Woodland Restoration on Landfill Sites: Earthworm Activity and Ecosystem Service Provision

by

Francis Edwin Ashwood

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Central Lancashire

July 2016

STUDENT DECLARATION

Concurrent registration for two or more academic awards

I declare that while registered as a candidate for the research degree, I have not been a registered candidate or enrolled student for another award of the University or other academic or professional institution.

Material submitted for another award

I declare that no material contained in the thesis has been used in any other submission for an academic award and is solely my own work.

Signature of Candidate: ______________________

Type of Award: Doctor of Philosophy

School: Forensic and Applied Sciences

ABSTRACT

The addition of composted greenwaste (CGW) into soil-forming materials during land reclamation may improve tree growth, alleviate certain negative soil properties and provide an effective waste management solution. CGW addition may also assist the establishment of sustainable earthworm populations, which in turn can further aid soil development through their burrowing and feeding activities. Despite these potentially mutual benefits, little research exists into CGW and earthworm interactions with trees on reclaimed land, and the aim of this thesis was to investigate such interactions. A large-scale field experiment and a nursery-based mesocosm experiment revealed the responses of the tree species Italian alder (*Alnus cordata*) and Norway maple (*Acer platanoides*) to CGW and earthworm addition in reclaimed soil. Findings revealed a synergistic effect of CGW addition and earthworm activity leading to significantly greater *A. cordata* and *A. platanoides* growth. CGW addition significantly increased levels of soil organic carbon and essential plant macro-nutrients, with earthworm activity increasing the accumulation of organic carbon into reclaimed soils. Additional laboratory-based research revealed the performance of four common UK earthworm species in reclaimed soil, and demonstrated that CGW can support earthworm establishment, and that the earthworms *Aporrectodea longa* and *Allolobophora chlorotica* are particularly suitable candidates for inoculation to reclaimed soil. These two earthworm species showed a preference for the foliar material of *A. cordata* over *A. platanoides*, but after two weeks, microbial degradation of leaf litter increased *A. platanoides* leaf palatability to these earthworms. These two tree species may therefore be capable of supporting earthworm populations on reclaimed landfill. A survey of a newly reclaimed site showed that natural colonisation of reclaimed land by earthworms can occur rapidly (within 2 years), where soil quality is sufficient and legacy soil materials are stockpiled and applied following best practice guidance. The studies in this thesis demonstrate methods for effectively improving woodland establishment and soil quality on reclaimed landfill, through CGW application and earthworm activity promoting soil development and encouraging tree growth.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisory team, Drs Kevin Butt, Kieron Doick and Elena Vanguelova for all of their guidance and support throughout my PhD. I am truly grateful to Kieron for taking me under his wing many years ago and investing in me, I hope it has paid off. Sincere thanks to Kevin for his generosity with advice; he taught me a great deal about being a researcher, as well as the importance of marching to the beat of your own drum. Many thanks to Elena for her advice and encouragement - her inspiring passion for research helped get me through some low points over the last three years. Thanks also to my Research Degrees Tutor Dr Chris Lowe at the University of Central Lancashire, for his time and honest advice and feedback throughout the project.

My thanks to the University of Central Lancashire (UCLan) and the Forestry Commission England's Thames Beat team for financially supporting this research project, and providing access to their sites and resources. Thank you to all of my colleagues at Forest Research who helped me set-up, monitor and analyse my experiments, I wouldn't have been able to do it without your help. Thanks to Siobhan Quigg at UCLan for her help and company in the field and the lab. Thanks also go to my fellow PhD students who provided advice and support along the way; I wish them the very best of luck for the future.

Finally, thank you to my brothers Karl and Kurt and their families, my friends, and to Helen. Without your support and considerable patience, I would never have been able to achieve this PhD.

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

GLOSSARY OF TERMS

Term	Definition
Aestivation	A state of animal dormancy, similar to hibernation, characterized by inactivity and a lowered metabolic rate, which is entered in response to high temperatures and arid conditions.
Amphimictic	Capable of interbreeding freely and of producing fertile offspring.
Anecic	Earthworms which create deep near-vertical burrows which are connected to the soil surface, and which may be inhabited for extensive periods of time. Anecic species principally feed on organic matter but also ingest large amounts of inorganic soil, vertically redistributing these throughout the soil profile.
Beat-up	Replacing trees that have died shortly after planting.
Comminution	The reduction of solid materials from one average particle size to a smaller average particle size, e.g. by crushing, grinding, or cutting.
De-faunated	Treated (e.g. through freezing) to destroy soil fauna, which might act as competitors or predators of earthworms.
Diapause	A delay in development in response to regular and recurring periods of adverse environmental conditions. In diapause, earthworms avoid moisture loss by curling into a knot within a mucus-lined cavity in the soil until conditions improve.
Endogeic	Earthworms which create temporary burrows which are often shallow and horizontally oriented in the soil; these earthworms ingest inorganic soil materials and organic matter, and produce organically enriched casts either within the soil layers or on the soil surface.
Epigeic	Earthworms which dwell in litter on the soil surface, whereby their feeding activities comminute organic matter into smaller particles.
Foliar	Fresh leaf material.
Geophagous	Feeding on soil.
Litter	Fallen, decomposing leaf material.

1. INTRODUCTION

1.1. General introduction

Globally, mineral extraction and a decline of heavy industry have resulted in an abundance of degraded land, which may be left contaminated and derelict (Bradshaw and Chadwick, 1980). Because of its links with environmental and social degradation, such 'Brownfield' land has increasingly become a scientific focus for restoration ecologists (Harris *et al.*, 1996; Perrow and Davy, 2002; Butt, 2008). Amongst other habitat types, brownfield sites can be regenerated into greenspaces such as public woodlands, which may benefit biodiversity and improve the delivery of ecosystem services from the site (Bullock *et al.*, 2011). However, the degree of ecosystem service provided by a regenerated habitat is dependent upon the quality of its creation and management. A key measurement for success in greenspace creation is vegetation establishment and growth (Doick *et al.*, 2009). Vegetation health is largely influenced by soil quality, which is a combination of chemical, physical and biological parameters (Scullion, 1992; Moffat and McNeill, 1994).

The soil-forming materials typically used during land reclamation generally contain low levels of plant nutrients and organic matter, and possess many physical and chemical properties which can inhibit the growth of vegetation and the activity of soil organisms (Bending *et al.*, 1999; Dickinson *et al.*, 2005). Previous reclamation activities which have used mineral wastes without proper consideration given to the amelioration of the material's physical and chemical limitations have often been unsuccessful (Bending *et al.*, 1999). In addition to generally detrimental substrate properties, the physical and environmental disturbance caused throughout reclamation processes can have significant negative impacts on soil macro- and micro-faunal communities, which differ in their ability to recover (Scullion, 1992). Vegetation growth and the provision of key soil functions and ecosystem services are often the functional outputs of biological processes within the soil, which can be negatively influenced by the physical and chemical properties typical to reclaimed soils (Moffat and McNeill, 1994; Kibblewhite *et al.*, 2008).

In recognition of their role in improving soil structure and fertility, earthworms have been the subject of research during land reclamation for over 50 years (e.g. van Rhee, 1969a; Curry and Cotton, 1983; Curry, 1988; Butt *et al.*, 1995). However, destructive activities such as mining may leave a soil material which is devoid of earthworms due to heavy physical disturbance or pollution, or may involve the introduction of poor quality soil-forming materials unsuitable for supporting earthworm populations (Scullion, 2007). The soil materials in such sites are typically unsuitable for earthworm establishment due to extremes of pH (particularly acidity), low organic matter content, unfavourable soil moisture conditions, high levels of compaction and metal toxicity (Curry and Cotton, 1983).

Research indicates that incorporating organic waste into soil-forming materials during land regeneration may improve tree growth and help alleviate many of the previously identified negative soil properties. The addition of organic matter to soil may also be important for establishing sustainable earthworm populations on reclaimed land (Lowe and Butt, 2002b, 2004). A range of organic waste types have been investigated for suitability to support earthworm growth, including composted green waste (CGW), papermill and boardmill sludge, sewage sludge, separated cattle solids and other animal wastes. However, these have largely been demonstrated for low-fertility mineral soils and less so for reclaimed soils. It has been argued that organic waste materials should always be considered for improving soil-forming materials for brownfield regeneration to community greenspace such as woodland (Moffat, 2006). However, despite the availability of research and guidance regarding the amendment of soil-forming materials with organic wastes (Moffat and McNeill, 1994; Forest Research, 2015), and it being an effective organic waste stream management solution (Scullion, 2007), such techniques are typically not used during the creation of community greenspaces (Ashwood *et al.*, 2014). As such, little research currently exists which investigates CGW and earthworm interactions with vegetation on reclaimed land, and demonstrates the potential benefits of considering these methods to raise soil quality for woodland establishment. Further research is required to build up a body of evidence into the interactions between earthworm species and CGW in reclaimed soils, and provide data on tree growth and soil development over time after CGW and earthworm addition.

1.2. Problem statement

The addition of CGW and earthworms is an effective way of improving reclaimed soil quality, subsequently benefitting the growth and survival of vegetation on reclaimed landfill. However, there is currently little knowledge regarding earthworm population dynamics and responses to CGW addition on reclaimed landfill sites, nor the influence of this interaction on tree performance. Addressing these knowledge gaps will enable more informed future landfill reclamation activity; providing more effective woodland creation, and soil and woodland ecosystem service provision on reclaimed land.

1.3. Research aims

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

1. the interactive effects of CGW addition and earthworm activity on tree growth and survival on reclaimed landfill,
2. the interactive effects of CGW addition and earthworm activity on the biological, physical and chemical quality of reclaimed soil under woodland,
3. the community dynamics of naturally and artificially introduced earthworms on landfill sites, and responses to tree establishment and CGW addition.

1.4. Thesis Structure

Chapter one positions this research project within the wider scope of land restoration 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, and concludes with a summary of the research gaps identified during the literature review process. A broad range of the methodological considerations adopted during the research project is 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 woodland, and the role of earthworms as ecosystem engineers is explained, with an overview provided of their ecology. Land reclamation and restoration is introduced, followed by a review of how reclaimed soil quality influences, and can be influenced by, earthworm activity. Land reclamation to woodland is then discussed, followed by a review of research into the effects of earthworms on woodland establishment on reclaimed land. The use of organic waste materials in land reclamation is then described, including the role that earthworms may play in assisting organic waste utilisation for reclamation projects. The 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 woodland ecosystem services

Ecosystem services have been defined as the benefits provided by ecosystems to humankind as well as other species (Millennium Ecosystem Assessment, 2005). Work has been conducted to categorise services, identify global supply and demand, and identify economic values and valuation methods of ecosystem services (Daily, 1997; Millennium Ecosystem Assessment, 2005; Blouin *et al.*, 2013). Four categories of ecosystem service have been defined: Regulating services cover disease, climate and flood regulation, and water purification; Supporting services include nutrient cycling, primary production, soil formation, pollination and other key processes which support biodiversity and ecosystems; Provisioning services are those which provide material goods such as food, fresh water, wood and fuel; and Cultural services provide humans with educational, recreational and aesthetic values (Millennium Ecosystem Assessment, 2005). Many of these ecosystem services are products of biodiversity and ecosystem structure. However, what is still lacking for many forms of service is quantification of how biodiversity relates to ecosystem service provision in a manner which can inform conservation and management targets (Kremen and Ostfeld, 2005; Blouin *et al.*, 2013). In order to reach management objectives, there is a need to identify the relationships that exist between ecological entities and ecosystem functions or services, and develop techniques for manipulating these (Blouin *et al.*, 2013).

The degree of ecosystem service provided by a regenerated habitat is dependent upon the quality of its creation and management. A key measurement for success in greenspace creation is vegetation establishment and growth (Doick *et al.*, 2009). Vegetation health is largely influenced by soil quality, which is a combination of a number of interacting chemical, physical and biological parameters (Scullion, 1992; Moffat and McNeill, 1994). The provision of soil functions and ecosystem services is often based on the outputs of biological processes within the soil, such as the transformation of carbon through interactions with soil organic matter; the cycling of essential plant nutrients; structural maintenance of the soil, and; regulating soil biodiversity. These processes play a large role in enabling the soil to fulfil key functions, and are influenced by complex biological interactions with physical and chemical aspects of the soil (Kibblewhite *et al.*, 2008).

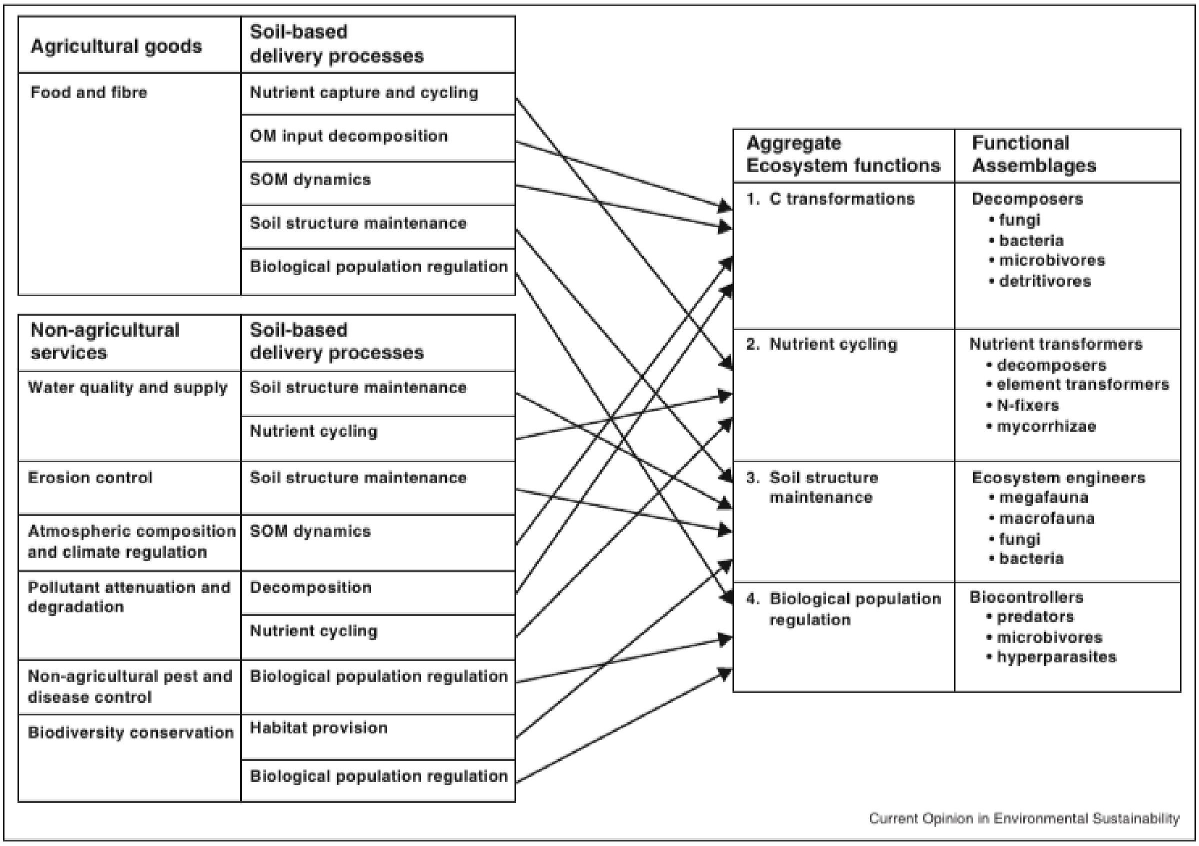


Figure 2.1. Relationships between the activities of the soil biota and a range of ecosystem goods and services that society might expect from soils. OM = organic matter; SOM = soil organic matter (modified after Figure 1.3.2 on p. 47 in Wall *et al*., 2012, and in Kibblewhite *et al.,* 2008).

Soil biological diversity has until recently been a poorly studied area by ecologists, however it is now becoming increasingly recognised that soil flora and fauna play key roles in the regulation of many ecosystem processes (Mittelbach *et al.*, 2001; Bardgett, 2005; Wall *et al.*, 2012; Blouin *et al.*, 2013). Primary production (for example tree growth in woodland) and the provision of soil functions and other supporting ecosystem services are often based on the functional outputs of biological processes within the soil (Moffat and McNeill, 1994; Millennium Ecosystem Assessment, 2005; Pulleman *et al.*, 2012). Many authors have summarised the services linked to soils (Swift *et al.*, 2004; Haygarth and Ritz, 2009; Dominati *et al.*, 2010; Wall *et al.*, 2012). Because services may be delivered by different functions of the soil ecosystem, soil biota are commonly grouped into "functional groups" according to their relationship to these services (Figure 2.1) (Lavelle *et al.*, 1995; Brussaard, 1998; Wall *et al.*, 2012). For the managers of ecosystems it is useful to identify whether all species of organisms are equally involved in the delivery of ecosystem services, or whether some organisms are more crucial than others (Blouin *et al.*, 2013). Ecosystem managers could then better focus their efforts on those particular ecological entities which have been demonstrated to be of high functional importance. Such species which have a disproportionately high impact on their habitat structure, other biota and ecosystem functions may be called "ecosystem engineers" (Jones *et al.*, 1994; Lavelle *et al.*, 1997; Wall *et al.*, 2012; Blouin *et al.*, 2013). In the soil, the major ecosystem engineers are ants, termites and earthworms (Wall *et al.*, 2012).

2.2.2. Earthworms as ecosystem engineers

Whilst comparatively low in numbers, their large body size means earthworms often exceed the biomass of all other soil biota of fertile soils in temperate zones (Lavelle and Spain, 2001; Edwards, 2004; Bardgett, 2005). As ecosystem engineers, earthworms are known to actively manipulate their environment: through burrowing and mixing of soil and organic matter they modify soil conditions and significantly influence the local vegetation and soil biota (Lavelle *et al.*, 1997; Scullion, 2007; Blouin *et al.*, 2013). Darwin (1881) first demonstrated the beneficial role of earthworms, with much research having since been conducted into their ecology and their role in the delivery of ecosystem services by the soil (Edwards and Bohlen, 1996; Blouin *et al.*, 2013). Earthworms are catalysts for two 'supporting services' as defined by the Millennium Ecosystem Assessment (2005): Soil formation (Darwin, 1881) and nutrient cycling (Edwards, 2004). These soil services are, however, prerequisites for a number of other services (Blouin *et al.*, 2013), all of which are discussed in section 2.3.2, in the context of reclaimed soils.

2.2.3. Earthworm ecology

Earthworms are soil invertebrates belonging to the phylum Annelida, (class Clitellata, subclass Oligochaeta), and can be found in forests, grasslands and agro-ecosystems across most of the world; except areas which experience climatic extremes, e.g. deserts and polar regions (Edwards, 2004). Earthworm biodiversity is widely variable between habitat types and locations, with some soil and habitat types often linked to species associations. Earthworm numbers are likewise highly variable, and are affected by factors such as soil type, moisture-holding capacity, temperature, pH, and, importantly, organic matter quantity and quality (Edwards, 2004). Typical earthworm community density for soils in grassland or woodland is around 400 m^{-2}. Soils in temperate areas typically contain less diverse earthworm communities than in warmer locations, with the former dominated by species of the family Lumbricidae (Lavelle *et al.*, 1999; Edwards, 2004). In temperate regions, earthworm activity is highly seasonal; with the majority of activity occurring in spring and autumn (Edwards, 2004). In winter and summer, and during other adverse conditions such as drought, some species are known to employ behavioural patterns which afford some protection; for example deeper burrowing and/or aestivation in mucus-lined cells (Edwards, 2004).

