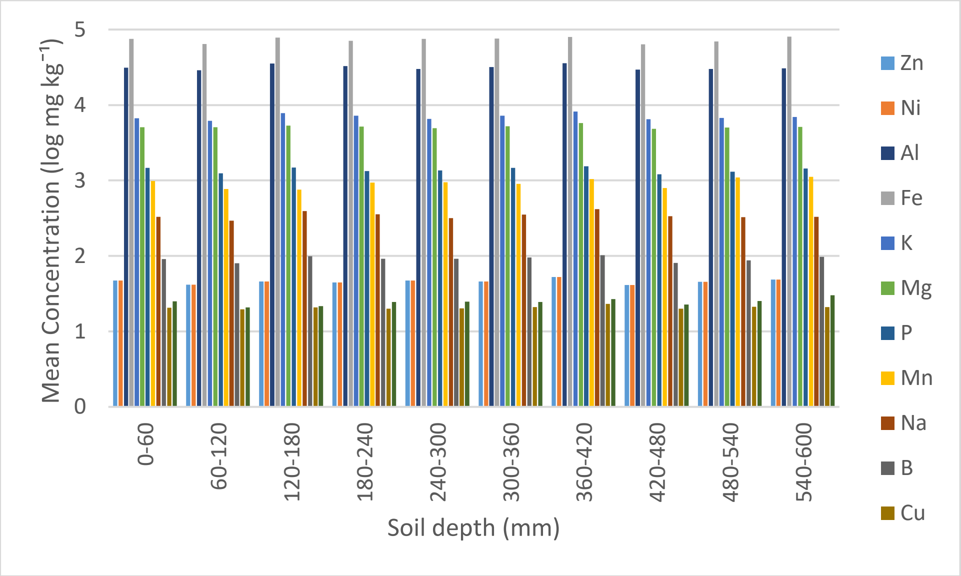


Figure IV.13. The mean TN concentration (log mg kg^{-1}) of macro- and micronutrients under midden soil at increasing depth. 0 mm = the soil surface; 600 mm = maximum depth of vessel.

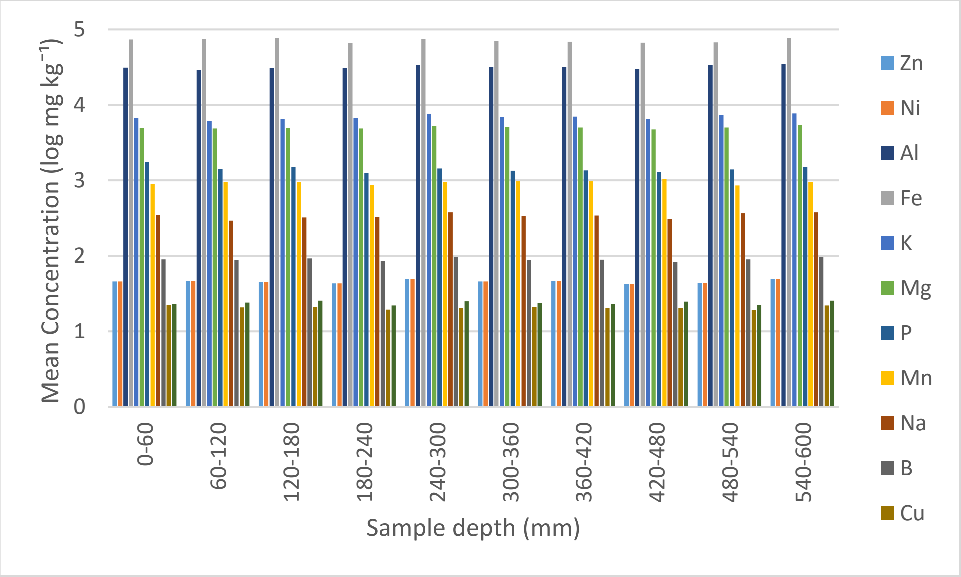


Figure IV.14. The mean TN concentration (log mg kg^{-1}) of macro- and micronutrients under control soil at increasing depth. 0 mm = the soil surface; 600 mm = maximum depth of vessel.