Earthworms are divided across three main ecological groupings (epigeic, anecic, and endogeic); each behaves differently in the soil ecosystem and may therefore have unique contributions to ecosystem processes and services (Bouché, 1977; Blouin *et al.*, 2013). All earthworm species consume organic matter, adding to its decomposition by soil micro-organisms; however these ecological groups differ in how they consume organic matter and facilitate its incorporation to the soil (Edwards, 2004). Epigeic earthworm species (e.g. *Eisenia fetida, Lumbricus castaneus*) dwell in litter on the soil surface, whereby their feeding activities comminute organic matter into smaller particles which facilitate microbial activity (Bouché, 1977; Edwards, 2004). Endogeic species (e.g. *Aporrectodea caliginosa*, *Allolobophora chlorotica*) create temporary burrows which are often shallow and horizontally oriented in the soil; these geophagous earthworms ingest inorganic soil materials and organic matter, and produce organically enriched casts either within the soil layers or on the soil surface (Bouché, 1977; Edwards, 2004). Anecic species (e.g. *Aporrectodea longa*, *Lumbricus terrestris*) create deep near-vertical burrows which are connected to the soil surface, and which may be inhabited for extensive periods of time (Bouché, 1977; Edwards and Bohlen, 1996). Anecic species principally feed on organic matter but also ingest large amounts of inorganic soil, vertically redistributing these throughout the soil profile; making these species of key importance in the process of pedogenesis (Edwards, 2004).

2.3. Land reclamation and restoration

2.3.1. Background to restoration ecology

Terms such as 'degraded', 'disturbed', 'damaged' and 'brownfield' are often used interchangeably to describe land which has been negatively influenced by human activities to the point where remedial action may be necessary to return it to a self-sustaining system (Bradshaw and Chadwick, 1980; Harris *et al.*, 1996). The UK government has defined brownfield land as "previously developed land", whereby: "Previously-developed land is that which is or was occupied by a permanent structure, including the curtilage of the developed land and any associated fixed surface infrastructure" (DCLG, 2011). This definition applies to land which has been used for waste disposal or mineral extraction until some form of land reclamation/restoration has taken place (DCLG, 2011). The extent to which this land can be returned to beneficial use is highly dependent upon the nature of the existing brownfield site (Bending *et al.*, 1999).

Although many brownfield sites may simply be derelict, some previous land uses may leave behind chemical contaminants in soils, e.g. former industrial sites and waste management sites such as landfills (DEFRA, 2009). Contaminated land is defined by DEFRA (2006) and part 2A of the EPA 1990 as "any land which appears to be in such a condition, by reason of substances on, in or under the land, that: a) significant harm is being caused or there is a significant possibility of such harm being caused; or b) pollution of controlled waters is being, or is likely to be caused". Common contaminants include solvents, hydrocarbons and heavy metals: The latter can have irreversible negative effects on vegetation health, and may directly affect the ability of soil organisms to survive and engage in vital ecosystem processes (Bending *et al.*, 1999; Hutchings, 2002; DEFRA, 2009). In the UK, it is a legal requirement for contaminated land to be remediated to a level which is fit-for-purpose, and generally it is the case that amenity soft end-uses such as woodland (section 2.4.1) require less extensive soil remediation than other land uses e.g. housing (Hutchings, 2002; DEFRA, 2006). In an urban context, such activities may be referred to as 'brownfield regeneration'. As a part of urban regeneration schemes, brownfield regeneration aims to complement the sustainable development of neglected urban areas by providing a desirable physical environment. This is reflected in the following EU Projects (2002) definition of brownfield regeneration: "Sustainable brownfield regeneration is the management, rehabilitation and return to beneficial use of brownfield base of land resources in such a way to ensure the implementation and continuity of satisfying peoples' needs for present and future generations in a non-degrading, environmentally friendly, economically viable, institutionally robust and socially acceptable manner". When brownfield land is regenerated to soft end-use such as woodland or grassland, the regeneration process adopts the principles and techniques of ecological restoration.

Ecological restoration has been defined as "…the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed" (Society for Ecological Restoration, 2004). Restoration was the term used by Bradshaw & Chadwick (1980) to refer to attempts to upgrade damaged land or restore a site back to its former habitat and enable it to regain its former biological potential. Harris *et al*. (1996) note that there is contention amongst restoration professionals regarding the concept of 'indigenous' ecosystems in this definition. For example, whilst it may be possible to characterise an indigenous ecosystem in North America (Jordan and Gilpin, 1987); it is far more difficult to do so in Europe, where land has undergone far more extensive change. As such, it is questionable whether restoration can actually be fully achieved, because natural systems undergo change over time and therefore the target land use is dependent on the scale at which the researcher decides to examine the ecosystem. Still, because the goal is to re-create former systems, land restoration has emerged as a significant area of focus for ecologists as a testing ground for exploring their understanding of ecological processes (Bradshaw and Chadwick, 1980; Jordan and Gilpin, 1987; DCLG, 1996; Perrow and Davy, 2002; Butt, 2008).

‘Reclamation’ was the original term used, before the development of the scientific discipline of restoration ecology in the 1980s, to refer to activities such as using degraded mining land for further economic exploitation, or draining wetlands (Harris *et al.*, 1996). Reclamation was subsequently redefined to cover when a new end-use or habitat type was envisaged for damaged land (Bradshaw and Chadwick, 1980). This definition has expanded over the years, generally now referring to the conversion of previously unusable land to a state where some benefit can be made from it (Harris *et al.*, 1996). At first this term was used for severely damaged or contaminated land which is converted into hard end-use (e.g. housing or car parking) or high-input soft end-uses (see section 2.4.1) (e.g. golf courses, sports pitches). However, a greater appreciation has since developed for the construction of valuable wildlife habitats on reclaimed sites (Andrews and Kinsman, 1990; Harris *et al.*, 1996). In this sense, reclamation differs from restoration in that the defined end-use may not be the indigenous habitat that existed on the site prior to human disturbance; however other restoration principles (e.g. ecological approaches) may be employed (Harris *et al.*, 1996). It should also be recognised that in some cases it may be of greater benefit to create new ecosystem types, for example ones which meet essential societal demands, or are more resilient to anticipated future pressures than the indigenous ones, e.g. resilience to invasive species and climate change (Bradshaw and Chadwick, 1980; Harris *et al.*, 1996). The extent to which land reclamation can be successfully achieved is highly dependent upon the nature of the existing site and the soil materials that are available for use (Doick and Hutchings, 2007) .

2.3.2. Reclaimed soil quality and earthworm influences on soil ecosystem services

Reclaimed soil quality is inevitably affected by the previous usage of the site, often resulting in a range of adverse soil properties (Moffat and McNeill, 1994). Industrial activities may lead to soil contamination, and mining or quarrying may result in significant physical alteration to soil structure (Moffat and McNeill, 1994; DEFRA, 2009). A common issue for brownfield sites, particularly ex-mineral workings and landfill sites is a shortage or total lack of topsoil available for use in reclamation (Maslen *et al.*, 2003). Topsoil is typically defined as the biologically-active, nitrogen-rich upper soil layers (Moffat and McNeill, 1994). In the absence of topsoil, vegetation establishment is typically carried out using subsoil or by using soil-forming materials (Bending *et al.*, 1999). In most cases, these soil-forming materials will consist of waste derived from mineral extraction or other man-made wastes from industrial activities (see Table 2.1).

Table 2.1. Soil-forming materials considered for use in land reclamation (adapted from Bending *et al*., 1999, pg.41).

Group	**Industry**	**Types**
Hard rock	Surface and deep mine coal	Colliery shales
		Hard rock overburden
	Stone quarries	Limestone and dolomite Sandstone
		Slate
		Igneous rock
	China clay	China clay waste
Soft rock	Sand and gravel	Sand and gravel
	Brick clay	Clay
	Superficial deposits	Drift
Man-made	Industrial wastes	Iron wastes
		Building rubble and arisings from construction projects
		Dredgings

Soil-forming materials can be considered equivalent to parent material, in which humans have conducted the initial stages of physical weathering, i.e. the starting point for soil development (Bending *et al.*, 1999). For the purposes of land reclamation, soil-forming materials have been defined as follows:

"Parent material for a new soil used as a substitute for, or supplement to, natural soils in the course of land reclamation. The material should, with appropriate surface treatment and the use of amendments as necessary during the period of aftercare, be capable of sustaining the required vegetation beyond this term by the implementation of normal management practices." (Bending *et al*., 1999, pg.28).

Although the complete development of a natural soil from soil-forming materials is better measured in a geological timescale (Bending *et al.*, 1999), given favourable conditions an acceptable degree of development can be achieved over decades (Bridges, 1961). However, the physical and chemical nature of the original material significantly influences the direction of soil development and the suitability of it to sustainably support flora and fauna (Bending *et al.*, 1999). Because of their raw and potentially hostile nature, soil-forming materials are generally considered inferior to natural soils in most regards; the materials generally contain low levels of plant nutrients and organic matter content, and possess many physical and chemical properties which can inhibit the growth of vegetation and the activity of soil organisms (Bending *et al.*, 1999; Dickinson *et al.*, 2005). Previous reclamation activities which have used mineral wastes without proper consideration given to the amelioration of the material's physical and chemical limitations have often been unsuccessful (Bending *et al.*, 1999). For example, when reviewing six failed greenspace regeneration projects, Doick *et al*. (2009) found that each was low in essential plant nutrients, and five sites had localised areas of vegetation failure due to compaction, low nutrient availability and low or high soil pH. In addition to generally detrimental substrate properties, the physical and environmental disturbance caused throughout reclamation processes can have significant negative impacts on soil macro- and micro-faunal communities, which differ in their ability to recover (Scullion, 1992). Vegetation growth and the provision of key soil functions and ecosystem services are often the functional outputs of biological processes within the soil (Moffat and McNeill, 1994; Kibblewhite *et al.*, 2008). These processes can be influenced by a number of undesirable physical and chemical properties typical to reclaimed soils (or 'soil-forming materials'), which can inhibit the activity of soil organisms (Kibblewhite *et al.*, 2008).

In recognition of their role in improving soil structure and fertility, earthworms have been the subject of research during land reclamation for over 50 years, e.g. (van Rhee, 1969a; Curry and Cotton, 1983; Curry, 1988; Butt *et al.*, 1995). Internationally, earthworms have been considered as potential soil improvement agents in a wide range of reclamation projects, ranging from the improvement of low fertility mineral soils for agricultural pasture in New Zealand (e.g. Hamblyn and Dingwall, 1945; Nielson, 1951) to grassland and orchards on reclaimed Dutch polders (van Rhee, 1969b; Rogaar and Boswinkel, 1978; Hoogerkamp *et al.*, 1983). Generally, in these reclamation projects the soil materials either already contained earthworms (albeit 'native' species, less effective at promoting soil development than European lumbricidae) or were devoid of worms but could be expected to undergo natural colonisation over timespans of years to decades (Scullion and Malik, 2000). However, destructive activities such as mining may leave a soil material which is devoid of earthworms due to heavy physical disturbance or pollution, or may involve the introduction of poor quality soil-forming materials unsuitable for supporting earthworm populations (Scullion, 2007). The soil materials in such sites are typically unsuitable for earthworm establishment due to extremes of pH (particularly acidity), lack of suitable food, unfavourable soil moisture conditions, and metal toxicity (Curry and Cotton, 1983).

Early colonisers of low-quality reclaimed soils are typically surface-dwelling or shallow-burrowing endogeic species, which may play a less active role in accelerating soil development than deeper-burrowing species (Zhang and Schrader, 1993). On reclaimed sites such as restored opencast coal workings, earthworm populations are low, and it may require 20 years or longer for deeper-burrowing species such as *L. terrestris* and *A. longa* to become established and for earthworm densities to reach levels typical of undisturbed soils, and for soil depths to become sufficient for these deeper-burrowing species (Armstrong and Bragg, 1984; Rushton, 1986; Scullion *et al.*, 1988; Scullion, 1994). It is therefore necessary that the soil environment is made suitable for earthworm colonisation and managed in a manner which supports soil biota (Scullion, 2007; Butt, 2008). UK studies into the use of earthworm action to improve the properties of manufactured soil materials during the restoration of brownfield sites are relatively few in number and have occurred over a range of scales and previous land uses (Butt, 1999b). A more recent critical review of UK case studies has highlighted a number of issues which have led to limited success in earthworm-focussed land regeneration projects, including inappropriate earthworm species selection, the use of excessively hostile substrates without amendment, and poor monitoring (Butt, 2008).

The principle physical, chemical and biological issues typical for reclaimed soils will now be discussed in context of the soil ecosystem services delivered and influenced by earthworm activity.

2.3.2.1. Soil formation

Natural soil development begins with the physical and chemical weathering of rocks or other parent material. This is followed by the development of a soil profile with soil structure, and in which secondary minerals (e.g. clay minerals and oxides) are produced, and a biological community develops which engages in the conversion of organic matter and plant nutrients (Bending *et al.*, 1999). Under natural conditions, a soil profile cannot be expected to develop from soil-forming materials in a timescale meaningful to human activities (Bending *et al.*, 1999). However, during reclamation there are opportunities to modify the environment to accelerate the process of soil formation, particularly studied in the context of colliery spoils (Crampton, 1967; Down, 1975; Bending *et al.*, 1999). Soil formation processes require mineral and organic components, and the activity of soil organisms. The addition of organic matter and the inoculation (introduction) of soil organisms may therefore provide opportunity to accelerate soil formation on reclaimed sites (Bending *et al.*, 1999).

Through the consumption, comminution and redistribution of organic matter throughout the soil profile, earthworms play a key role in soil formation (Edwards, 2004). The promotion of microbial activity by earthworms accelerates humic organic matter breakdown and stabilisation (Edwards, 2004). Membership of specific ecological groups (section 2.2.3) influences the manner in which earthworm species contribute to these process, for example through different deposition of casts within the soil profile (Curry, 1988; Edwards and Bohlen, 1996).

van Rhee (1969a, 1969b) utilised earthworm activity to accelerate the process of soil maturation on reclaimed polders in the Netherlands. A number of inoculation trials took place on polders reclaimed to grassland and orchards, with earthworm populations establishing much more rapidly in the former (750 m^{-2} and 140-250 m^{-2}, respectively, after eight years), potentially due to pesticide use in the orchards (van Rhee, 1969a; Curry and Cotton, 1983). Polders where earthworms were present developed mull soils in comparison to the mor soils in earthworm-free polders nearby (Curry and Cotton, 1983). Beneficial effects of earthworm activity on polder soil formation included improved aggregation and aeration, and increased leaf litter incorporation (van Rhee, 1969a, 1969b; Rogaar and Boswinkel, 1978). Overall, the earthworm species used were *A. caliginosa, A. chlorotica, A. longa, L. rubellus* and *L. terrestris*, and of these *A. caliginosa* was found to be a particularly successful coloniser of polders and important for soil formation in these sites (Curry, 1988).

2.3.2.2. Soil structure

Most soil-forming materials initially lack a formal structure, due to an absence of organic matter and biological components (Bending *et al.*, 1999). The main physical soil structural properties of interest during brownfield reclamation are soil strength, bulk density, compaction, particle size distribution and topsoil depth (Nortcliff, 2002; Moffat, 2003). Moffat & McNeill (1994) identified three key physical soil factors that directly and indirectly (by affecting a range of other physical soil properties) influence tree growth on brownfield soils: soil structure, texture and stone content.

Soil texture refers to the particle size distribution within the soil; the size and arrangement of soil particles are of principle importance for determining physical soil quality (Nortcliff, 2002; Bardgett, 2005). Brownfield soils often consist of extreme textures, which can reduce ease of handling, increase risk of compaction, and lead to reduced nutrient retention and water-holding capacity (section 2.3.2.3); therefore restricting vegetation growth (Moffat and McNeill, 1994).

Earthworms ingest soil mineral particles, mixing them intimately with organic matter during passage through the gut, and creating water-stable aggregates which improve soil structure (Edwards, 2004). It has been estimated that up to 50% of the aggregates in upper soil layers are due to earthworm activity in this manner (Kubiëna, 1953). Marashi and Scullion (2003) investigated the influence of earthworms on soil aggregation over a period of 8 years under grassland on a restored opencast coal site in South Wales. Before inoculating the site, surveys revealed dominance of the early colonising shallow-working species *L. rubellus* and *A. chlorotica* and an absence of deeper-burrowing species such as *L. terrestris and A. longa*. Inoculations then increased the populations of *L. terrestris, A. longa* and *A. caliginosa*. Within 3 years, inoculated areas of the reclaimed site contained populations similar to undisturbed soils, and over 5-6 years, soil stable aggregation, plant productivity and root growth all increased (particularly associated with the activity of *A. longa*).

Brownfield sites are also characterised by very stony soils, for example, ex-mineral workings will often possess and may also contain boulder-type materials (Doick and Hutchings, 2007). Soils with a high stone content are likely to present issues with tree rooting and anchorage (Moffat and McNeill, 1994). Through burrowing and feeding activities, earthworms move large quantities of soil from lower soil levels and deposit it on the surface, thereby reducing surface stone content of soil (Darwin, 1881). Values for this turnover of soil range from 2 to 250 tonnes ha^{-1} $year^{-1}$, which are equivalent to a 1 to 50 mm thick layer of soil per year (Edwards, 2004).

One of the most common negative soil physical attributes of brownfield soils is compaction, caused during physical reclamation activities (Dickinson *et al.*, 2005). A minimum recommended bulk density of reclaimed soils intended to support trees is 1.5 g cm^{-3} for the upper 50 cm soil profile, and <1.7 g cm^{-3} for the lower profile, to enable tree roots to exploit soil moisture at depth (Bending *et al.*, 1999). Because compaction destroys the soils natural structure and reduces its porosity, a number of undesirable issues can arise. For example, soil organism mobility and root penetration becomes limited, soil aeration is restricted, and there may be a reduction in the water-holding capacity of the soil (Moffat and McNeill, 1994; Wall *et al.*, 2012). Furthermore, compacted soils suffer issues with drainage and may rapidly lose moisture during warm weather, which can affect the survival and activity of soil biota (Moffat and McNeill, 1994). Moreover, under wet conditions compacted soil may lead to water-logging and anaerobic soil conditions, which can negatively affect soil biodiversity. The activity of anaerobic soil bacteria can result in by-products harmful to vegetation, e.g. carbon dioxide and alcohols (Moffat and McNeill, 1994).

Through their burrowing behaviour, earthworms create channels in the soil which facilitate air circulation and water infiltration, and reduce soil compaction (Butt, 1999b). Furthermore, mineral soil and organic matter become mixed through the ingestion and excretion of soil materials on the surface and throughout the soil column, and soil crumb structure is improved (Edwards and Bohlen, 1996; Butt *et al.*, 1999).

2.3.2.3. Water regulation

Another key physical soil property of interest during brownfield reclamation is soil water-holding capacity (Nortcliff, 2002; Moffat, 2003). Water-holding capacity of reclaimed soils can vary widely, depending on the types of soil-forming material used, and is influenced by particle size distribution in the soil (Bending *et al.*, 1999). Particle size distribution also affects water infiltration rates to soil, and cation-exchange capacity, which can influence the behaviour of nutrients in the soil (e.g. coarse china clay sands are freely draining with low cation exchange capacities, leading to nutrient leaching) (Bending *et al.*, 1999).

Through the formation of water-stable soil aggregates and burrows, earthworms have been demonstrated to improve soil porosity, drainage and moisture-holding capacity (Stockdill, 1966; Edwards and Lofty, 1977; Edwards, 2004). Burrows from different ecological groupings are positioned at different angles and depths within the soil profile, improving infiltration and soil drainage, and also water retention (Carter *et al.*, 1982; Edwards, 2004).

Whilst increased water infiltration and retention are generally beneficial soil properties, in some cases they may lead to negative conditions if site management is not properly considered. For example, Marashi and Scullion (2003) found that inoculation of earthworms to a restored mine site increased soil porosity throughout the top 20 cm of soil. However, the increased porosity led to greater soil water-logging and artificial drainage was unable to effectively remove the excess water from the soil profile. Likewise, Stockdill (1982) observed a reduced surface bearing strength due to waterlogging from increased water infiltration into earthworm burrows, particularly during the colonisation phase of earthworm introduction (Scullion, 1994). Under water-logged conditions, there is potential for treading damage (e.g. poaching from animal activity) to undo the other soil structural improvements brought about by earthworm activity (Scullion, 1994; Marashi and Scullion, 2003; Baker *et al.*, 2006). Furthermore, a tropical compacting species of earthworm (*Millsonia anomala*) has been found to increase drought stress in rice plant (*Oryza sativa*) (Blouin *et al.*, 2007).