Appendix V. Supplementary results from Chapter 8.

Table V.1. Multiple comparisons output (Tukey HSD) of the differences in *L. terrestris* biomass, *L. terrestris* density, mean soil removed (g $0.25m^{-2}$) and reduction in straw mass (g $0.25m^{-2}$) between the sites (1 = Ojainen; 2 = Kotkanoja; 3 = Kiikoinen). OJAINEN

Test Statistics[a]

	Soil_removed	Lt_total_density	Lt_total_biomass	Lt_middens	Total_earthworm _density	Soil_moisture	Straw_removed
Mann-Whitney U	9.000	.000	.000	.000	.000	8.000	1.000
Wilcoxon W	24.000	15.000	15.000	15.000	15.000	23.000	16.000
Z	-.733	-2.795	-2.785	-2.668	-2.635	-.940	-2.402
Asymp. Sig. (2-tailed)	.463	.005	.005	.008	.008	.347	.016
Exact Sig. [2*(1-tailed Sig.)]	.548[b]	.008[b]	.008[b]	.008[b]	.008[b]	.421[b]	.016[b]

a. Grouping Variable: Lt_expected

b. Not corrected for ties.

Table V.1. Multiple comparisons output (Tukey HSD) of the differences in *L. terrestris* biomass, *L. terrestris* density, mean soil removed (g $0.25m^{-2}$) and reduction in straw mass (g $0.25m^{-2}$) between the sites (1 = Ojainen; 2 = Kotkanoja; 3 = Kiikoinen). KOTKANOJA

Test Statistics[a]

	Soil_removed	Lt_total_density	Lt_total_biomass	Lt_middens	Total_earthworm _density	Soil_moisture	Straw_removed
Mann-Whitney U	25.000	5.500	5.000	.000	12.000	3.000	2.000
Wilcoxon W	61.000	41.500	41.000	36.000	48.000	39.000	38.000
Z	-.737	-2.908	-2.962	-3.467	-2.110	-3.050	-3.151
Asymp. Sig. (2-tailed)	.461	.004	.003	.001	.035	.002	.002
Exact Sig. [2*(1-tailed Sig.)]	.505[b]	.003[b]	.003[b]	.000[b]	.038[b]	.001[b]	.001[b]

a. Grouping Variable: Lt_expected

b. Not corrected for ties.

Table V.1. Multiple comparisons output (Tukey HSD) of the differences in *L. terrestris* biomass, *L. terrestris* density, mean soil removed (g 0.25m^{-2}) and reduction in straw mass (g 0.25m^{-2}) between the sites (1 = Ojainen; 2 = Kotkanoja; 3 = Kiikoinen). KIIKOINEN

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Soil_removed	Between Groups	242.064	1	242.064	10.166	.013
	Within Groups	190.492	8	23.811		
	Total	432.556	9			
Lt_total_density	Between Groups	705.600	1	705.600	50.043	.000
	Within Groups	112.800	8	14.100		
	Total	818.400	9			
Lt_total_biomass	Between Groups	178.084	1	178.084	12.050	.008
	Within Groups	118.227	8	14.778		
	Total	296.311	9			
Lt_middens	Between Groups	4.900	1	4.900	2.513	.152
	Within Groups	15.600	8	1.950		
	Total	20.500	9			
Total_earthworm_density	Between Groups	2250.000	1	2250.000	45.872	.000
	Within Groups	392.400	8	49.050		
	Total	2642.400	9			
Soil_moisture	Between Groups	2.025	1	2.025	.706	.425
	Within Groups	22.944	8	2.868		
	Total	24.969	9			
Straw_removed	Between Groups	.197	1	.197	.016	.902
	Within Groups	97.986	8	12.248		
	Total	98.184	9			

Appendix VI: Conferences and awards.

Table VI.1. Conferences attended during research program

Date	Name of Conference	Presentation
September 2019	BSSS Early Career Research Conference, Sheffield	Oral poster presentation
June, 2019	Graduate Research School Conference, UClan	Poster presentation
November 2018	Myerscough Research Conference, Myerscough College	Poster presentation
September 2018	Biezczady Earthworm Conference, Rzeczow, Poland	Oral poster presentation
June 2018	ISEE International Earthworm Congress, China	Poster presentation
December 2016	BES Annual Meeting, Edinburgh	Poster presentation
November 2016	Myerscough Research Conference, Myerscough College	Oral presentation
December, 2015	BES Annual Meeting, Liverpool	Poster presentation
April 2015	Graduate Research School Conference, UCLan	Oral presentation

The action of anecic and endogeic earthworm species on incorporation of wheat straw into agricultural soils

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Introduction

Anecic earthworms such as *Lumbricus terrestris* may play a pivotal role in reduced tillage agricultural soils, where their feeding behaviour enables incorporation of surface applied organic matter into the soil profile, enhancing degradation and nutrient cycling. This work is part of a wider research project exploring the incorporation of organic matter into agricultural soils by earthworm species. This laboratory study was designed to investigate whether the behavioural relationships between *L. terrestris* and *A. caliginosa* enhances wheat straw incorporation into soil in agricultural environments and if there are any mutualistic benefits between anecic and endogeic species.

Methodology

This experiment examined 3 earthworm treatments (2 x *L. terrestris*; 3 x *A. caliginosa*; and a combination of 1 x *L. terrestris* and 3 x *A. caliginosa*) and incorporation of wheat straw into soil.

Three straw treatments were used with lengths of 40, 20, and 1 cm, plus a control with no straw applied. These lengths were chosen to reflect measurements taken from a harvested field.

Mature earthworms were set up in 3 L polyethylene-bound units of soil (diam. 10 cm; height 18 cm) and 10 g of wheat straw was cut into selected particle sizes and surface applied. There were 5 replicates per treatment (5 reps. x 3 earthworm x 4 straw). Units were incubated at 15 °C in 24 h darkness for 8 weeks.

At experimental end, remaining surface straw was removed, dried at 105 °C and had mass determined. Soil samples were taken at 3 depths (0-6, 6-12, and 12-18 cm) and nutrient concentration and C/N ratio analysed. Recovered earthworms had their masses determined and soil was checked for cocoons.

Results and Discussion

Of the earthworm combinations examined, *L. terrestris* monocultures incorporated the largest straw mass into soil (6.36 ± 0.60 g of 1 cm particle size; Figure 1), had 100% survival rate and produced cocoons. Although *A. caliginosa* incorporated straw into soil under all treatments, survival rates were lower (80, 60 and 93% with 40, 20 and 1 cm respectively). Straw incorporation under mixed species treatments was low (<1 g), however *A. caliginosa* survival was higher and average mass loss was lower over the period of study. This suggests there could be mutualistic benefits between *L. terrestris* and *A. caliginosa*. *L. terrestris* incorporated wheat straw at lower depths throughout the 8 week study between all particle size treatments, where *A. caliginosa* and mixed species wheat straw incorporation depth was negatively influenced by particle size (Figure 2).

Figure 1. The total mean mass (g) of wheat straw removed from the soil surface over an 8 week period.

Figure 2. The mean maximum depth wheat straw was located in the soil profile between particle size treatments.