Earthworm populations are significantly influenced by soil moisture content, it is a key environmental factor which affects earthworm activity and reproduction (Satchell, 1967; Lee, 1985; Holmstrup, 1994). In field conditions, most earthworms are active at water tensions around field capacity level (-10 kPa), but above -100 kPa activity reduces (Curry, 2004). Laboratory investigations have generally found that soil moisture content of 25-30% is preferable for a range of temperate Lumbricid species (Berry and Jordan, 2001; Lowe and Butt, 1999, 2005). Many species possess behavioural adaptations to survive periods of drought, for example *A. longa* will attempt to move to lower and moister soil levels, failing this, they enter into diapause (a resting stage) (Edwards *et al.*, 1995). In diapause, earthworms avoid moisture loss by curling into a knot within a mucus-lined cavity in the soil until conditions improve. Evans and Guild (1947) reported similar behaviour in endogeic species such as *A. caliginosa* and *A. chlorotica*. These behaviours may be lacking in juvenile earthworms, suggesting they may be more prone to drought stress (Curry, 2004).

2.3.2.4. Organic matter and nutrient cycling

In the majority of cases, the physical and chemical deficiencies of soil-forming materials for supporting vegetation are due to a lack of soil organic matter (OM) (Bending *et al.*, 1999). Guidance on the use of soil-forming materials recommends the addition of bulk OM to provide conditions which favour re-colonisation by soil organisms and the processing of OM into humic layers (Bending *et al.*, 1999; Moffat, 2006). Mineralisation rates of added OM will often be lower in reclaimed soils than in their natural counterparts, due to poor aeration and extremes of pH and drought (Fresquez and Lindemann, 1982; Bending *et al.*, 1999). Reclaimed soil materials which contain little OM before amendment will have poor microbial diversity and population density, which can also lead to poor initial mineralisation rates; however, given favourable conditions, this can be overcome within two years (Sopper, 1993; Bending *et al.*, 1999).

Earthworm distribution is strongly influenced by the OM distribution in soil, as poor quality and quantity of OM is associated with low earthworm numbers (Edwards and Bohlen, 1996). Addition of OM to reclaimed soils in the form of organic waste materials may influence the establishment of viable earthworm populations (Lowe and Butt, 2002b). Earthworms play a key role in the comminution and redistribution of OM through the soil profile, and the promotion of microbial activity by earthworms accelerates humic OM breakdown and stabilisation (Edwards, 2004). Earthworms are capable of incorporating very large quantities of OM into the soil; in natural ecosystems such as temperate forests, earthworms may have the capacity to incorporate the total annual litter fall (Raw, 1962; Satchell, 1967; Shipitalo and Le Bayon, 2004).

In the absence of earthworms, soils may build up a surface mat of decomposed OM (Kubiëna, 1953). Hoogerkamp *et al.* (1983) observed the removal of the surface organic mat by inoculated earthworms in polder grassland, noting complete incorporation into the soil within 3 years of earthworm presence. After 8-9 years, a 5-8 cm deep A_1 soil horizon had developed and soil had increased in aeration, pore volume, C:N ratio, water infiltration and available moisture (Hoogerkamp *et al.*, 1983; Curry, 1988). However it may be the case that the beneficial results obtained from earthworm activity are enhanced by the exploitation of these resources by the earthworms, and as such the findings may not reflect the true extent of their activities in more organically stable soil conditions (Scullion and Malik, 2000).

Soil-forming materials are typically deficient in essential plant nutrients compared with natural soils. Most geologically-derived soil-forming materials are deficient in nitrogen to the point where they are unable to provide sufficient plant-available nitrogen to support tree establishment (Bending *et al.*, 1999). Phosphorus availability largely depends upon the type of soil-forming material being used; some limestones and other minerals may contain sufficient quantities to support vegetation, however most materials are lacking in sufficient phosphorus (Bending *et al.*, 1999). Other essential plant nutrients such as calcium, potassium and magnesium can be variable in availability; and some materials may contain excessively high or low levels of single nutrients (e.g. London clay may contain very high levels of magnesium, while china clay may be deficient in potassium). This can cause antagonistic effects whereby an excess of one nutrient prevents plant uptake of others (Bending *et al.*, 1999). Antagonistic effects may also occur for minor plant nutrients; however these are generally present in most reclaimed soil materials at suitable levels for vegetation establishment (Bending *et al.*, 1999).

Earthworm feeding activity reduces the carbon: nitrogen ratio of OM, and results in the conversion of most of the nitrogen into the nitrate or ammonium form (Edwards, 2004). Likewise, essential plant nutrients such as potassium and phosphorus are converted into forms available to plants (Edwards, 2004). Research indicates that earthworm-mediated mineralisation of OM and improvement in nutrient availability, and subsequent improvements in plant growth, are likely to be greater in nutrient-poor soils (Jana *et al.*, 2010). Using two types of soil; an organic and mineral nutrient-poor soil plus a more nutrient-rich one, Jana *et al.* (2010) investigated the interactions between *A. caliginosa* and the model plant cress species *Arabidopsis thaliana*. In the poor soils, earthworm activity led to an increase in above-ground plant biomass and significant increase in soil nitrate content; in the nutrient-rich soil no significant effect was detected on above ground plant biomass in the presence of earthworms. Jana *et al.* (2010) concluded that in nutrient-limited soil materials, earthworm-induced mineralisation is likely a determining factor for plant growth. In higher quality soils, plants may be less noticeably affected by nutrient limitation and hence any benefits from earthworm activity harder to detect (Brown *et al.*, 2004).

Brownfield sites often display extremes of soil pH due to activities such as disposal of concrete and other building waste (Dickinson *et al.*, 2005). Extremes of pH negatively affect tree growth, particularly in native tree species, which are normally intolerant of these conditions (Dickinson *et al.*, 2005; Doick *et al.*, 2009). In general, materials possessing extremes of pH (<4.0 or >8.0) should not be considered for woodland establishment (Bending *et al.*, 1999; Forest Research, 2015). Stockdill (1959, 1966) found that introduction of *A. caliginosa* was unsuccessful in acidic, poor fertility unimproved tussock grassland; however, when added to improved upland pasture, it was associated with a reduction of the surface organic mat and an improvement in soil structure and nutrient cycling.

2.3.2.5. Climate regulation

Through their burrowing, feeding and casting activities, earthworms have a large influence on the greenhouse-gas (GHG) balance of soils (Blouin *et al.*, 2013). However, there is contention over whether earthworm activity results in soils becoming a net sink or source of GHGs (Lubbers *et al.*, 2013). Earthworms have been shown to increase organic matter incorporation into soil, storing it in the form of compact stable aggregates, thus sequestering C by preventing its release as carbon dioxide (CO_2) gas (Guggenberger *et al.*, 1996; Lavelle *et al.*, 2006; Don *et al.*, 2008). Earthworms may also facilitate carbon sequestration through earthworm-influenced increases in primary production (section 2.3.2.8) and therefore carbon fixation by plants (Blouin *et al.*, 2013). Whilst earthworms may encourage the sequestration and stabilisation of carbon in soil aggregates, there is some indication that they increase the emission of CO_2 and nitrous oxide (N_2O), two main GHGs. For example, in a meta-analysis of 36 studies, Lubbers *et al.* (2013) found that earthworms are responsible for producing a net increase of CO_2 emissions by 33% through aerobic respiration. The same meta-analysis reviewed 12 studies and found that earthworm presence led to a 37% increase in N_2O emissions. Earthworm-mediated increase in N_2O emissions from crop residues, for example, occurs due to the mixing of residues into the soil. This alters residue decomposition from an aerobic (and low denitrification) pathway to one with significant denitrification and N_2O production (Rizhiya *et al.*, 2007). Whilst these studies give insight into the complexities of earthworm activity influences on soil GHG balances, there is insufficient research into the long-term, landscape-scale effects of earthworm activity from which to draw conclusions (Blouin *et al.*, 2013).

2.3.2.6. Cultural services

Earthworms provide a number of cultural services. The burial of objects by earthworm casting was observed and measured by Darwin (1881), and the role of earthworms in the burial (and subsequent protection) of archaeological artefacts has been widely investigated and quantified (Wood and Johnson, 1978; Stein, 1983; Armour-Chelu and Andrews, 1994; Yeates and der Meulen, 1995). Earthworms provide a cultural and recreational service in the form of fishing bait, although in North America, careless disposal of such bait has likely contributed to the spread of invasive European earthworm species such as *L. terrestris* into native ecosystems (Callaham *et al.*, 2006; Kilian *et al.*, 2012). Blouin *et al.* (2013) identified that earthworms provide a cultural service as tools for educating environmental awareness both in classrooms and the home; where both adults and children can learn about recycling of organic wastes into fertile organic plant substrate via worm bins.

2.3.2.7. Terrestrial and soil food webs

Through interactions which stimulate and promote soil microbial activity, and as hosts to pathogens and parasites, earthworms play an important role in soil food webs. Earthworms are also a key component in the diet of many terrestrial vertebrate and invertebrate predators (Edwards and Bohlen, 1996). As described further in the following section, earthworms interact with soil micro-organisms in a number of ways, such as stimulating microbial activity and redistributing micro-organisms throughout the soil (Lavelle and Spain, 2001). Through the production of casts, earthworms provide a substrate rich in nutrients and OM, which support greater populations of bacteria and fungi than the surrounding soil (Tiwari and Mishra, 1993). However, some earthworms also ingest micro-organisms as a food source (Edwards and Fletcher, 1988).

Large numbers of earthworms are eaten by mammals such as badgers (*Meles meles*), hedgehogs (*Erinaceus europaeus*), shrews (*Sorex* spp.), red foxes (*Vulpus vulpes*), and moles (*Talpa europaea, Parascalaps breweri, Condylura cristata*) (Macdonald, 1983; Kruuk and Parish, 1985; Edwards and Bohlen, 1996). Many species of birds feed upon earthworms, including thrushes (*Turdus ericetorum*), starlings (*Sturnus vulgaris*) and Blackbirds (*Turdus merula*). Earthworms also form part of the diet of a variety of invertebrate species, including carabid and staphylinid beetles and their larvae, e.g. the slug-feeding carabid beetle *Pterostichus melanarius* (Edwards and Bohlen, 1996; Symondson *et al.*, 2000). In the UK and elsewhere, land development operations legally require the relocation of reptiles, however very little is known about most reptiles' typical diets, and therefore the suitability of receptor sites (which may include previously reclaimed sites) cannot be guaranteed (Brown *et al.*, 2012). In an investigation into the prey DNA component derived from analysis of slow worm (*Anguis fragilis*) faeces, Brown *et al.* (2012) identified that earthworms were the majority of the slow worm's diets, ranging from 6 to 9 species per slow worm, and with earthworm species found from across all three earthworm ecological groupings (sensu Bouché, 1977). By forming an important link in food webs, earthworms can further assist the restoration of an ecosystem beyond simply the effects of their activity on soil development.

2.3.2.8. Primary production (earthworm-plant interactions)

The objective for land reclamation activities, particularly mineral workings, is usually to restore the land to its original habitat and level of ecological productivity; however other after-uses may be considered, depending on factors such as availability of sufficient soil or soil-forming materials. Reclamation to agricultural use is possible, however this is usually to arable land or grassland rather than crops unless sufficient topsoil is available (Bending *et al.*, 1999). Positive effects on plant growth after earthworm addition have been demonstrated for agricultural crops and grasses; typically in existing agricultural fields, as summarised by Scheu (2003) in a review of 67 plant-earthworm interaction studies. Early agricultural field trials in New Zealand mainly involved the inoculation of European Lumbricidae earthworms to improve production on sown pasture (Curry and Cotton, 1983). Hamblyn and Dingwall (1945) and Nielson (1951) attributed the introduction of *A. caliginosa* via turf transfer to an increase in pasture land stock carrying-capacity and improved cover of high fertility vegetation. In Australia, beneficial results were obtained for sown pasture on irrigated sandy loam soil which was inoculated with *A. caliginosa* and *Microscolex dubius* (a Megascolecid species); however the same inoculation type was unsuccessful on clay soils (Barley and Kleinig, 1964; Noble *et al.*, 1970).

On a reclaimed peat extraction site in Ireland, Curry and Boyle (1987) set up a field microplot experiment to investigate the effect of earthworms on herbage production of perennial ryegrass and white clover. No clear effect on herbage was detected in the first year for plots containing earthworms compared with control plots; however, the second and third year total herbage yield was 25 and 49% greater than controls, respectively. Control microplots did not remain free of earthworms, however, and soil heterogeneity made it difficult to determine effects of earthworms on soil properties. Controlled glasshouse experiments in buckets were subsequently carried out over a period of 20 months to emulate the field experiment (Curry and Boyle, 1987). Results demonstrated 89% higher herbage yield in buckets with earthworms which also received cattle manure, when compared with earthworm-free controls receiving the same fertiliser application. Because of the inherent difficulties in ensuring earthworm-free controls in the field, most investigations into earthworm influence on plant growth have been carried out as pot trials (McColl *et al.*, 1982).

Earthworms directly affect plant growth through the production of plant-growth promoting-substances (Nielson, 1951, 1965; Gavrilov, 1963). Extracts from the tissues of *A. caliginosa, L. rubellus, E. fetida, A.longa, D. rubida*, and *L. terrestris* have shown significant effects on plant growth (Nielson, 1965). Springett and Syers (1980) proposed that *L. rubellus* casts contained an auxin-like compound, which has subsequently been confirmed for casts of the species *A. caliginosa* and *Aporrectodea rosea* (Muscolo *et al.*, 1999). Other research has concluded that earthworm activity produces a hormonal effect on plants (Tomati *et al.*, 1983; Edwards and Burrows, 1988). Research conducted by Jana *et al.* (2010) has linked the activity of *A. caliginosa* to phytohormone-like compound production by earthworm-stimulated bacteria in the soil. This led to increased soil nitrate content and increased above-ground plant biomass production, and these effects were very significant in low mineral nutrient and OM soil. This research therefore has implications for experiments involving reclaimed soil materials, which are typically low in nutrients and OM content.

Earthworms may indirectly affect plant growth through dispersal interactions with soil organisms which have mutualistic or symbiotic relationships with plants (Edwards and Bohlen, 1996). Root-colonising micro-organisms (such as pseudomonads, rhizobia and mycorrhizal fungi) have minimal ability to disperse through the soil unaided; and their dispersal has been shown to be improved via earthworm activity (Doube *et al.*, 1994; Stephens *et al.*, 1994; Edwards and Bohlen, 1996; Montecchio *et al.*, 2015). Rouelle (1983) associated increased root nodules on soybean with the presence of *L. terrestris*, and demonstrated that *Rhizobium japonicum* is able to pass through the digestive systems of *L. terrestris* and *E. fetida* to produce root nodules on soybean. Similar findings by Doube *et al.* (1994) showed the ability of *A. longa* and *A. trapezoides* to vertically distribute *Rhizobium meliloti* from surface OM into the soil and increase root nodulation. Mycorrhizal fungal spores and hyphal fragments have been observed to spread, following passage through the gut of the earthworm *Pontoscolex corethrurus* (Reddell and Spain, 1991). It is reasonable to expect that any micro-organism effects on plant growth, as mediated by earthworm activity, should be more marked in nutrient-poor materials such as reclaimed soils than in fertile soils where mineral nutrient resources are more available (Laossi *et al.*, 2010).

2.4. Woodland establishment on reclaimed sites

2.4.1. Restoration to woodland end-use

Hard or soft end-use are the common terms used to describe the outputs of land reclamation activities. These two land use options differ in terms of the biological and physical engineering component, with hard end-uses having little-to-no biological, but a high engineering component (e.g. housing, industry, roads) (Harris *et al.*, 1996). Alternatively, biological components are integral to soft end-uses, which may also have varying levels of engineering required. Soft end-uses can be further divided into either 'productive' or 'amenity' uses (Table 2.2). Early restoration philosophy dictates that the soft end-use chosen for a site should consider the requirements of the local area and fit-in with surrounding land uses to ensure the site integrates with local ecological systems (Bradshaw and Chadwick, 1980). For the purposes of this thesis, the two main forms of end-use

Table 2.2. Forms of productive and amenity soft end-use (adapted from Harris *et al*., 1996).

Soft end-use type	Use
Productive	Arable or grassland agriculture, economic forestry, energy crop plantations, horticulture.
Amenity	Community woodlands/forests, country parks, nature reserves, campsites, golf courses, urban greenspaces, and landscaped areas of hard end-uses.

considered are community woodlands and amenity forests. These are both amenity end-uses; however, in some cases they may include an element of productive end-use such as short-rotation coppice energy plantation.

Brownfield sites can be regenerated into greenspaces such as community woodlands, which may provide improved biodiversity, contribute toward climate change mitigation and adaptation, and help alleviate local social deprivation (Doick *et al.*, 2009). Greenspace may be defined as an "area of grass, trees, or other vegetation set apart for recreational or aesthetic purposes in an otherwise urban environment" (Oxford English Dictionary, 2013). Turning brownfield sites into greenspaces may benefit biodiversity and improve the delivery of ecosystem services from the site (Bullock *et al.*, 2011). Restoration standards are typically less stringent for woodland establishment than for agriculture, and woodland establishment is usually possible on reclaimed soil materials with a wider range of chemical properties (Bending *et al.*, 1999). However, there are published minimum standards for reclaimed soil materials used for forestry end-use, and these should be adhered to in order to obtain better results (Table 2.3).

2.4.2. Tree effects on earthworms

Tree litter has been shown to influence soil faunal populations (Swift *et al.*, 1979), and so different tree species influence soil quality and soil faunal communities differently through the quality and quantity of their leaf litter (Pigott, 1989; Muys *et al.*, 1992; Reich *et al.*, 2005). The chemical composition of litter appears to strongly influence earthworm feeding behaviour, particularly aspects such as C:N ratio and the content of nitrogen, calcium, lignin and polyphenols (Satchell and Lowe, 1967; Hendriksen, 1990; Rajapaksha *et al.*, 2013). Additionally, leaf size and shape has been found to affect earthworm litter consumption (Satchell and Lowe, 1967). Generally, higher N and Ca content and a lower C:N ratio have been associated with increased palatability of leaf litter to earthworms (Reich *et al.*, 2005; Rajapaksha *et al.*, 2013). In a leaf litter choice chamber experiment, Rajapaksha *et al.* (2013) found that leaf litter from the least preferred tree species, sweet chestnut

(*Castanea sativa*), contained particularly low levels of nitrogen and calcium, and highest C:N ratio of all tree species investigated: alder (*A. glutinosa*), common ash (*F. excelsior*), silver birch (*Betula pendula*), sweet chestnut (*Castanea sativa*), sycamore (*Acer pseudoplatanus*), and an exotic eucalyptus species (*Eucalyptus nitens*). Other factors may affect leaf palatability to earthworms besides those already discussed, and indeed other chemical factors such as content of lignin and tannins have been identified as contributing to earthworm preference (Hendriksen, 1990). It has been suggested that litter selection by earthworms can be affected by the state of leaf litter decomposition or weathering (Satchell and Lowe, 1967; Hendriksen, 1990).

Table 2.3. Minimum soil and soil-forming material standards for reclamation to forestry (Forest Research, 2015) (adapted from Moffat *et al.*, 1992; Moffat and McNeill, 1994; Bending *et al.*, 1999).