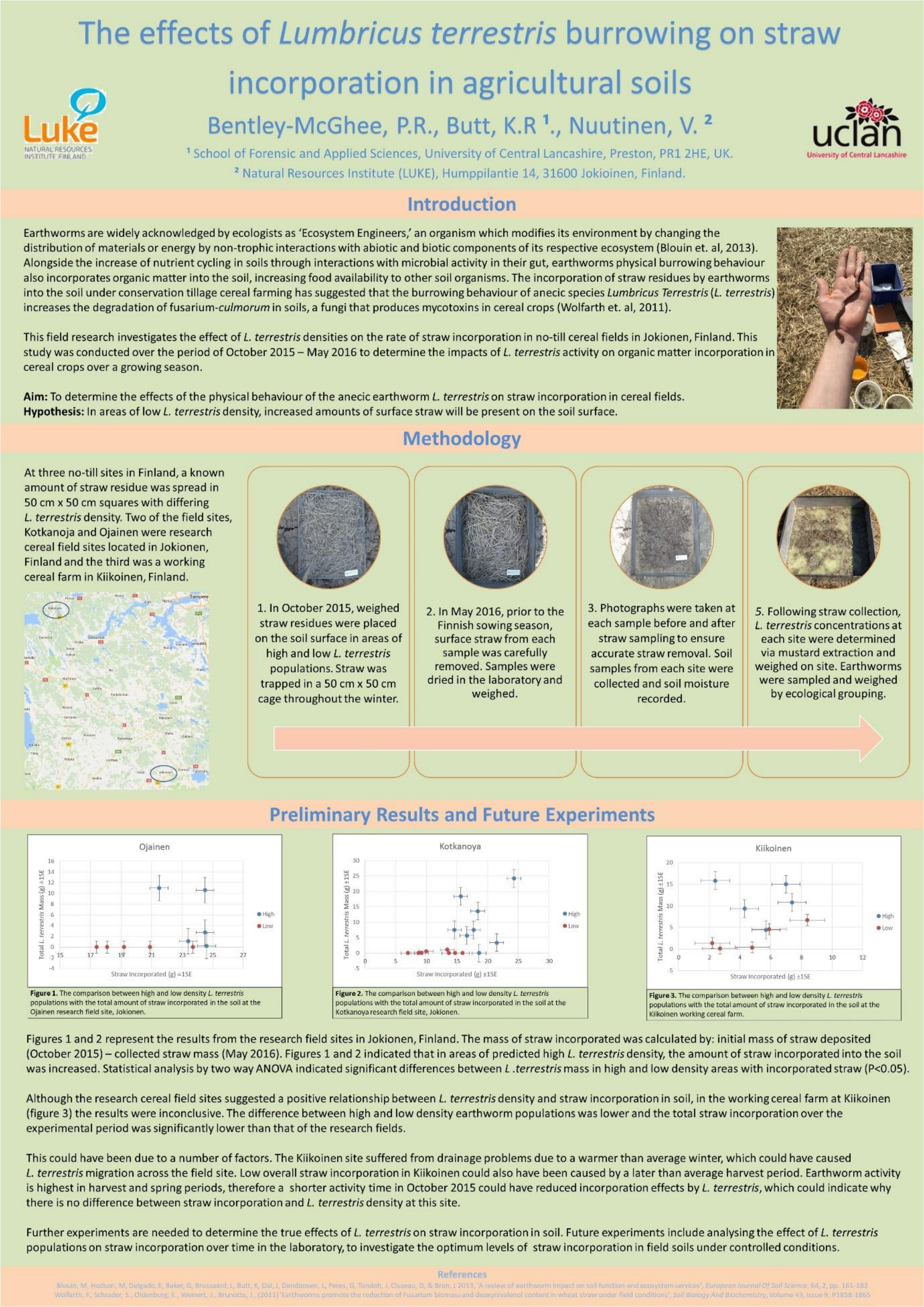
Figure 3. The mean C-N ratio of soil (sampled at 10 cm depth) between particle size treatments and earthworm species after 8 week inoculation of surface applied wheat straw.

A 2-way ANOVA of the straw removal, particle size and earthworm species indicated a significant difference between straw surface removal and particle size under *L. terrestris* monocultures ($p < 0.05$), however there was no significant relationship between particle size, straw removal and earthworm populations in the other treatments. Nutrient levels increased under earthworm treatments compared with controls. Analysis of nutrient concentration against soil depth indicated that there was a significant difference between nutrient concentration and depth within *L. terrestris* monocultures ($p < 0.05$), where nutrient concentration decreased with depth. The nutrient concentration under *A. caliginosa* interactions did not change with soil depth. *A. caliginosa* also had a higher mean C/N ratio for all particle size treatments against other earthworm combinations (Figure 3). This could imply that earthworm species interactions and/or earthworm numbers influence nutrient concentration in agricultural soils.

This experiment suggested that the presence of *L. terrestris* increased the incorporation rate of wheat straw and may provide some mutualistic benefits with *A. caliginosa*; however, this requires further exploration in field settings. Further research will investigate the relationship between *L. terrestris* and *A. caliginosa* species behaviour and community dynamics on wheat straw incorporation to explore the implications of this on sustainable agricultural soils.

11th International Symposium on Earthworm Ecology: June 2018 – Shanghai, China

Figure VI.1. Poster presented at the International Earthworm Congress, Shanghai, 2018.



The effects of *Lumbricus terrestris* burrowing on straw incorporation in agricultural soils

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Introduction

Earthworms are widely acknowledged by ecologists as 'Ecosystem Engineers,' an organism which modifies its environment by changing the distribution of materials or energy by non-trophic interactions with abiotic and biotic components of its respective ecosystem (Blouin et. al, 2013). Alongside the increase of nutrient cycling in soils through interactions with microbial activity in their gut, earthworms physical burrowing behaviour also incorporates organic matter into the soil, increasing food availability to other soil organisms. The incorporation of straw residues by earthworms into the soil under conservation tillage cereal farming has suggested that the burrowing behaviour of anecic species *Lumbricus Terrestris* (*L. terrestris*) increases the degradation of fusarium-*culmorum* in soils, a fungi that produces mycotoxins in cereal crops (Wolfarth et. al, 2011).

This field research investigates the effect of *L. terrestris* densities on the rate of straw incorporation in no-till cereal fields in Jokionen, Finland. This study was conducted over the period of October 2015 – May 2016 to determine the impacts of *L. terrestris* activity on organic matter incorporation in cereal crops over a growing season.

Aim: To determine the effects of the physical behaviour of the anecic earthworm *L. terrestris* on straw incorporation in cereal fields.
Hypothesis: In areas of low *L. terrestris* density, increased amounts of surface straw will be present on the soil surface.

Methodology

At three no-till sites in Finland, a known amount of straw residue was spread in 50 cm x 50 cm squares with differing *L. terrestris* density. Two of the field sites, Kotkanoja and Ojainen were research cereal field sites located in Jokionen, Finland and the third was a working cereal farm in Kiikoinen, Finland.

1. In October 2015, weighed straw residues were placed on the soil surface in areas of high and low *L. terrestris* populations. Straw was trapped in a 50 cm x 50 cm cage throughout the winter.

2. In May 2016, prior to the Finnish sowing season, surface straw from each sample was carefully removed. Samples were dried in the laboratory and weighed.

3. Photographs were taken at each sample before and after straw sampling to ensure accurate straw removal. Soil samples from each site were collected and soil moisture recorded.

5. Following straw collection, *L. terrestris* concentrations at each site were determined via mustard extraction and weighed on site. Earthworms were sampled and weighed by ecological grouping.

Preliminary Results and Future Experiments

Figure 1. The comparison between high and low density *L. terrestris* populations with the total amount of straw incorporated in the soil at the Ojainen research field site, Jokionen.

Figure 2. The comparison between high and low density *L. terrestris* populations with the total amount of straw incorporated in the soil at the Kotkanoya research field site, Jokionen.

Figure 3. The comparison between high and low density *L. terrestris* populations with the total amount of straw incorporated in the soil at the Kiikoinen working cereal farm.

Figures 1 and 2 represent the results from the research field sites in Jokionen, Finland. The mass of straw incorporated was calculated by: initial mass of straw deposited (October 2015) – collected straw mass (May 2016). Figures 1 and 2 indicated that in areas of predicted high *L. terrestris* density, the amount of straw incorporated into the soil was increased. Statistical analysis by two way ANOVA indicated significant differences between *L .terrestris* mass in high and low density areas with incorporated straw (P<0.05).