Parameter		Standard	Comments on method
Texture		No limitation; however, the placement of materials of different texture on site should be related to site factors, e.g. topography	Texture (% sand, silt and clay) should be determined by pipette method. Preferred textures include materials with >25% clay
Bulk density (after placement)		<1.5 g^{-} cm^{-3} to at least 50 cm depth <1.7 g^{-} cm^{-3} to at least 50 cm depth	
Stoniness	Clay or loam	< 40 % by volume of materials greater than 2 mm in diameter and <10 % by volume of material greater than 100 mm in diameter	Measure mass of stone > 2 mm and >100 mm in a known mass / volume of soil, divide each value by 1.65 to calculate the volume
	Sand	<25% by volume of material greater than 2 mm in diameter and < 10 % by volume of material greater than 100 mm in diameter	
pH		Must be within the range 4.0 to 8.0	Based on a 1:2:5 soil: $CaCl_2$ (0.01 M) suspension
Electrical conductivity		<0.2 S m^{-1}	Based on a 1:1 soil: water suspension
Iron pyrite content		<0.05 %	British Standard 1016 method
Topsoil nutrient and organic content		N >200 kg N ha-1 P >16 mg l-1 (ADAS Index 2) K >121 mg l-1 (ADAS Index 2) Mg >51 mg l-1 (ADAS Index 1) Organic matter content >10%	N determination using the Dumas method P and organic matter determination K and Mg determination
Specific metal and organic contaminants		These should fall between the Soil Guideline Values (DEFRA and EA, 2002) for residential without plant uptake and industrial / commercial. Where no SGVs are available acceptable limits should be derived using a risk-based approach for human health.	Determination according to substance using a method comparable with the SGVs being used. Approval should be sought from Forest Research on the guideline concentrations being used before soil placement begins.

Earthworms have been demonstrated to prefer decomposed litter over fresh litter, and this increased palatability has been linked to fungal and bacterial colonisation and activity on the leaf material (Satchell and Lowe, 1967; Wright, 1972; Cooke and Luxton, 1980; Cooke, 1983; Hendriksen, 1990). However, in a laboratory experiment Butt (2011a), used dried green *Betula pendula* leaves as feedstock for *L. terrestris*, and found that switching from dried senesced leaves to green leaves resulted in increased *L. terrestris* mass and significantly increased cocoon production. This was attributed to the larger nitrate content in green leaves enabling more rapid protein synthesis for growth and reproduction. After 5 months the feedstock was switched back to dried fallen leaves, resulting in a reversal of this trend (Butt, 2011a)

Trees may also be expected to influence certain earthworm species through associations with their roots. For example, endogeic earthworm species such as *A. chlorotica* dwell within the rhizosphere, forming close associations to the root systems of plants (Sims and Gerard, 1999). Therefore, root chemistry might be expected to affect these species either directly or indirectly e.g. through root exudates, or modifying local soil pH (Dakora and Phillips, 2002; Rajapaksha *et al*., 2014).

2.4.3. Effects of earthworms on tree growth, and woodland establishment on reclaimed soils

Research has been conducted into the effects of earthworm activity on plant growth, but timescales of years are often required to show any effect through earthworm-influenced soil development (Edwards and Bohlen, 1996). Field experiments are relatively few in number compared with pot experiments, with the latter often leading to inconclusive results due to experimental errors, such as being unable to determine whether positive results are due to earthworm activity or the release of nutrients from dead and decaying earthworms (Edwards and Bohlen, 1996). However, positive effects on plant growth after earthworm addition have been demonstrated for agricultural crops and grasses; typically, in existing agricultural fields (section 2.3.2.8).

There has been extensive research into the effects of earthworm activity on plant growth, however these studies primarily focussed on agricultural crop plants (see review of 67 such articles by Scheu, 2003). Comparatively, there has been very little research into the influence of earthworms on tree health and growth (e.g. Marshall, 1971; Haimi *et al*., 1992; Muys *et al*., 2003; Moffat *et al*., 2008; Rajapaksha *et al*., 2014). Additionally, most studies have focused on species with short life cycles (annual or biennial) that are often herbaceous: earthworm interactions with long-lived trees is less researched (Avendaño-Yáñez *et al*., 2014). However, in the few studies undertaken, promising results have been achieved. Results include 26% increased growth in 2-year-oak (*Quercus robur*) and 37% in green ash (*Fraxinus pennsylvanica*) using live earthworms in pot trials, and significantly increased weight of black spruce (*Picea mariana*) seedlings when earthworms were added to the soil (Marshall, 1971; Muys *et al*., 2003; Welke and Parkinson, 2003). Beech (*Fagus sylvatica*) seedlings, when grown in forest soils with either an individual *Octolasion lacteum* or no earthworms, showed clear differences in growth and allocation of carbon and nitrogen (Wolters and Stickan, 1991). The beech seedlings grown in soils containing earthworms exhibited greater nitrogen incorporation and growth of stems compared to those grown in control soils. Whilst no effect was found on total root production, earthworm presence increased the proportion of large to small roots (Wolters and Stickan, 1991).

Using partially sterilised coniferous forest soils, Haimi *et al.*, (1992) investigated the effects of *L. rubellus* on young (9 cm high) birch (*Betula pendula*) seedlings in a microcosm experiment. The seedlings exhibited significantly faster growth in the presence of *L. rubellus*, with longer stems and 33% greater leaf biomass than those in earthworm-free soil after 51 weeks. Height was also influenced by the presence of earthworms. After 51 weeks, earthworm-treated seedlings reached a mean height of 36 cm, whilst earthworm-free control seedlings grew to a mean height of 21 cm. Stem biomass was observed to be higher in the presence of earthworms, however root biomass was shown to decrease compared with controls. Nitrogen content of leaves was almost twice as high for seedlings in the earthworm-added soil than those grown in control soils, although some of this may be explained by increased nitrogen content of the soils due to earthworm mortality. It was concluded that this can only partially explain the increased nitrogen content, and that earthworm activity contributes toward increased plant performance in forest soil.

Rajapaksha *et al.* (2014) used a field-based mesocosm experiment to investigate the effects of a combination of *L. terrestris* and *A. chlorotica* on two short-rotation forestry (SRF) tree species. One-year old birch (*B. pendula*) and eucalyptus (*E. nitens*) seedlings were grown in tubes containing de-faunated Kettering loam soil, with half of the tubes receiving the combined earthworm inoculate, and half as earthworm-free controls. OM was added to the soil surface in all tubes, in the form of leaves of the host plant. Eucalyptus demonstrated a 25% increase in total biomass and a 27% increase in foliar nitrogen concentration in earthworm-containing soils compared to controls. However, no significant earthworm effect was found for foliar nutrient content or biomass of birch. Rajapaksha *et al.* (2014) concluded that whilst there is evidence for a beneficial earthworm-SRF interaction, further study is required to investigate the interaction effects between different earthworm species and SRF tree species, preferably over longer timeframes than the 1-year duration of their study.

There has been little research into the influence of earthworms on forest tree health and growth. In a systematic review of the existing body of research at the time, Butt (1999) found only 11 documented field-scale brownfield regeneration projects utilising earthworm inoculation, and of these only 3 were to a woodland end-use (with the majority to grassland or pasture). Most research to date has been in the form of microcosm/mesocosm experiments using pot trials under controlled environmental conditions. Furthermore, most experiments have investigated the effects of earthworms on plant growth using grassland or pasture soils; very few have looked into tree growth in forest soil, and fewer still have investigated tree growth using reclaimed soil materials.

In an early experiment into tree growth on reclaimed soil, van Rhee (1977) observed a clear influence of earthworms on the root growth of apple trees on reclaimed Dutch polders. Previously earthworm-free polder soil was inoculated with *A. caliginosa* and *L. terrestris* at a rate of 500 m^{-2}, which led to an increase of up to 140% in fine root (<0.05 mm diameter) density, along with an increase in density of thicker roots and a 70% increase in soil aggregate stability. Vimmerstedt (1983) conducted greenhouse studies using soil cores from acidic colliery shale spoils, inoculated with *L. terrestris* and used to grow red oak (*Quercus rubra*) seedlings from acorns; followed by harvesting and earthworm removal after four months and re-use of the soil fraction (<2 mm) for planting rooted cuttings of eastern cottonwood (*Populus deltoides*), which had height measurements taken over the course of one growing season. No significant difference was found in size, biomass or leaf N or K contents for red oak in earthworm-containing spoil against controls. The leaves of seedlings grown in earthworm-free spoils displayed higher concentrations of P, Mg and Ca than those with earthworms (0.20, 0.42 and 1.13 compared to 0.17, 0.33 and 0.93%, respectively). Cottonwood-rooted cuttings grew to 29 cm in earthworm-worked spoil materials, compared to 22 cm in soils with had not previously contained earthworms. The author suggests that these inconclusive results demonstrate the need for rigorously designed field-experiments investigating these relationships on reclaimed land such as mining spoils. In a field investigation, Vimmerstedt (1983) introduced *L. terrestris* to two areas of mining spoil (one calcareous, one acidic) in Ohio which had been re-vegetated with common alder (*Alnus glutinosa*) and black locust (*Robinia pseudacacia*) but not topsoiled. On calcareous spoils, *L. terrestris* were larger in biomass and had higher population densities under *A. glutinosa* than *R. pseudacacia*. After 13 years, *L. terrestris* populations survived in both spoils, and were found to have incorporated leaf litter from both tree species into the spoil; however, a preference was shown for *A. glutinosa* litter. Field soils containing no earthworms were found to have significantly greater levels of P than those containing earthworms.

Ma *et al.* (2003, 2006) conducted a series of pot-based investigations into the effects of the litter feeding, burrowing earthworm *Pheretima guillelmi* on growth of a woody leguminous tree species (*Leucaena leucocephala*) on top-soil amended metalliferous mine tailings in China. Both tree and earthworm growth and survival were recorded in varying dilutions of mine tailing and soil amendment; *L. leucocephala* grew on tailings with 25% w/w volume soil amendment, whilst *P. guillelmi* failed to survive and/or actively burrow in treatments containing less than 50% soil amendment. Earthworms were found to increase plant productivity by 10-30%, marginally increase P and N in soils, and increase phosphorus uptake to above-ground plant tissues by about 10% compared to controls (Ma *et al.*, 2003). However, increased plant mass driven by earthworms also led to increased Pb and Zn metal uptake by the plants (an increased uptake of between 16% and 53%).

An ecosystem restoration experiment conducted by Muys *et al.* (2003) saw a mixed red oak (*Q. rubra*) and common beech (*Fagus sylvatica*) forest in Belgium cut down on an area of compacted and acidified clayey loam soil, and reforested with ash (*Fraxinus excelsior*). Treatment combinations of inorganic fertiliser and a combination of anecic and endogeic earthworm species (*L. terrestris, A. longa, A. caliginosa, A. rosea* and *Allolobophora limicola*) were applied in a randomised block design, including a control group containing neither treatment. After two years, the trees in the control plots failed to survive. Fertilised-only trees grew faster than fertilised trees with earthworms for the first two years, and a reversal in this trend was observed from year 4 onwards. This suggests that earthworms potentially encouraged sustained fertiliser response from the trees, although this could not be experimentally confirmed (Muys *et al.*, 2003). Biomass of leaves, branches and trunks were higher in the fertiliser and earthworm treatment, although not statistically significant; as were foliar concentrations of N, P, Na and K (with the opposite trend of Ca and Mg). No significant differences were detected in exchangeable nutrient concentrations between treatments (i.e. due to earthworm activity). By the end of the ten-year experiment, the anecic earthworm species *L. terrestris* and *A. longa* could no longer be found within the experimental plots and were presumed to have failed to colonise.

In one of the few UK field studies investigating earthworm use in reclamation to woodland, Craven (1995) inoculated the site of a former steelworks with *L. terrestris* through use of the Earthworm

Inoculation Unit (EIU) technique of Butt and Frederickson (1995), to assist short-rotation coppice (SRC) willow (*Salix sp.*) and poplar (*Populus sp.*). In total, 8,000 *L. terrestris* were obtained commercially and placed into EIUs containing sewage sludge and colliery spoil (a similar material to the substrate of the site) and inoculated into the site. After 2 years, no earthworms were located on the site and parallel laboratory trials indicated that the earthworm species selection for this experiment was unsuitable for the soil conditions on the site (Craven, 1995; Bain *et al.*, 1999).

In 1992, Butt *et al.* (1997, 1999) set up what was perhaps the largest earthworm inoculation and tree planting experiment on reclaimed land in Britain to date (Butt, 2008). A 2 ha area of a reclaimed landfill site at Calvert in Buckinghamshire with a clayey soil-forming material cover was inoculated with EIU treatments containing either mixed or monocultures of *A. longa* and *A. chlorotica* (Butt *et al.*, 1997). In the second phase of the experiment, a year following inoculation, alder (*Alnus glutinosa*) and sycamore (*Acer pseudoplatanus*) were experimentally planted on the site. Performance of trees and earthworms then took place until 2003. After 11 years, 100% mortality was recorded for sycamore, which was replaced naturally by a cover of perennial ryegrass and clover. A significant effect was found for alder on earthworm populations compared to those in the former sycamore locations (mean earthworm density and biomass under alder was 198 m^{-2} and 33.9 g m^{-2} whilst those under former sycamore was 118 m^{-2} and 20.7 g m^{-2}, respectively). Results showed that *A. longa* was significantly affected by tree presence, however *A. chlorotica* was not. No effect of earthworm inoculation treatment was detected for tree performance, likely due to low soil OM content and the highly compact nature of the soils (Butt, 2008). Four naturally colonising species (*A. rosea, L. castaneus, L. rubellus,* and *Eiseniella tetraedra*) were recorded within the experimental plots, alongside the two experimental species; a significantly greater number of these were found in the presence of trees. Damage to the site in the form of road construction terminated this experiment, however it demonstrated that the presence of alder had a positive effect on sustainable earthworm community development (Butt *et al.*, 2004; Butt, 2008).

Studies into the utilisation of earthworms during the restoration of brownfield sites to woodland are few in number (Butt, 1999b). Critical review of UK case studies has highlighted a number of issues which have led to limited success in earthworm-focussed land regeneration to woodland projects, particularly due to inappropriate earthworm species selection and the use of excessively hostile substrates without amendment (Butt, 2008). In general there is limited understanding of the dynamics of naturally and artificially colonised earthworm populations under reclaimed soils regenerated to woodland (Boyer and Wratten, 2010; Eijsackers, 2011). Investigations into the relationship between earthworm activity and plant (especially tree) growth have considered a limited number of species of both, and very few have investigated the influence of reclaimed soil. To better understand the relationships involved, more field-scale and pot-trial studies are required which investigate a wider variety of earthworm and plant species and, importantly, soil conditions (Edwards and Bohlen, 1996; Edwards, 2004).

2.5. The use of organic waste materials during land reclamation

2.5.1. Application of organic wastes in land reclamation

Since the rate of soil development is largely determined by the rate of OM accumulation into the soil, it is logical to consider adding additional OM from external sources as a means of accelerating the soil development process (Bending *et al.*, 1999). Soil-forming materials undergo biological, chemical and physical modifications following amendment with organic wastes, which are predominantly the results of soil organisms processing additional plant or animal residues (e.g. roots and litter) into humified organic materials (Bending *et al.*, 1999). Because soil OM decomposes over time, the objective with adding organic amendments is to increase soil organism and plant productivity to a point where their inputs of organic residues are at rate which can maintain soil OM levels (Bending *et al.*, 1999).

Research indicates that incorporating organic waste into soil-forming materials during land regeneration may improve tree growth and help alleviate many of the previously identified negative soil properties, as summarised in Table 2.4 (Foot *et al.*, 2003; Forest Research, 2015). Guidance literature on the use of soil-forming materials for land regeneration asserts that whilst inorganic fertilisers can have some benefits, the use of organic waste materials is far more effective at raising soil-forming material fertility for woodland establishment (Bending *et al.*, 1999). Moffat (2006) argued that organic waste materials should always be considered for improving soil-forming materials for brownfield regeneration to community greenspace such as woodland.

Table 2.4. The main advantages and disadvantages of using organic wastes as soil improvers (adapted from Forest Research, 2015).

Advantages	**Disadvantages**
Chemical • Stabilise or increase pH • Most immobilise metal contamination • Act as slow release fertiliser (nitrogen and phosphorus) Physical • Decrease bulk density in compacted soils • Increase soil porosity • Increase soil water holding capacity • Increase hydraulic conductivity • Reduce surface crusting leading to improved water infiltration Biological • Increase micro-organism activity	• Can immobilise plant-available nutrients if carbon: nitrogen (C: N) ratio is above 25:1 • Possible heavy metal contamination • Presence of potentially harmful organisms (plant, animal, human pathogens) in insufficiently composted materials • If C:N is below 10, amendments are more likely to contribute to nutrient leaching from the amended soil

Despite an existing body of research and guidance regarding the amendment of soil-forming materials with organic wastes, and it being an effective organic waste stream management solution (Scullion, 2007), such techniques are typically not used during the creation of community greenspaces (Ashwood *et al.*, 2014). This is often reflected in schemes that have displayed failed vegetation establishment (Doick *et al.*, 2009).

2.5.2. Earthworms and organic waste materials

Research indicates that on restored sites, the addition of OM to soil may be important for establishing sustainable earthworm populations (Lowe and Butt, 2002b, 2004). A range of organic waste types have been investigated for suitability to support earthworm growth, including composted green waste, papermill and boardmill sludge, sewage sludge, separated cattle solids and other animal wastes. However, these have largely been demonstrated for low-fertility mineral soils and less so for reclaimed soils (Edwards and Bohlen, 1996; Piearce and Boone, 1998; Bain *et al.*, 1999; Butt *et al.*, 2004; Lowe and Butt, 2004). Not all organic waste materials may provide a suitable medium for earthworm growth, as some materials may require processing to remove certain harmful properties (e.g. pathogens or heavy metals in sewage sludge, as previously described) (Edwards and Bohlen, 1996; Bain *et al.*, 1999; Bending *et al.*, 1999). However, it has been shown that certain species will actively incorporate and mix organic waste materials into soils, enhancing mineralisation and benefiting soil fertility (Piearce and Boone, 1998). The addition of earthworms may therefore be an effective way of enhancing the benefits of utilisation of organic wastes by vegetation and other soil fauna during land regeneration. There is also a suggestion that the physical blending of OM with soil mineral components through the action of earthworms may provide carbon sequestration benefits (Scullion and Malik, 2000; Scullion, 2007).

Location of organic waste application within the soil profile can influence the establishment of earthworm populations. Lowe and Butt (2002) investigated the foraging ability of two anecic earthworm species previously used in reclamation experiments, and found that *A. longa* exhibited greater capacity to forage for OM within the soil profile than *L. terrestris*. These findings may help explain the outcomes of a field experiment in which *A. longa* out-performed *L. terrestris* in reclaimed soils in which Separated Cattle Solids (SCS) had been incorporated rather than applied to the surface (Butt *et al.*, 1997; Lowe and Butt, 2002b). This suggests that decisions on earthworm species selection and method of organic waste application to the soil cannot be made independently, however there is still very little research which enables informed choices to be made in this regard.

2.5.3. Forms of organic waste and their application in land restoration to woodland, with examples of earthworm utilisation

A wide range of organic waste materials have been considered for use in land regeneration over the past two decades, with an accompanying body of research into the effects of their application to land (Wolstenholme *et al.*, 1992; Sopper, 1993; Moffat, 2006; SNIFFER, 2010). Each form of waste material possesses unique advantages and disadvantages for soil development, but also from regulatory, economic and supply perspectives; as such the selection of a waste type must be based on the specific conditions and objectives of each reclamation project. The main historic and contemporary forms of organic waste considered for land reclamation will now be given specific focus, and examples given where possible of their use alongside earthworm research for reclaimed soils.

Sewage sludge (biosolids)

Sewage sludge is perhaps the most historically used form of organic waste in land reclamation, however it has lost favour in modern reclamation projects due to potentially high pathogen content and odour issues (Bending *et al.*, 1999). Multiple forms of sewage sludge are available: these are liquid, cake, and thermally-dried pelletised forms (Moffat, 2006). Sludges may be treated through anaerobic digestion or the addition of alkaline materials, with digested sludge generally recommended in the literature to overcome pathogen and odour issues. The form of sewage sludge (e.g. liquid, cake, pelletised) is determined primarily by the extent of water removal and solids content during processing, and this affects how these materials can be applied to soil during land reclamation (Bending *et al.*, 1999; Moffat, 2006).