Although the research cereal field sites suggested a positive relationship between *L. terrestris* density and straw incorporation in soil, in the working cereal farm at Kiikoinen (figure 3) the results were inconclusive. The difference between high and low density earthworm populations was lower and the total straw incorporation over the experimental period was significantly lower than that of the research fields.

This could have been due to a number of factors. The Kiikoinen site suffered from drainage problems due to a warmer than average winter, which could have caused *L. terrestris* migration across the field site. Low overall straw incorporation in Kiikoinen could also have been caused by a later than average harvest period. Earthworm activity is highest in harvest and spring periods, therefore a shorter activity time in October 2015 could have reduced incorporation effects by *L. terrestris*, which could indicate why there is no difference between straw incorporation and *L. terrestris* density at this site.

Further experiments are needed to determine the true effects of *L. terrestris* on straw incorporation in soil. Future experiments include analysing the effect of *L. terrestris* populations on straw incorporation over time in the laboratory, to investigate the optimum levels of straw incorporation in field soils under controlled conditions.

References

Blouin, M, Hodson, M, Delgado, E, Baker, G, Brussaard, L, Butt, K, Dai, J, Dendooven, L, Peres, G, Tondoh, J, Cluzeau, D, & Brun, J 2013, 'A review of earthworm impact on soil function and ecosystem services', *European Journal Of Soil Science*, 64, 2, pp. 161-182
Wolfarth, F., Schrader, S., Oldenburg, E., Weinert, J., Brunotte, J., (2011) 'Earthworms promote the reduction of Fusarium biomass and deoxynivalenol content in wheat straw under field conditions', *Soil Biology And Biochemistry*, Volume 43, Issue 9, P1858-1865

Figure VI.2. Poster presented at BES Annual Meeting, 2016.

Certificate

Higher Education
Academy

This is to certify that
Peter Bentley

has achieved the status of
Associate Fellow
of The Higher Education Academy

in recognition of attainment against the
UK Professional Standards Framework for
teaching and learning support in higher education.

Recognition reference:
PR169893
Date of recognition
22/07/2019

Alison Johns
Chief Executive Advance HE

Nigel Carrington
Chair of the Board of Directors Advance HE

Figure VI.3. Associate Fellow of the HEA certificate, awarded in June 2019.

Appendix VII: Ethical Approval.

uclan
University of Central Lancashire

27th November 2014

Kevin Butt & Peter Bentley
Grenfell-Baines School of Architecture, Construction & Environment
University of Central Lancashire

Dear Kevin & Peter,

Re: STEMH Ethics Committee Application
Unique reference Number: STEMH 285

On the basis of the information contained in the Research Degrees Application form, the STEMH ethics committee does not envisage any insoluble ethical issues arising that might make the proposed project non-viable for ***PhD via MPhil route***. The committee therefore has no objection to the project **'Earthworms as ecosystem service providers in sustainable agro-ecosystems'** proceeding to research programme approval / registration.

It appears from the Research Degrees Registration application, that the project will not require full ethics committee approval. This is because it does not mention the inclusion of human (or animal) research participants or their data and seems not to have any significant ethical issues. If any phase of the research includes human (or animal) research participants or their data, or significant ethical issues are identified by you or your supervisory team, a full proposal application will need to be submitted to and approved by the committee. If this occurs, please ensure that you quote the unique reference number (above) on your application form. (You may then also find it convenient to make separate proposal applications for different stages of the project, especially if the design of the later stages is highly dependent on the findings from the earlier stages.)

Yours sincerely

Colin Thain
Chair
STEMH Ethics Committee

NB - Ethical clearance is contingent on any health and safety checklists having been completed, and necessary approvals as a result of gained.

Figure VII.1. Copy of Ethical approval confirmation for research project.