Sewage sludge is a suitable material for improving reclaimed soil quality, as it is contains essential plant nutrients in available and slow-release forms, has a C:N ratio suitable for soil organic nutrient cycling, and contains OM; allowing it to improve the physical structure and water-retention of reclaimed soil materials (Wolstenholme *et al.*, 1992; Bending *et al.*, 1999). Thermally-dried sludge has much higher solids content and, like sludges treated by alkaline stability, may have alkaline properties which can reduce the acidity of some reclaimed soils (Bending *et al.*, 1999). Like other forms of organic waste, sewage sludge may concentrate heavy metals and other endocrine-disrupting compounds; therefore the guidance for land reclamation is to use these materials at a level appropriate to prevent harmful accumulation of these in soils (Nason *et al.*, 2007). Sewage sludge is generally available free-of-charge; however, its application to land may require a permit under waste regulations (e.g. The Environmental Permitting (England and Wales) Regulations 2010).

Butt (1999a) conducted pot trials and a field investigation into the effects of thermally dried sewage granules on earthworms and vegetation growth on grassland. Sewage granule application rates were determined by nitrogen application limits to agricultural land (250 kg N ha^{-1}) (Council of the European Union, 1991). Generally, greater application of sewage granules led to significantly greater plant production in the field. However, conflicting results were found between the pot and the field experiment, in terms of earthworm survival. In the pot trials, negative effects were observed for all anecic species, and surface-dwelling epigeic species did not appear to have sufficient OM to significantly increase in number. Conversely, the anecic species *A. longa* and *L. terrestris* populations increased under granule application in field conditions. Butt (1999a) suggests that for some organic wastes, pot experiments may be an unreliable indicator of earthworm performance in natural systems, due to issues such as sterilisation of soil and therefore absence of interaction with plants and fauna, and a build-up of ammonia and salts, to which earthworms are sensitive.

Composted Green Waste (CGW)

CGW is growing in popularity for use in land reclamation activities in the UK, due to the nitrates directive reducing the use of these materials in agriculture, and national legislation restricting disposal of biodegradable waste to landfill (Nason *et al.*, 2007). Benefits of CGW as a soil improver are that it is a source of slow-releasing nitrogen, has a high bulky organic content thereby improving soil physical structure and water retention, and contains plant nutrients (Foot *et al.*, 2003; Moffat, 2006; Forest Research, 2015). In the UK, CGW is available either as waste material (not quality-assured) or as a quality-assured product (PAS 100 Standard). Quality-assured CGW guarantees an acceptable level of metals and other contaminants and therefore confidence in performance as a soil improver. Because it is a product it does not require waste permits, however it carries a cost of purchase (Nason *et al.*, 2007; WRAP, 2011). As such, there may be a cost-benefit for reclamation practitioners to use non-quality assured CGW materials.

Foot *et al.* (2003) conducted a field experiment investigating the effect of CGW incorporation on soil development and establishment of sycamore (*Acer psuedoplatanus*) and Italian alder (*Alnus cordata*) on a capped landfill. Deep incorporation and high rates of application (500 t ha^{-1}) of CGW were demonstrated to be beneficial to tree establishment, with intimate mixing of CGW within the rhizosphere found to be important to ensure tree roots can access available nutrients in the compost and have access to open fissures to encourage root penetration. The authors suggest repeated application of CGW to the soil surface every 3 to 4 years to ensure adequate nutrition for trees until the age of 10.

Despite the growth in popularity of CGW for land reclamation (Nason *et al.*, 2007), little research exists which investigates its interaction with earthworm populations in reclaimed soils. Lowe and Butt (2004) investigated the effects on soil quality and earthworm populations of surface application and soil rotavation using CGW on a restored landfill cap which had previously received earthworm inoculation with *A. longa* and *A. chlorotica*. Results suggest that surface application alone led to the greatest population densities and masses (densities of 331 and 276 worms m^{-2} and masses of 95.6 and 50.9 g m^{-2} during sampling two and four years after establishment, respectively). Comparatively, rotavation alone had a negative effect on numbers and masses (111 and 128 worms m^{-2} and 35.7 and 11 g m^{-2}, respectively) which were lower than those recorded in the control treatment. Monitoring at four years recorded a sharp decline in the population of *A. longa* in the experimental plots, associated with the complete removal of surface OM and subsequent inter-specific competition for limited food resources (Lowe and Butt, 2004).

Moffat *et al.* (2008) reported Common alder (*Alnus glutinosa*) survival rates of 75% after 10 growing seasons on a clay-capped landfill following a one-time surface application of a CGW mulch mat. They reported 50% survival of sycamore under the same conditions after 3 growing seasons, and 25% survival of Norway maple on uncultivated (but CGW mulched) soil after 15 growing seasons on the same experimental site (Butt et al., 1999; Moffat *et al.*, 2008). Foot *et al*. (2003) found that deep mixing of CGW within the soil profile is preferable to surface application for early successful tree establishment of Italian alder and sycamore. Foot *et al.* (2003) found sycamore height to be significantly greater with CGW application, and greatest Italian alder height was recorded when CGW was incorporated into the soil to 0.6 m depth, although they did not find this relationship to be statistically significant. The improvement of tree growth by CGW was attributed to provision of nitrogen to the N-limited sycamore, and the encouragement of an open-structure in the soil, enabling deeper root penetration and subsequently greater opportunity for nitrogen fixation by alder (Foot *et al*., 2003).

These results suggest that surface application of organic wastes may be beneficial to the establishment of anecic earthworm species, however the findings of Foot *et al.* (2003) indicate that mixing CGW within the soil column is preferable for early successful tree establishment. Further research is needed which investigates the long-term effect of surface application of organic wastes, followed by incorporation into the soil profile and rhizosphere by soil-dwelling earthworms.

Laboratory-based research by Lowe and Butt (2002) suggests that certain anecic species (i.e. *A. longa*) may be better able to forage within the soil profile for OM than others, and the use of this species in land reclamation may present an opportunity to use compost mixed into soil material for tree and earthworm establishment.

Compost-like outputs (CLO)

CLO is produced from household biodegradable waste undergoing mechanical treatment followed by composting or digestion, and is reportedly increasing in production in the UK (Nason *et al.*, 2007). Whilst these materials are high in OM and plant nutrients, they may also contain physical and chemical contaminants (Bending *et al.*, 1999). For this reason, CLO is heavily regulated and commonly disposed of in landfill; however it has potential for use in land reclamation (Nason *et al.*, 2007). Possibly due to its lack of current acceptability for use as a soil improver, no research has been identified which investigates the influence of CLO on earthworm activity in land reclamation.

Alternative organic wastes

Many other forms of organic waste have potential application in land reclamation, including by-products of anaerobic digestate processes, and composted materials such as papermill sludge, spent mushroom, seaweed and abattoir wastes (Bending *et al.*, 1999; Hutchings, 2002; Forest Research, 2015). In particular, spend mushroom compost has proven beneficial as a soil improver; although it may be undesirable due to high metal concentrations (Bending *et al.*, 1999).

Cattle manure has been an extensively studied form of organic waste for earthworm research, with some investigations having taken place on reclaimed land. On a reclaimed cutover peat bog in Ireland, Curry (1988) identified a significant effect of earthworms on grass growth (25% in the second year, and 49% in the third year) when cattle slurry was applied to the soil surface, compared with earthworm-free controls. On a reclaimed landfill where *A. chlorotica* had been inoculated, sterilized cattle dung was applied to two small (2 m^2) areas (Butt, 1999b). Sampling three months after manure application revealed a significantly greater number of earthworms in the manured soil than in control areas which received no manure.

Paper mill residues have also been investigated for their potential to support earthworms for land reclamation activities. In controlled conditions Butt (1993) found that solid paper mill residues mixed with spent brewer's yeast can provide a sufficient food source for *L. terrestris*, a species recognised for soil improvement and use in land reclamation projects. Piearce and Boone (1998) treated sandy arable soil with paper sludge at a rate of 200 t ha^{-1} and found that under very dry summer conditions the treated soils supported a greater abundance of *A. caliginosa* and *Octolasion cyaneum* than adjacent untreated soil. Furthermore, *L. terrestris* was found to draw the material into their burrows, suggesting that paper mill sludge may enhance long-term soil fertility (Piearce and Boone, 1998).

2.6. Gaps in the literature

From review of the literature, the following areas have been identified as requiring further investigation:

- In general, there is limited understanding of the dynamics of naturally and artificially colonised earthworm populations under reclaimed soils regenerated to woodland.
- Investigations into the relationship between earthworm activity and plant (especially tree) growth have considered a limited number of species of both, and very few have investigated the influence of reclaimed soil.
- To better understand the relationships involved, more field-scale and pot-trial studies are required which investigate a wider variety of earthworm and plant species and, importantly, soil conditions. A combination of pot trials and associated field studies will help overcome the constraints on each (e.g. difficulty in maintaining earthworm-free controls in the field, and the arising of un-natural conditions in the pot trials).

- Further research is required to build up a body of evidence into the interactions between earthworm species and organic wastes in reclaimed soils, particularly composted green waste, and provide more data on tree growth and soil development over time after CGW and earthworm addition. An investigation into the placement of organic waste materials in the soil profile and the effects on the above is needed.

3. GENERAL MATERIALS AND METHODS

3.1. Introduction

This chapter introduces the range of methodological considerations made during this research project, and describes how the focus of the thesis was developed from investigating a broad to a narrow set of conditions and ecological interactions. The direction of this thesis was determined by the findings from the literature review, and the availability of suitable study sites and resources.

3.2. Study sites and the Thames Chase Community Forest

Thames Beat are the current land managers for the Thames Chase Community Forest (TCCF) on behalf of the TCCF Trust. The TCCF is a 104 km^2 area of countryside along the border between Essex and London. The natural landscape in this area has been widely impacted by urban sprawl, industrial development, mineral extraction and subsequent landfill. Sand, gravel and clay extraction from the Thames Terraces, followed by landfill operations, has affected around 16% of the forest area of the TCCF landscape since the turn of the 20th century. The Forestry Commission is involved in restoring landfill sites within the Community Forest, with a target of recovering the 16% of degraded land in the area (Thames Chase, 2015). Restoration varies in quality across the TCCF, with the London Borough of Barking and Dagenham containing the earliest and most poorly restored sites, and the standard improving eastward to Essex. Whilst some areas have had limited end-use options due to hazardous waste disposal, a number of former mineral extraction sites have received inert construction materials and this has provided opportunities for woodland expansion (Thames Chase, 2015). Where sites have been restored from landfill to woodland end-use, there was opportunity to use these sites for conducting research to address the aims of this thesis.

There was significant interest from Thames Beat for the restored sites in the TCCF to be utilised for research; this provided the opportunity to research the ecological systems of a number of former mineral extraction and landfill sites reclaimed to woodland, spanning a range of different site histories, reclamation standards and woodland tree species mixes. Given the tight timeframes of a

PhD project, only a small number of the available sites in the area could be chosen for an in-depth investigation. Sites were assessed with reference to their site history (including time since reclamation), restoration standard, soil type/quality and tree species present providing the appropriate conditions to address the research gaps summarised at the end of the previous chapter. Further considerations included site accessibility, availability of space and logistical support by the Forestry Commission. Two community woodland sites were chosen: Ingrebourne Hill and Little Gerpins. These are described, along with the rationale for their selection.

3.2.1. Ingrebourne Hill Community Woodland

3.2.1.1. Background

Ingrebourne Hill Community Woodland is a 54 ha area of land in Rainham, Essex, UK (Nat Grid Ref: TQ 52572 83192) (Figure 3.1). The area has been reported as receiving 1,500 to 1,600 hours sunshine per annum, an annual rainfall of <600 mm and a mean daily maximum temperature of >14°C (Met Office, 2015b). This site is a former gravel extraction and inert and putrescible waste disposal landfill, which underwent clay capping of approximately 0.8 to 1.8 m depth, followed by placement of inert, screened construction waste materials as soil substrate of 0.5 to 0.8 m depth. Poor initial restoration led to a second restoration project, converting the site into a Country Park during the 1990s by Ingrebourne Valley Ltd., a locally-based land reclamation company. Towards the end of the decade, the Forestry Commission (FC) entered into a long-term management lease of the site as part of the Thames Chase Community Forest. In 2007, the Department for Communities and Local Government funded development of the site beyond its existing planning regulations. The site comprises two areas: Phase I (ca. 20 ha) which received inert waste as capping and restoration materials from 1997 to 2003; Phase IIA (ca. 20 ha) in two approximately equal halves. The southern half received inert waste as capping and restoration materials from 1995 to 2004, and the northern half between 2000 and 2007; Phase IIB (ca. 14 ha) is still undergoing reclamation (as of 2016).

Figure 3.1. Location of Ingrebourne Hill Community Woodland, a regenerated landfill site in Rainham, London and the location of the experimental area (image source: google maps).

Discussions with the site management team in April 2013 resulted in the identification of a fenced-off area in Phase IIA of the site (Figure 3.1) which was due to be re-planted, but which could otherwise be utilised as a location for a field experiment. This area has previously experienced almost complete tree mortality, suspected to be due to high levels of compaction, unsuitable tree species selection and competition by weeds (Doick and Willoughby, 2011). The site provided a unique opportunity for a field experiment to be set up within the early stages of this research project, providing a potential monitoring period of $\geq$2 years.

3.2.1.2. Soil materials

The soils at Ingrebourne Hill comprise generally of sandy clay loam materials, with a high stone content (Heaven and Richardson, 2007). A range of waste and building materials are found in the upper soil layer, including brick and tile fragments, concrete, porcelain and plastics, glass, rebar and other metals. Despite being loose and with a low bulk density at the time of placement between 2004 and 2007, a later soil survey found that soil was heavily compacted in all planting blocks investigated. This led to shallow rooting depth (of around 0.5 – 0.6 m depth) and drought conditions at the soil surface (Doick and Willoughby, 2011). Chemical analysis of soil samples revealed that in general there are no issues with metal content in the soils, although copper was slightly above normal soil concentrations (100 mg kg^{-1}) in two sampling sites. All of the metal contents were within the soil guideline values for non-residential uses (Doick and Willoughby, 2011). Soils across the site are relatively low in nitrogen and carbon (C:N ratios values ranged 22-34 (average of 29), and extractable-N concentrations ranged 0.05-0.85 mg l^{-1} (average 0.19 mg l^{-1}), with 11 of the 24 samples at or below the minimum acceptable level of 0.1 mg l^{-1} for tree growth as defined by Forest Research (2015) (Doick and Willoughby, 2011). Soils were found to be slightly to moderately alkaline, with a pH range of 7.1-8.3 and average of 7.9 (Doick and Willoughby, 2011). Soil organic matter (OM) concentrations ranged from 3.5-5.2% and averaged 4.0%, which are at the lower limit recommended for tree establishment in land regeneration (Forest Research, 2015). Additionally, qualitative observations were made of known biological indicators of soil quality during the digging of soil pits. Of the 24 soil pits, earthworms (species unknown) were found in only one pit, indicating potentially poor soil biological quality (Doick and Willoughby, 2011). The low earthworm populations found on the site as well as the low soil carbon and nitrogen suggested that this location would be a suitable candidate for an earthworm inoculation experiment and addition of organic waste, which would address all three of the research areas set out at the beginning of this chapter.

3.2.1.3. Tree species selection for Ingrebourne Hill

Failure of a large proportion of trees (species including field maple (*Acer campestre*), hazel (*Corylus avellana*), hornbeam (*Carpinus betulus*), hawthorn (*Crataegus monogyna*), wild cherry (*Prunus avium*) and rowan (*Sorbus aucuparia*)) to get established across the site had led to a plan for extensive re-planting in early 2013 using the tree species Italian alder (*Alnus cordata*) and Norway maple (*Acer platanoides*), following the proposed species mix for the newly restored Little Gerpins site (section 3.2.3). These species planned for use in future land reclamation to woodland sites in TCCF, following species mix selection assessment conducted by Forest Research (2011), and are described further in section 3.3. The availability of tree stock for planting onsite coincided with the timeframe for which a field experiment could be set up in the available area for the purposes of this research project.

3.2.2. Little Gerpins Community Woodland

3.2.2.1. Background

Little Gerpins is a 17 ha former landfill site in Rainham, East London, that has been restored to community woodland (Figure 3.2). The site history includes quarrying for sand and gravel followed by backfilling with waste materials in the late 1950s and early 1960s (Forestry Commission England, n.d.). The site subsequently became derelict, and by 1981 differential settlement and shallow depth of soil led to the site being classified as damaged land. For the following 30 years the site was mainly used as rough pasture (Figure 3.3), until it was acquired by Ingrebourne Valley Ltd in 2005 with a view to regenerate the site (Forestry Commission England, n.d.). Planning permission was granted in 2009 and on-site restoration works began the following year. In 2012 the groundworks were completed, and following tree planting the site was opened to the public in the spring of 2013 (Forestry Commission England, n.d.). The Forestry Commission manages the site as an amenity and recreation-based community woodland, and the site acts as a green corridor in the Thames Chase Green Infrastructure plan, linking the nearby Ingrebourne Hill woodland site to surrounding greenspaces (Forestry Commission England, n.d.).

Figure 3.2. Location of Little Gerpins community woodland, Rainham, National Grid Reference: TQ 54929 84214 (image source: Google maps).

A site walkover in April 2013 during the tree-planting stage of site restoration revealed little evidence of obvious earthworm activity (i.e. cast production), indicating that this site would be suitable to survey for earthworm colonisation activity at a later date. Adjacent woodland to the East, and agricultural land to the North and West may provide a source for earthworm natural colonisation; however, these were not accessible for surveying. Similarities in restoration practice and tree species selection between Little Gerpins and Ingrebourne Hill ranked this site as suitable for experimental comparisons between the two sites.

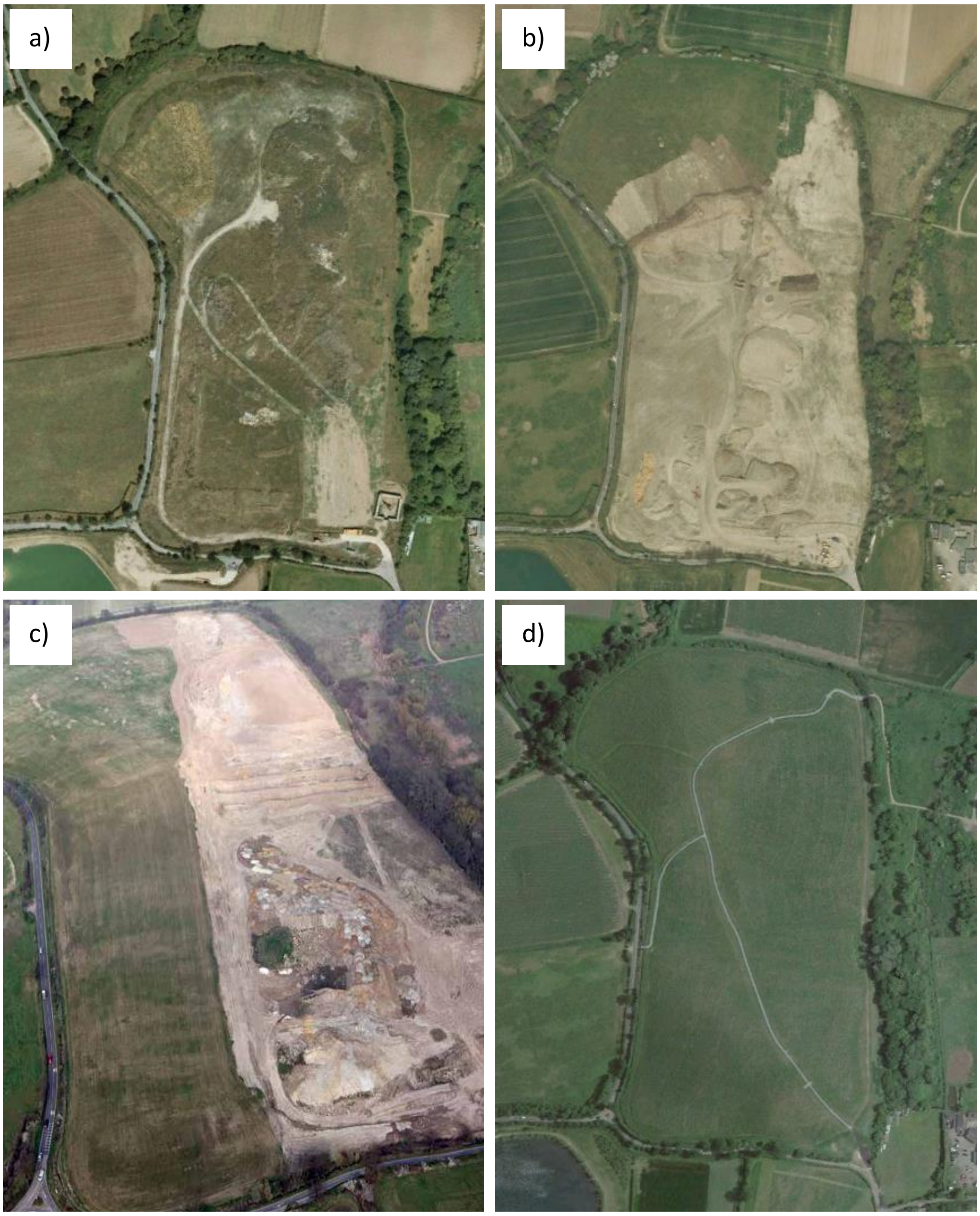


Figure 3.3. Little Gerpins site restoration phases: (a) derelict rough pasture pre-reclamation (2005), (b) early stages of reclamation (2010), (c) western half of the site completed (2011), (d) site fully reclaimed and afforested (2015) (image source: Google Earth).

3.2.2.2. Soil materials

The most recent site cover (in 2011) was reclaimed in two distinct phases (Figure 3.3); the western half of the site was re-soiled using the historic topsoil material (which was stockpiled onsite in 4 m high piles, for 3 years prior to re-use) to an even depth of 150 mm, and the eastern half was completed one year later, and received imported topsoil material from a local agricultural field which had been previously intensively used for crop (mainly wheat) production (Clark, Pers. Comm.). Background soil surveys were conducted at locations across the site in 2011, the results of which are presented in Table 3.1. The soil specification for Little Gerpins comprised roughly 2 m of sandy clay capping material, 0.5 m of inert subsoil clayey material and 0.2 m sandy clayey, gravelly topsoil material with average of 14.7% clay content (WD Environmental, n.d.). Soil specifications and best practice guidance by Forest Research (2015) suggest a guideline minimum of 1.2-1.5 m soil depth. Given the dry local climate and the dry nature of the soil-forming materials used, a preferred depth of 1.8-2.0 m loose-tipped subsoil material was adopted wherever possible in the regeneration to minimise drought conditions (Forestry Commission England, n.d.). Following reclamation, a free-draining, average root zone of 1.8 m was reported for the areas of the site which were investigated. This, however, resulted in dry soil conditions, which were exacerbated during the summer (Forestry Commission England, n.d.).

Table 3.1. Results of soil analysis for the stockpiled topsoil on site in 2011 (adapted from WD Environmental, n.d.).

Soil parameter	Topsoil depth after spreading (mm)	pH	Electrical conductivity (μS/cm^{-1})	Moisture content (%)	Loss on ignition (%)	Mg (mg/kg)	Total K (mg/kg)	Total N (mg/kg)	P (mg/kg)
Value	150	8.28	606	6.57	4.34	2,675	2,731	914	696

3.2.2.3. Tree species selection for Little Gerpins

The area has been reported as receiving 1,500 to 1,600 hours sunshine per annum, an annual rainfall of <600 mm and a mean daily maximum temperature of >14°C (Met Office, 2015b). As such, tree species selection for this site was focused largely on those which are suited to dry soil conditions and a warm climate. In 2012, around 12 ha of new woodland (approx. 27,500 trees and shrubs) was planted onsite in single-species planting blocks, along with 5 ha of grassland (Forestry Commission England, n.d.). A primary management objective for the Little Gerpins site is the creation of Short Rotation Forestry (SRF), to generate long-term income to support site management. As such, tree species selection for the site considered those species which may coppice rather than require replanting. It was also considered that, under the 2050 high emission climate change scenario, trees which have already established on the site are likely to regenerate easier than new planting (Forest Research, 2011). In some instances, certain species are being trialled for SRF suitability on the site and due to the dry nature of the soil materials on-site, tree species selection focussed on drought-tolerant species. Furthermore, because the soil profile includes heavily compacted layers of dry inert waste below the topsoil layer, it was important that drought tolerance in these species was not associated with those which grow deep roots to reach groundwater (Forest Research, 2011). The dominant tree species in the woodland blocks are Italian alder (*Alnus cordata*), comprising 55% of the overall woodland planting mix. Norway maple (*Acer platanoides*) and Sycamore (*Acer pseudoplatanus*) each makes up 10% of the species mix (for the complete species mix see Table 3.3).

3.2.3. Forestry Commission's Headley Nursery

The Headley Nursery enclosure is situated in Bordon, Hampshire (Figure 3.4). In the period 1981-2010, the area had a mean annual maximum and minimum temperature of 14.1^{o}C and 6.4^{o}C, respectively, and a mean annual rainfall of 755.5 mm (Met Office, 2015a). The area is characterised as sandy humo-ferric podzol (Jarvis *et al.*, 1983), with the nursery site situated on an area of sandy soil.

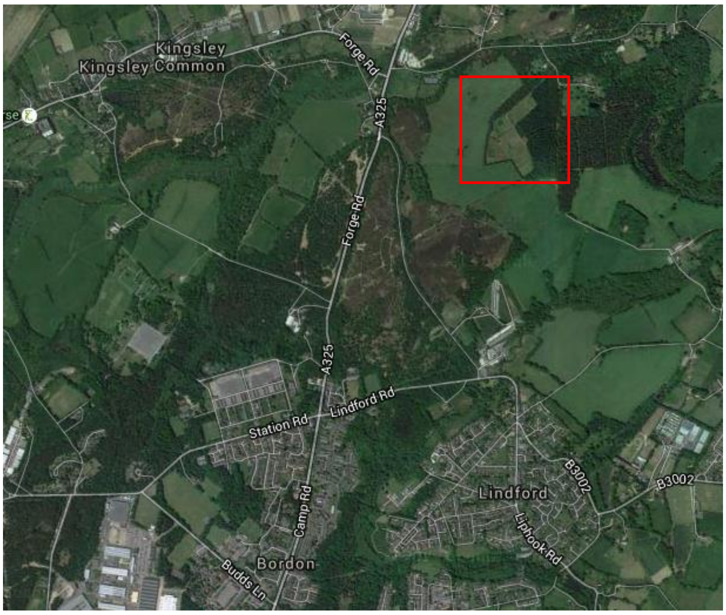


Figure 3.4. Location of Headley Nursery, Bordon, National Grid Reference: SU 80925 37969 (Image source: Google maps).

This site is managed by Forest Research, and has been used for a number tree-based field experiments (McKay *et al.*, 1999; Moffat, 2000; Moffat *et al.*, 2001; Broadmeadow *et al.*, 2005; Rajapaksha *et al.*, 2014). The site is research-dedicated, and provides access to equipment and facilities such as drip-feed irrigation and electric rabbit-proof fencing. The sandy soil present at the nursery site supports a low native population of epigeic earthworm species only, as described by Rajapaksha *et al.* (2014). The site's soil chemical characteristics were assessed by Moffat (2000), and are reproduced in Table 3.2.

Table 3.2. Headley Nursery soil properties (reproduced from Moffat, 2000).

Property	Value
pH (1:2.5 water)	5.35
ADAS extractable P (mg l^{-1})	73
ADAS extractable K (mg l^{-1})	54
ADAS extractable Mg (mg l^{-1})	127
Bray P (μg g^{-1})	74
Mineralisable N (μg g^{-1})	2.47

Table 3.3. Summary of Thames Chase and Forest Research experimental sites, locations, tree species, soil materials and site history (sources: WD Environmental, n.d.; Land Research Associates, 2007; Doick and Willoughby, 2011; Forest Research, 2011).

Site Name	**Location (Nat. grid ref.)**	**Tree species present (% of planting mix, if known)**	**Soil materials**	**Restoration history**
Ingrebourne Hill Community Woodland	Rainham, London (TQ 52572 83192)	Tree replanting (beat-up) mix largely consisting of Italian alder and Norway maple. Original planting of silver birch, oak, cherry, hazel, field maple, hawthorn, rowan	Screened construction waste materials as soil substrate. Shallow, compacted and high pH.	Gravel extraction and inert and putrescible waste disposal landfill, which underwent clay capping. Poor initial restoration led to a second restoration project, converting the site into a country park during the 1990s.
Little Gerpins Community Woodland	Havering, London (TQ 54929 84214)	Italian alder (55%), Norway maple (10%), Sycamore (10%), small-leaved lime (5%), Silver birch (2%), Grey alder (2%), Sweet chestnut (2%), White poplar (2%), Grey poplar (2%), Rowan (2%)	Historic site topsoil and imported soil from agricultural land. Soil is comparable to, though higher quality than, Ingrebourne Hill.	Quarrying for sand and gravel followed by backfilling with waste materials in the late 1950s and early 1960s. The site underwent dereliction and use as rough pasture. Restored to community woodland in 2013
Headley Nursery	Bordon, Hampshire (SU 80925 37969)	N/A, heathland	Sandy heathland soils	Managed heathland, cleared for experimental purposes.

3.3. Tree species selection for this research project

One of the aims of this project was to investigate the relationship between earthworm activity and the performance of previously unconsidered tree species, particularly those relevant to a land reclamation setting. Of the range of tree species used in land restoration which were suitable for this investigation, two were selected; as the timeframes for a PhD project only allowed for a small number of species to be investigated thoroughly in a complex experimental design involving treatments. Italian alder (*Alnus cordata*) and Norway maple (*Acer platanoides*) were considered most relevant to current land restoration in the UK. The rationale for these species choices follows.

3.3.1. Italian alder (*A. cordata*)

A. cordata is the most common tree species for new planting at the Little Gerpins site and in the re-planting operations at Ingrebourne Hill, making up over 50% of the planting mix for both. Tolerant of high pH, dry soils and low soil nitrogen levels (due to the N-fixation abilities of alder species), *A. cordata* is considered suitable for planting on reclaimed soil materials and is recommended for planting on industrial spoils (Hibberd, 1986). This species has demonstrated good performance on similar sites, leading to increased confidence in the use of Italian alder for land restoration (Forest Research, 2011). However, there is little local use of this species in the TCCF, and as such the use of this species has been experimental. There is currently a paucity of research into the interaction between *A. cordata* and soil biota, particularly on reclaimed land. Research by Rajapaksha *et al*. (2013) identified that the litter of a related species, common alder (*Alnus glutinosa*) was highly palatable to the earthworm species *A. chlorotica*, *A. caliginosa*, *L. terrestris* and *A. longa*. On a restored landfill site, Butt (2004) found that the presence of *A. glutinosa* led to significantly increased earthworm populations and mass, compared with sycamore (*Acer psuedoplatanus*) which had poor survival rates and was largely replaced by grass cover. Given the lack of knowledge about its ecological performance on restored woodland, and its growing popularity for restoration projects, Italian alder was considered an important species to investigate during this research project.

3.3.2. Norway maple (*A. platanoides*)

A. platanoides is the second-most abundant tree species for new planting at both the Little Gerpins site and in the re-planting operations at Ingrebourne Hill, alongside sycamore (*A. pseudoplatanus*). Norway maple is considered a suitable species for highly alkaline soils, and is recommended for dry sandy soils, but not industrial spoils (Hibberd, 1986). This species has unknown suitability for SRF, and is also experimental in its inclusion in TCCF planting mixes (Forest Research, 2011). There is little knowledge regarding *A. platanoides* interaction with soil biota on reclaimed land. The related maple species, sycamore, has already been investigated for use in land restoration, where it was found to have poor survival rates (Butt *et al.*, 2004). Rajapaksha *et al*. (2013) found sycamore leaf litter to be poorly palatable to four common UK earthworm species: *A. chlorotica*, *A. caliginosa*, *L. terrestris* and *A. longa*. Norway maple is therefore considered an appropriate species for this project, as it is poorly understood from a restoration ecology perspective, and could yield results directly comparable to previous research with Sycamore (Butt *et al.*, 2004; Rajapaksha *et al.*, 2013).

3.4. Soil materials for microcosm and mesocosm experiments

Earthworm activity is strongly affected by soil type, as well as factors such as moisture-holding capacity, temperature, pH, and, importantly, organic matter quantity and quality (Edwards, 2004). Research has identified that generally, larger earthworm populations are supported by loamy soils than other soil types (Bouché, 1977). For this reason, loam/clay soils, and in particular pre-sterilised 'Kettering' or 'Boughton' loam have been widely used as a standard substrate for earthworm research and ecotoxicology experiments (Butt *et al.*, 1994b; Lowe and Butt, 2005; Arnold *et al.*, 2008), to provide a sterile and consistent experimental control soil medium. Because of the wealth of existing data for earthworm activity using Kettering loam as a microcosm experiment substrate, it was used as a soil media in the microcosm experiments of this project, to provide conditions against which results for earthworm activity in reclaimed soil treatments could be compared.

In land restoration, soil materials are generally very poor in quality and unsuitable to support soil fauna without amelioration (Bending *et al.*, 1999; Dickinson *et al.*, 2005). As such, it would be inappropriate to consider the use of Kettering loam as a substitute for reclaimed soil material in laboratory or nursery-based earthworm experiments, as this is unlikely to yield results comparable to field conditions. This was recognised during development of the Earthworm Inoculation Unit technique, for which field soil is collected and utilised when possible (Butt, 2011b). Field soils are collected, frozen to destroy native earthworms present, and earthworm competitors/predators, and then allowed to thaw before earthworm addition (Butt, 2011b). This methodology was employed to create a standardised reclaimed soil media for use in the nursery and laboratory experiments of this thesis.

3.5. Earthworm species selection

The earthworm species used in the laboratory and field experiments throughout this project were selected based on the results of surveying at the field sites described above, and, following the literature review, consideration of the following criteria; species ecological function (section 2.2.3) (Bouché, 1977), tolerance of reclaimed soil conditions (e.g. Curry and Cotton, 1983; Zhang and Schrader, 1993) and suitability for addressing this project's research objectives (Zhang and Schrader, 1993). Particular consideration was paid to the outcomes of previous earthworm inoculation experiments and the earthworm species employed, to identify which species are under-researched and those which may be unsuitable for inoculation and research on reclaimed land in the UK (Butt, 1999b, 2008). In total, four species were selected for use in experiments, with varying degrees of research in UK mine/landfill restoration to greenspace (Table 3.4) These species are all native to and widespread throughout the UK, and represent the two ecological groups (anecic and endogeic) which are most appropriate for research into soil development (Zhang and Schrader, 1993). Furthermore, literature exists on the laboratory culture and maintenance of these species for microcosm and mesocosm experiments (Lowe and Butt, 2005).

Table 3.4. Summary of the four earthworm species selected for use in experiments, with general information on their ecology.

Earthworm species	**Common names**	**Ecological group and description**	**References**
Aporrectodea longa	Black-headed worm, long worm	Anecic (deep-burrowing); feeds on surface leaf litter; surface-casting; common to gardens, cultivated soil, pasture and woodlands; soil pH range 6.7- 9.4	Marfleet, 1985; Butt *et al.*, 1993, 1997, 2004
Lumbricus terrestris	Lob worm, Dew worms, Night crawler	Anecic (deep-burrowing); feeds on surface leaf litter; surface-casting, producing middens; common to grasslands and woodlands; soil pH range 6.2 - 10	Marfleet, 1985; Butt *et al.*, 1993; Scullion, 1994; Craven, 1995; Butt, 1999
Allolobophora chlorotica *(green morph)*	Green worm, stubby worm	Endogeic (shallow-burrowing); geophagous, feeding on mineral soil; common in gardens, grassland and woodland, where it is often co-dominant with *A. caliginosa*; soil pH range 4.5 - 8.2. Two morphs may be separate species.	Butt *et al.*, 1997, 2004; Lowe and Butt, 2008
Aporrectodea caliginosa	Grey worm	Endogeic (shallow-burrowing); Geophagous, feeding on mineral soil; common in gardens, and cultivated land, where it is often co-dominant with *A. chlorotica*; soil pH range 5.9 - 11.1	Marfleet, 1985; Butt *et al.*, 1993

3.6. Earthworm sampling and laboratory techniques

Earthworms for use in laboratory experiments were collected using a combination of digging and hand-sorting of soil, followed by application of mustard vermifuge to the soil pit where necessary. Digging and hand-sorting of soil is the simplest method of earthworm collection, albeit labour intensive, and the methodology employed depends upon the objective of the collection activity (Butt and Grigoropoulou, 2010). For collection of earthworms for use in experiments/ inoculation, soil is dug from the target area and placed onto a plastic sheet where it is processed by hand and earthworms collected. For earthworm population surveying, where quantification is required of the earthworm density in a given area, a standardised amount of soil is removed at each sampling event (e.g. at regular intervals along a transect line), digging to a consistent soil depth and using a quadratic frame of known dimensions (in the case of this research, a 0.1 m^2 quadrat and a digging depth of 15 cm was used) (Butt and Grigoropoulou, 2010). However, digging and hand-sorting alone may only enable collection of epigeic and endogeic earthworm species, which live close to the soil surface (Butt and Grigoropoulou, 2010). Where anecic earthworm species are also required, and to give a more accurate sample of the earthworm community for surveying activities, application of a vermifuge solution should accompany digging and hand-sorting (Pelosi *et al.*, 2009).

Vermifuges are liquid expellents which cause skin irritation to earthworms, driving them from their burrows to the surface where they can be collected. Application is achieved either by pouring vermifuge solution broadly over the soil surface or into already dug soil pits and allowing the vermifuge to percolate into the burrows, or via more targeted application (e.g. injection directly into burrows using a syringe) (Butt and Grigoropoulou, 2010). A variety of chemicals have been used as vermifuges, with a standard being a dilute formaldehyde solution (formalin) (International Standards Organisation, 2006). However, other vermifuges have been explored as formaldehyde is reportedly carcinogenic and may negatively impact soil fauna and ground vegetation (Eichinger *et al.*, 2007).

A suspension of mustard powder in water has been demonstrated as a cheap and effective vermifuge, with 50 g per 10 litres of water proving sufficient to expel deep burrowing worms (Butt, 2000). Other mustard solutions or mustard extracts such as Allyl isothiocyanate (AITC) have been demonstrated to provide reliable indices of earthworm abundance in soils, although these may be more expensive than obtaining traditional mustard powder (Lawrence and Bowers, 2002; Zaborski, 2003).

An additional method of earthworm extraction is electrical soil stimulation, using apparatus such as that developed by Thielemann (1986). This involves inserting 8 steel electrodes into the soil in a 0.2 m^2 circular pattern, and applying varying frequencies of electrical current to drive earthworms from their burrows. Because this method is non-destructive and non-toxic it is useful for earthworm sampling at ecologically and aesthetically sensitive locations. However, this technique has not been widely used, potentially due to the apparatus being prohibitively expensive (Eisenhauer *et al.*, 2008; Butt and Grigoropoulou, 2010). As such, a combination of digging and hand-sorting, followed by application of vermifuge to the exposed soil pit is currently considered the most effective method of earthworm extraction (Pelosi *et al.*, 2009).

For the laboratory and nursery studies employed in this research, adult *A. longa*, *A. chlorotica* and *A. caliginosa* were collected via digging and hand-sorting on agricultural pasture at Walton Hall Farm, Preston, UK (Nat. Grid Ref: SD 55050 28100). Adult *L. terrestris* were collected from mixed deciduous woodland at Alice Holt forest, Farnham, UK (Nat. Grid Ref: SU 80246 42818), via targeted application of mustard vermifuge to middens using 100 ml syringes (see above). Following extraction using a vermifuge, earthworms were immediately and thoroughly washed with fresh cold water and transported to the laboratory for storage. All earthworms were kept in 750 ml plastic 'Tupperware' style vessels from Lakeland Plastics, containing sterile Kettering loam soil at 25% moisture content. They were then incubated at 15^{o}C for a minimum of 28 days prior to experimental use, to allow equilibration from field conditions (Fründ *et al.*, 2010). During this time, an excess of dried and re-wetted horse manure was provided as a food source; or a mixture of leaf litter from both Italian alder and Norway maple in the case of earthworms used for leaf litter preference experiments, to prevent experimental bias (Rajapaksha *et al.*, 2013).

Earthworm reproductive output is a recognised measure of earthworm health and activity in microcosm and mesocosm experiments (Lowe and Butt, 1999, 2002a). Cocoon production rate is determined through the separation of cocoons from experimental soil materials, and calculation of average cocoon production per earthworm over a known time frame, usually presented as cocoons earthworm^{-1} year^{-1} (Lowe and Butt, 2005). To separate cocoons from soil, used experimental soils are wet-sieved through a series of soil sieves appropriate to the size of the cocoons targeted (smallest mesh diameter of 1 mm) (Sims and Gerard, 1999).

4. AN EARTHWORM COLONISATION SURVEY OF A NEWLY RECLAIMED AFFORESTED LANDFILL SITE

4.1. Introduction and objectives

Through their burrowing and feeding activities, earthworms incorporate organic matter into soil and improve soil structure, and as such these organisms are regarded as ecosystem engineers (Lee, 1985; Lavelle *et al.*, 1997). The benefits of earthworm activity on soil development are likely to be enhanced in soil-forming materials such as those typically found on reclaimed landfill and ex-mining sites (Jana *et al.*, 2010), and colonisation by earthworms may therefore be particularly beneficial for woodland establishment on reclaimed land (Brun *et al.*, 1987; Marinissen and van den Bosch, 1992). To facilitate earthworm colonisation, it is necessary that the soil environment is made suitable for earthworm survival and managed in a manner which supports soil biota (Scullion, 2007; Butt, 2008). However, compromises in the quality of restoration due to time and cost constraints often result in the use of hostile subsoil materials (e.g. low organic matter content, high pH, highly compacted), lacking resident fauna (Butt *et al.*, 1999). In this situation, earthworm colonisation of a site must occur naturally by dispersal of earthworms from surrounding land or by passive or active introduction by human or animal activity (Eijsackers, 2011).

Natural earthworm colonisation of restored landfill has received some attention (Brockmann *et al.*, 1980; Judd and Mason, 1995; Butt *et al.*, 1999), although many more investigations have been made into the colonisation of newly-created land or previously earthworm-free natural habitats and unrestored mining sites (Hoogerkamp *et al.*, 1983; Marinissen and van den Bosch, 1992; Pizl, 2001; Eijsackers, 2011). Early colonisers of low-quality reclaimed soils are typically surface-dwelling (epigeic) or shallow-burrowing (endogeic) species, due to high reproductive rates and strong powers of dispersal (Judd and Mason, 1995). However, these may play a less active role in accelerating soil development than deeper-burrowing species (Zhang and Schrader, 1993). On newly reclaimed sites earthworm populations are low, and it may require 20 years or longer for deeper-burrowing species (with lower reproductive rates) such as *Lumbricus terrestris* and *Aporrectodea longa* to become established, and for soil depths to become sufficient for these deeper-burrowing species (Armstrong and Bragg, 1984; Rushton, 1986; Scullion *et al.*, 1988; Scullion, 1994; Pizl, 2001). However, in general there is still limited understanding of the dynamics of naturally and artificially-colonised earthworm populations under reclaimed soils regenerated to woodland (Boyer and Wratten, 2010; Eijsackers, 2011).

The aim of this survey was to quantify earthworm colonisation rates of a reclaimed landfill site regenerated to woodland. Specific objectives were to measure:

1. the effect of soil compaction and above-ground vegetation type on earthworm community composition,
2. the influence of surrounding land use as a source of earthworms for natural colonisation, and,
3. the influence of topsoil origin on earthworm colonisation.

4.2. Materials and methods

4.2.1. Study site

The location for this survey is the 'Little Gerpins' 2011 extension to Bonnetts Wood, Rainham (section 3.2.2). Little Gerpins is a 17 ha former landfill site that has received secondary reclamation through the importation of soil-forming materials. A site walkover in April 2013 during the tree-planting stage of site restoration revealed little visual evidence of earthworm activity (i.e. surface cast production), indicating that this site would be suitable to survey for earthworm colonisation activity. The survey was subsequently undertaken over two days in October 2014.

4.2.2. Survey design

Twenty-five transect lines of 20 m length were placed, at intervals of 50 m along the perimeter fence, radiating from the fence at 90° (toward the centre of the site). The transect lines began at pre-determined GPS points (marked using a Garmin Colorado 300 portable GPS device) on the site to ensure accuracy of site boundary coverage. Sampling was carried out at 5 m intervals along each transect, where: 0 m was the site boundary, 5 m always fell in the centre of a bridal path which ran the length of the site, and 10 m onwards marked the tree planting zones (Figure 4.1).

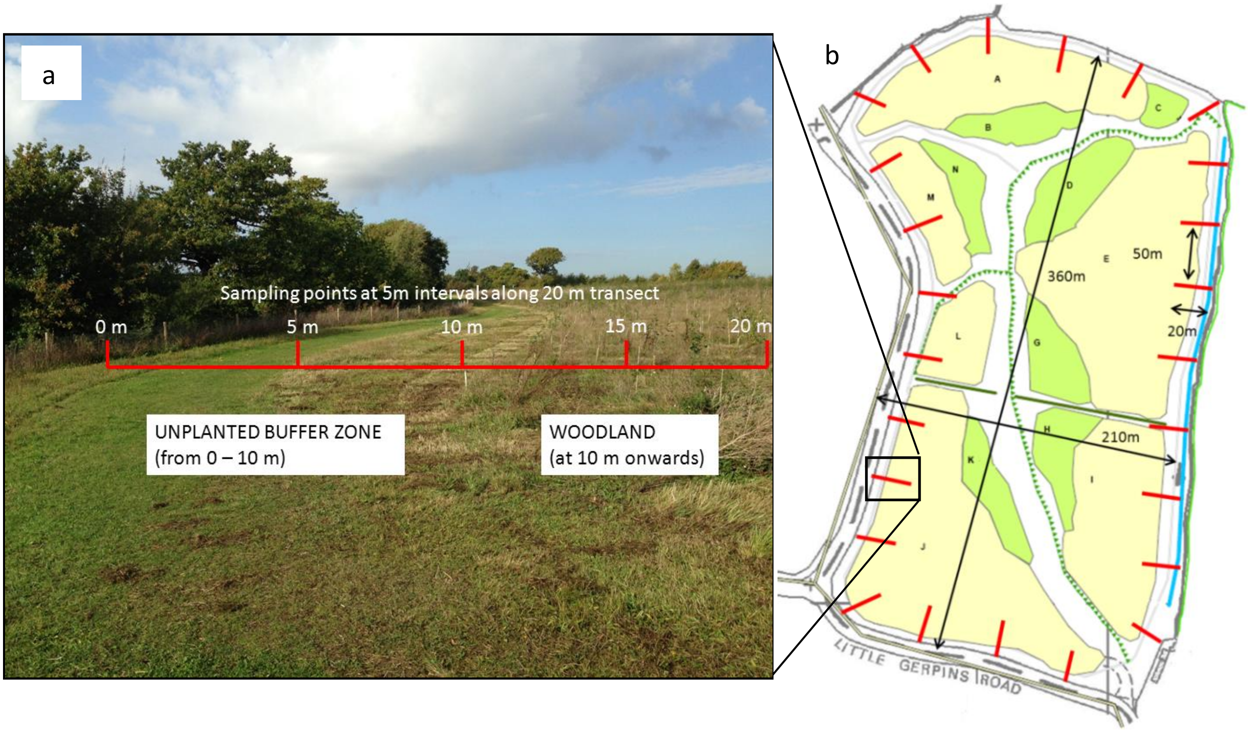


Figure 4.1. Layout of transects at Little Gerpins. (a) A typical transect on site (b) arrangement of transect lines around the site (indicated in red).

4.2.3. Measurements

At 5 m intervals along each transect, a 0.1 m^2 quadrat (31.6 cm X 31.6 cm) was placed on the soil surface (centred on the interval point). At each sampling point, the earthworm community composition, biomass and population density, soil compaction (using a digital penetrometer), soil moisture content (using a Delta-T probe), estimated cover of leaves, grass, and forbs within each quadrat (using five categories: none present; 1–25% cover; 26–50% cover; 51–75% cover; 76–100% cover, following the criteria developed by Cameron and Bayne, 2009), distance to nearest tree and species of the nearest tree were recorded. Prior to use, the digital penetrometer was calibrated following the manufacturer instructions, using a series of known weights resting on the penetrometer head unit and observing the load-cell readings, establishing the relation between cone resistance and load reading.

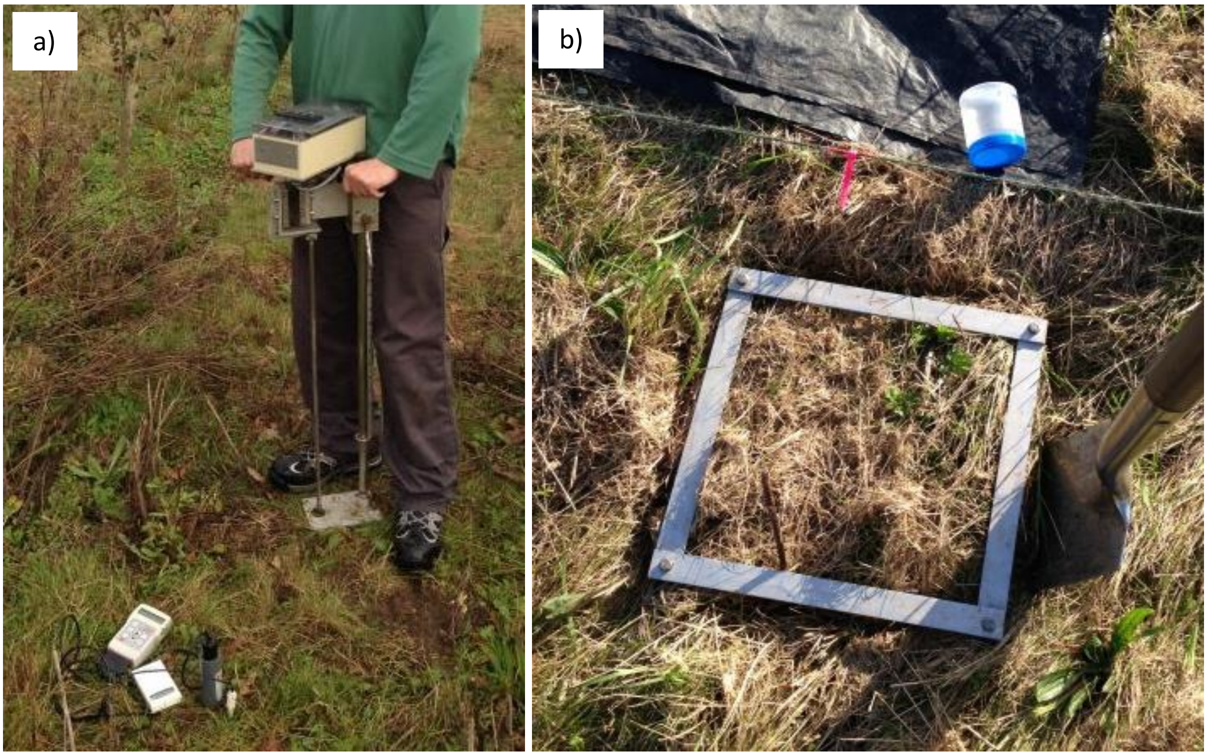


Figure 4.2. Examples of measurements taken at each sampling point; (a) Reading compaction throughout the soil profile with a digital penetrometer, and measuring soil moisture content using a Delta-T probe, (b) sampling earthworms using a 0.1 m^2 quadrat.

4.2.4. Earthworm sampling

The area underneath each quadrat was dug to 15 cm depth, and the topsoil removed and hand-screened for earthworms. A vermifuge suspension, with a concentration of 50 g mustard powder to 10 litres tap water (Butt, 2000), was then applied to the pit, left to infiltrate and re-applied if all vermifuge was absorbed by the soil. The pit was then monitored for 5 minutes to allow earthworms to emerge. Earthworms were collected and placed directly into pre-labelled plastic bottles containing 4% formaldehyde solution, and transported to the laboratory for identification. All adult worms were identified to species level following the identification key of Sims and Gerard (1999).

Initially, where earthworms were found at the 20 m sampling point on each transect (the innermost sample point to the site), an additional sampling point was added 5 m further into the site along the transect, and this process repeated until earthworms were no longer found. This approach was subsequently abandoned due to time constraints after earthworms were located 30 m into the site along some of the early transects sampled.

4.2.5. Statistical analysis

Earthworm diversity and abundance was mapped onto the site, using ArcGIS. Statistical analysis was performed using the freeware statistical software R, version 3.2.2., "Fire Safety" and the R Studio desktop software, version 0.99.486 (R Core Team, 2015; RStudio Team, 2015). Data were first tested for normality using the Shapiro-Wilk test, which is suited to smaller sample sizes. Where data did not follow a normal distribution, Kruskal-Wallis non-parametric one-way analysis of variance was applied, followed with multiple Mann-Whitney U (Wilcoxon rank-sum) tests on pooled data. These statistical models were applied to data on earthworm community density and species richness, ground vegetation cover and soil compaction, across and between transects, as described in section 4.2.3.

4.3. Results

4.3.1. Earthworm populations

A total of 2,582 individual earthworms were collected over two days, revealing a mean community density of 207 earthworms m^{-2} across the sampled area. Seven earthworm species were recorded, representing all three earthworm ecological types (*sensu* Bouché, 1977), these were; *Lumbricus festivus* and *Lumbricus castaneus* (epigeic), *Aporrectodea caliginosa*, *Aporrectodea rosea* and *Allolobophora chlorotica* (endogeic), *A. longa* and *L. terrestris* (anecic). During collection, all earthworms appeared healthy and all life stages were observed (including cocoons), with adults and juveniles accounting for 41 and 59 percent of total earthworms, respectively. The results showed an even distribution of earthworms from the site boundary to 20 m into the site.

Mean earthworm densities (m^{-2}) along transect lines from the inner site borders are presented in Figure 4.3. The endogeic species *A. chlorotica* had the highest recorded density, with an average of 58 m^{-2}, followed by *L. festivus* with a density of 9 m^{-2}. The highest earthworm community density was 1,110 m^{-2} (of which 790 were juveniles), which was recorded 15 m into the site along a transect on the Western side of the site. Across a number of sampling points along the three transects at the South-Eastern corner of the site (the last area restored on-site), no earthworms were recorded. No effect was found of sampling location (along transect) on earthworm densities (Figure 4.3), however *A. chlorotica* density was notably reduced at 5 m sampling points compared with the other sampling points (Kruskal-Wallis nonparametric one-way ANOVA, H = 8.613, df =4, p = 0.072).

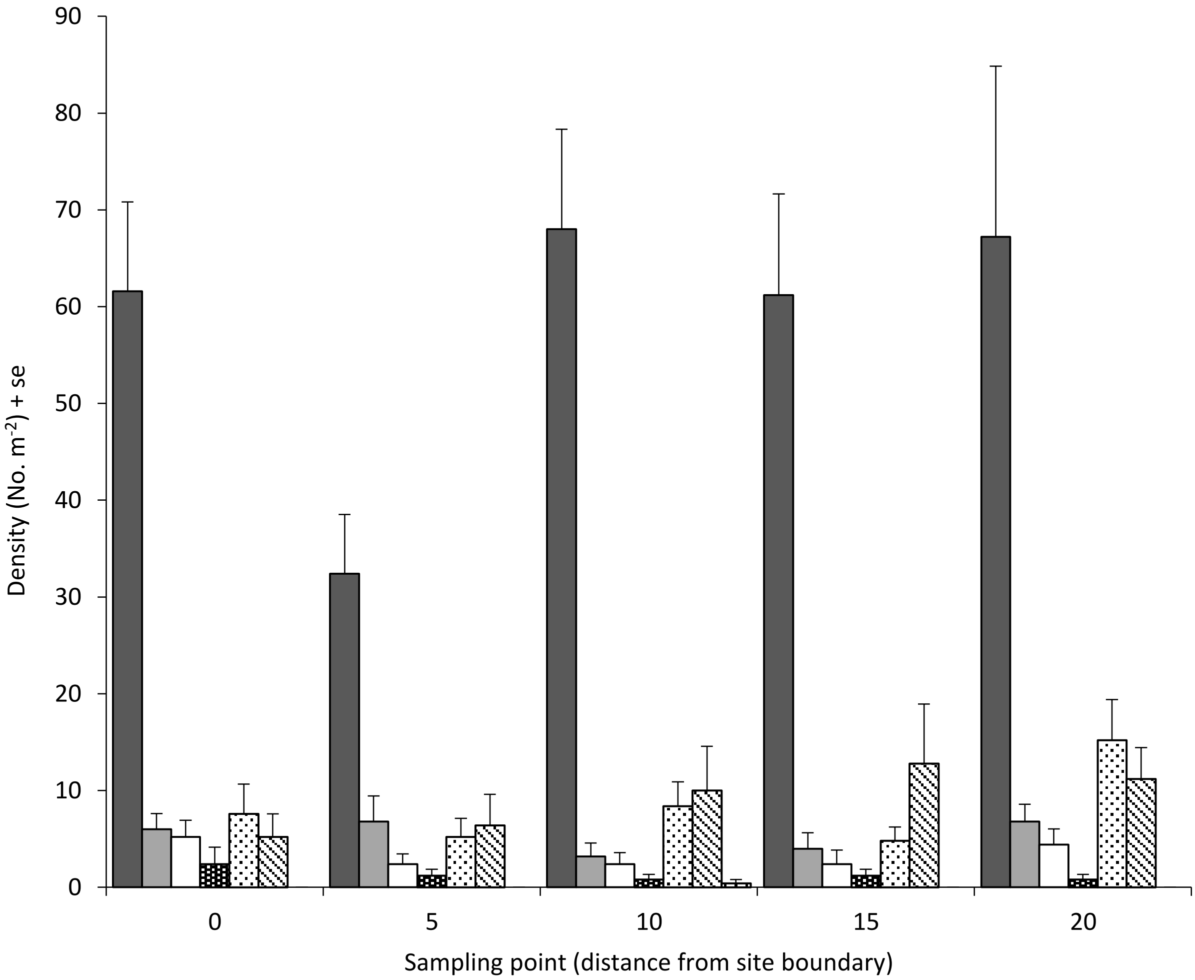


Figure 4.3. Mean earthworm density (+ SE) recorded under a 0.1 m^2 quadrat at 5 m intervals along a 20 m transect away the site edge (n = 25). Earthworm species: *A. chlorotica* *A. longa* *A. caliginosa* *A. rosea* *L. castaneus* *L. festivus* *L. terrestris*.

Mean intra-transect species richness was significantly lower at the 0 m sampling point compared with the 10, 15 and 20 m sampling points (Kruskal-Wallis nonparametric one-way ANOVA, H = 10.61, Adjusted for ties = 11.28, df = 4, p = 0.024, confirmed with multiple Mann-Whitney U tests). A significant inter-transect effect was found for earthworm community density (Kruskal-Wallis one-way nonparametric ANOVA, df = 24, p < 0.001, confirmed with multiple Mann-Whitney U tests). Transects along the west side of the site were compared against the East side, due to difference in soil origin and time since restoration. Most earthworm species were found to have significantly greater densities on the West side of the site compared with the East side (Table 4.1). These results are graphically presented in Figure 4.4, where species density data are mapped over the site using ArcGIS 10.2.

Table 4.1. Mean earthworm species density and richness (m^{-2}) for the West side (n = 65) and East side (n = 60) of the site (± SE). Different letters indicate significant differences (Kruskall-Wallis non-parametric ANOVA, df = 1, *p <0.05 ** p < 0.01 ***p <0.001).

Earthworm species and parameter	West side	East side
A. chlorotica	64.0 ± 6.4 ^{a*}	51.7 ± 8.2 ^{b}
A. caliginosa	4.9 ± 1.1 ^{a**}	1.7 ± 0.6 ^{b}
A. longa	7.5 ± 1.3 ^{a**}	3.0 ± 0.9 ^{b}
A. rosea	2.2 ± 0.8 ^{a*}	0.3 ± 0.2 ^{b}
L. festivus	16.2 ± 3.3 ^{a***}	1.5 ± 0.6 ^{b}
L. castaneus	10.0 ± 1.9 ^{a}	6.3 ± 1.6 ^{a}
L. terrestris	0.0 ± 0.0 ^{a}	0.2 ± 0.2 ^{a}
Community density	274.8 ± 22.8 ^{a***}	132.7 ± 18.4 ^{b}
Species richness	2.8 ± 0.2 ^{a***}	1.6 ± 0.2 ^{b}

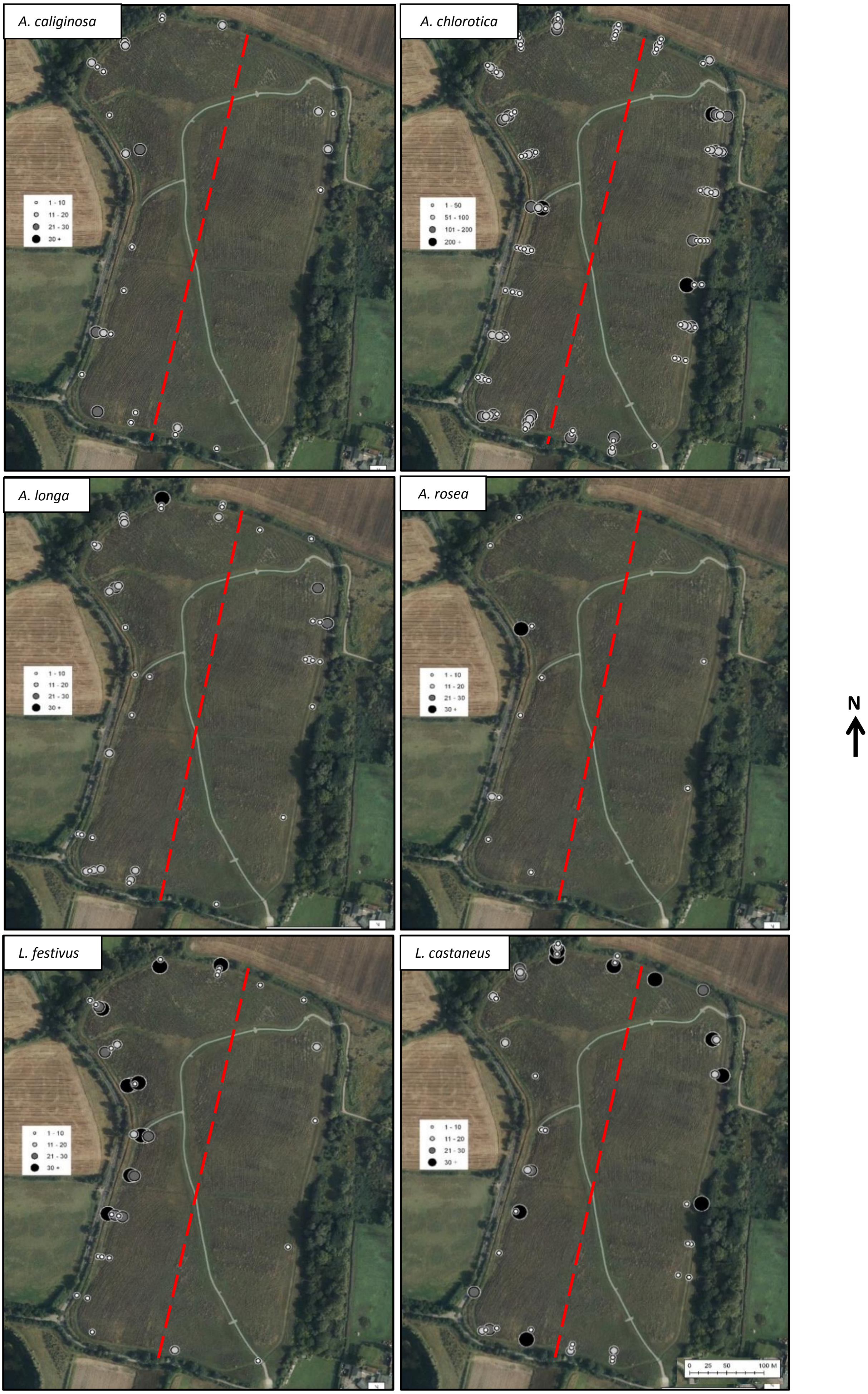


Figure 4.4. Earthworm species density data mapped over an OS map of Little Gerpins using ArcGIS 10.2. Dotted red line indicates the divide between West and East sides of the site. © Crown copyright and database right (2015) Ordinance Survey (100021242).

Earthworm abundance was lower at the 5 m sampling point (average of 149 m^{-2}, compared with $\geq$206 m^{-2} at all other points), where soil was significantly more compacted down to a 30 cm depth (Kruskall-Wallis non-parametric ANOVA, H=25.50, df =4, p = < 0.001, confirmed with multiple Mann-Whitney U tests) than surrounding areas (Figure 4.5). Species richness was not markedly reduced in the more compacted 5 m sampling points, and the anecic (deep-burrowing) species *A. longa* was found in similar densities at this distance as at other sampling points (Figure 4.3).

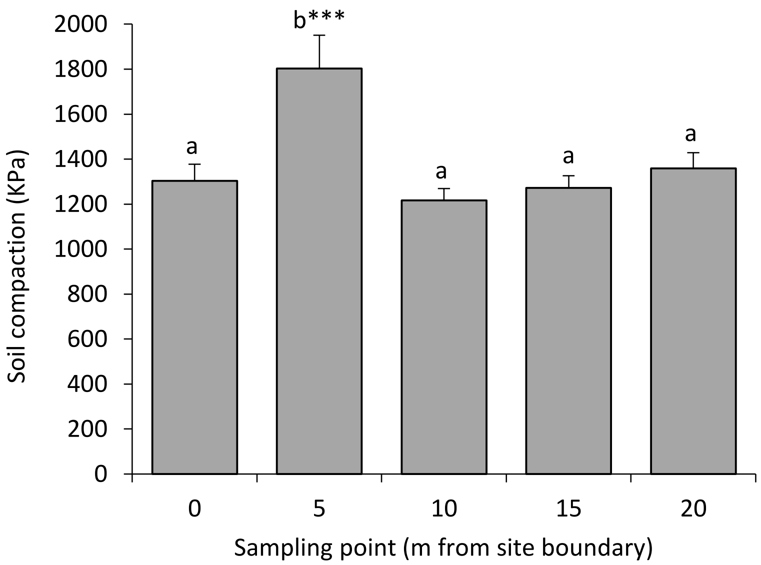


Figure 4.5. Mean soil compaction (± SE) to 30 cm depth at 5 m intervals along a 20 m transect away the site edge (n = 25). Different letters indicate significant differences, ***p <0.001.

4.3.2. Environmental variables

A. longa density was found to be significantly higher in areas of 100% forb cover compared with areas of reduced (<75%) forb cover (Kruskall-Wallis one-way nonparametric ANOVA, df = 4, p <0.05). Significantly greater densities of *L. festivus* were located in samples which were within 1 m of Norway maple trees, against samples which were close to Italian alder or no other tree species (Kruskall-Wallis one-way nonparametric ANOVA, df = 3, p <0.01). No other statistically significant relationships were found between any earthworm species density, overall community density or species richness, and the tree type or ground vegetation cover. Higher soil moisture content was found to significantly increase earthworm community density (Kruskall-Wallis one-way ANOVA, df = 1, p <0.001).

4.4. Discussion

4.4.1. Earthworm colonisation

Earthworm colonisation rates have been measured and modelled for a range of species and land-use types (Marinissen and van den Bosch, 1992). On a reclaimed landfill, Butt *et al.* (1999) recorded spread of *A. longa* and *A. chlorotica* at 6.4 and 5.6 m yr^{-1}, respectively. A comparable colonisation rate of 5 m yr^{-1} was found by Judd and Mason (1995) for the species *A. caliginosa* and *L. rubellus* on a one year old restored landfill site in Essex. The results of the study presented in this chapter showed an even distribution of seven earthworm species from the site boundary to at least 20 m (30 m on some transects, and maybe further) into the site within two years of site restoration. This would represent a colonisation rate which greatly exceeds the observed typical natural earthworm movement rates in reclaimed temperate soils of 4-6 m yr^{-1} (Marinissen and van den Bosch, 1992; Judd and Mason, 1995; Butt *et al.*, 1999). Earthworm population density was 207 earthworms m^{-2}, which is much higher than the earthworm density of 66.7 m^{-2} recorded by Pizl (2001) at a similarly aged afforested reclaimed site, and the approximately 80 m^{-2} found during October sampling by Judd and Mason (1995) at their four year old reclaimed site. These results indicate that the high earthworm community density of the site is unlikely to have purely arisen from natural colonisation from surrounding land. Significant differences in earthworm richness and community density between the Western and Eastern sides of the site were found, suggesting that the different soil resources used in the final restoration and/or time difference in restoration are key factors influencing the earthworm community of the site, discussed further below.

The dominant species found at Little Gerpins were the endogeic *A. chlorotica* and epigeic *L. festivus,* accounting for 68% and 11% of the adult earthworms collected, respectively. *A. chlorotica* is recognised as a coloniser of reclaimed sites (Curry and Cotton, 1983), and may be particularly successful in those with high clay content (Butt *et al.*, 1999; Sims and Gerard, 1999). Less recognised, however is *L. festivus*, which has not been widely recorded as a typical early coloniser of reclaimed sites (Curry and Cotton, 1983). Another typical early coloniser found at Little Gerpins was *A.*

caliginosa. However, *A. rosea* was found in similar numbers and this is considered a late colonising species (Dunger, 1989; Pizl, 2001). Judd and Mason (1995) found that *A. chlorotica* formed the bulk of the community at a 12 year old site (66% of all worms), whilst at the four year site, *L. rubellus* was the dominant species (53.8%). Interestingly, the epigeic *L. rubellus* was not found during the survey of Little Gerpins. This species has been recorded from a wide range of habitats, and is considered to be a common early colonist of landfill sites; with tolerance of a broad pH range, high dispersal ability and reproductive rate (Evans and Guild, 1947; Brockmann *et al.*, 1980; Sims and Gerard, 1999), and is often found to be numerically dominant in earthworm communities shortly after restoration (Dunger, 1989; Butt *et al.*, 1999; Pizl, 2001). The absence of this species, along with the presence of the anecic *A. longa* and *L. terrestris* (considered to be late colonisers of reclaimed sites, e.g. Curry and Cotton, 1983; Dunger, 1989; Butt *et al.*, 1999) may be further evidence for the earthworm community at Little Gerpins arising from the stockpiled soil material rather than natural colonisation from external sources. The combination of early and late colonising species on the site would seem to reflect a developed community structure, perhaps the original site community prior to reclamation activities, which naturally colonised the site from the surrounding land during the 30 years as rough pasture. The species present at Little Gerpins would appear to match those typically found in established pasture woodland (e.g. *A. chlorotica*, *A. caliginosa*, *A. longa*, *A. rosea*, *L. terrestris*) (Eggleton *et al.*, 2009). If this is the case, then the atypical community composition for an early site observed at Little Gerpins may be explained by facilitative interactions between the dispersing species following re-introduction to the site when stockpiled soil was re-applied to the surface of the site. For example, Butt *et al.* (1999) found that *A. chlorotica* exhibited greater dispersal ability when it was introduced to a reclaimed site alongside *A. longa*. This site was restored grassland after landfilling in the 1960s, then left as derelict pasture for a period of at least 30 years before the backfilling activity took place in 2010.

It is proposed that the high earthworm numbers on the West side are the result of earthworm re-colonisation via earthworms and cocoons in the stockpile of the original soil materials from the site.

At Little Gerpins, as with many reclaimed sites, the soil used for providing a final tree-planting substrate on the west side came from reserves of the site's topsoil stockpiled prior to the secondary reclamation activities. The East side received imported soil from a local crop field. Stockpiles often contain very low levels of earthworms and other soil fauna and re-colonisation of the site by earthworms may rely exclusively on dispersal from surrounding areas (Boyer *et al.*, 2011). However, it has been suggested that this process may be much slower than the natural expansion of earthworm populations on a site from individuals surviving in the stockpile (Wanner and Dunger, 2002).

Research into earthworm community survival in soil stockpiles indicates that sustainable communities can exist in the top 0.2 m of soil, however anaerobic conditions and compaction negatively affect earthworm survival below 1 m depth (Harris *et al.*, 1989; Boyer *et al.*, 2011). As such, any earthworm community surviving in a stockpile are likely to be challenged by the anaerobic conditions in lower layers. The proportion of anaerobic soil in a stockpile is related to the size of the pile itself; lower stockpiles will have a smaller proportion of compacted and anaerobic soil (Boyer *et al.*, 2011). In the UK, the recommended maximum height of stockpiled topsoil varies widely in reclamation guidance material. Moffat and McNeill (1994) advocated a maximum of 5 m height for topsoil piles, however Forest Research (2015) recommend topsoil stockpiles do not exceed 1.5 m; both advise that piles are constructed with minimum compaction and seeded with grass. Optimal stockpile height is variable between sites, depending on soil texture, but as an example Boyer *et al.* (2011) advised a maximum height of 2-3 m for the mine in their study. At Little Gerpins, the soil was stockpiled using bulldozer machinery to a height of 4 m, and stored on site under a grass sward for at least 3 years. Under these conditions earthworms may have survived in the surface of the stockpiles and acted as a reservoir for re-colonisation of the site. It has been demonstrated that earthworm survival rates in stockpile surface soil can be sufficient to act as a source for earthworm re-colonisation (Armstrong and Bragg, 1984; Pizl, 2001). It can take between 10 and 30 years for populations to recover following stockpiling and re-spreading of soil, although community composition may be altered from that which existed prior to reclamation, and this is benefited by soil quality (Scullion *et al.*, 1988; Pizl, 2001; Boyer and Wratten, 2010).

Another possible method of earthworm introduction to reclaimed sites is passive transport of cocoons or juveniles (Pizl, 2001). Examples of this include the introduction of earthworms in the soil around roots of the vegetation planted on afforested sites (Dunger, 1989). Pizl (2001) attributed passive transport of earthworms in tree stock from a nursery as a main factor in earthworm establishment at afforested reclaimed sites in the Czech Republic. It is possible that earthworms were brought into the Little Gerpins site during tree planting. However, as both sides of the site were tree-planted at the same time this would not explain the significant differences in earthworm density observed between the West and East sides of the site.

The effect of soil compaction on earthworm species abundance and behavioural adaptability was demonstrated by reduced community density along the bridal path bordering the inner perimeter of the site, which was significantly more compacted than surrounding areas. The anecic species *A. longa* showed no reduction in density at these highly compacted points, indicating behavioural plasticity in depth and orientation of burrowing by this species, as suggested by Butt *et al.* (1999).

Vegetation presence accelerates earthworm colonisation of reclaimed land, through plant roots enhancing earthworm burrowing ability and the provision of food in the form of decaying plant material (Springett *et al.*, 1998; Boyer and Wratten, 2010). Tree leaf litter palatability to soil fauna has been shown to strongly influence soil faunal population development (Swift *et al.*, 1979; Pigott, 1989; Muys *et al.*, 1992; Reich *et al.*, 2005; Rajapaksha *et al.*, 2013). Butt *et al.* (1999) found that, after 11 years, the presence of common alder had a significant positive effect on earthworm density and mass on a reclaimed landfill site. The young age (2 years) of the alder and other trees at Little Gerpins may explain the limited evidence of relationships with the earthworm community, since the trees may have not had sufficient time to provide sufficient quantities of litter or establish root networks. Repeated monitoring in a number of years may help to identify clearer relationships between earthworm and tree species. Soil moisture content was found to have a significant positive influence on earthworm community density; soil moisture is one of the main drivers for earthworm activity (Lowe and Butt, 2005) and this has been previously documented for earthworm populations in reclaimed soils and is expected to fluctuate seasonally (Curry and Cotton, 1983; Lee, 1985; Judd and Mason, 1995). On this site, soil moisture content is likely to be a function of site topography and high levels of soil compaction. Whilst Little Gerpins is an open site, soil moisture content is unlikely to have been influenced by organic matter inputs to the soil in the form of leaf litter, as the young trees (total age of three years) were not fully established at the time of sampling (Dobson and Moffat, 1993; Bending and Moffat, 1997).

Earthworm inoculation (e.g. Butt, 1992) may be a useful method for accelerating soil development on sites which are poorly restored and therefore have low residual earthworm populations. However, this may only be successful if acceptable restoration standards are met from the outset (Butt *et al.*, 2004). The research presented in this chapter indicates that earthworm inoculation may not be necessary on sites where soil quality is given due consideration and legacy soil materials are stockpiled and applied following best practice guidance, as natural colonisation by soil fauna can occur rapidly. Future research to better understand earthworm colonisation might include: more extensive surveying across the Little Gerpins site, to investigate the earthworm community at the centre of the site and across the divide between the two different sides (and therefore topsoil types), and in the adjacent land; detailed physical and chemical analysis of the two different soil resources in-situ; an investigation into earthworm presence in soil attached to roots of nursery trees, as a form of earthworm introduction to reclaimed sites; and an economic quantification and cost-benefit analysis of the ecosystem service benefit of using good standard topsoil and restoration practice, versus poor restoration and low soil ecosystem service output for a number of years following restoration.

4.4.2. Summary of chapter findings

The findings of this research were as follows, summarised against the chapter research objectives:

1. High levels of soil compaction were associated with reduced earthworm abundance; however, the presence of the anecic species *A. longa* in compacted areas indicates lifestyle adaptability in this species.
2. Natural colonisation of reclaimed land by earthworms can occur rapidly, where soil quality is given due consideration and legacy soil materials are stockpiled and applied following best practice guidance.
3. Topsoil origin significantly affected earthworm population densities; with higher densities found in areas where the original site topsoil (from rough pasture land use) was applied, versus imported soil from intensive agricultural land.

5. A FIELD EXPERIMENT INVESTIGATING COMPOSTED GREENWASTE, EARTHWORM AND TREE INTERACTIONS ON A RECLAIMED LANDFILL SITE

5.1. Introduction and objectives

Creation of a suitable soil resource is essential for sustainable greenspace establishment, in order to provide necessary soil chemical and physical conditions and restore normal soil biological functions (Scullion, 1992). There is increasing industrial and scientific interest in improving the soil materials used in reclamation projects, particularly through the addition of organic matter from waste streams, such as composted green waste (CGW) (Moffat, 2006; Nason *et al.*, 2007; Forest Research, 2015). However, under the the Nitrates Directive (91/676/EEC), inappropriate limitations may be set on the maximum amount of CGW applied during land regeneration projects, since these sites typically require much greater organic matter addition than allowed under the Directive (SNIFFER, 2010). To date, limited research has been conducted into the effect of CGW at any application rate on tree growth on reclaimed land (e.g. Foot *et al.*, 2003; Moffat *et al.*, 2008) (this thesis, section 2.5.3).

Research indicates that on restored sites, the addition of organic matter to soil may be important for establishing sustainable earthworm populations (Lowe and Butt, 2002b, 2004), and a range of organic waste types, including CGW, have been investigated for suitability to support earthworm growth (Edwards and Bohlen, 1996; Piearce and Boone, 1998; Bain *et al*., 1999; Lowe and Butt, 2004; Butt *et al.*, 2005) (section 2.5.2). It has been shown that certain earthworm species will actively incorporate and mix organic waste materials into soils, improving mineralisation and benefiting soil fertility (Piearce and Boone, 1998). The addition of earthworms may therefore be an effective way of enhancing the benefits of organic waste utilisation by vegetation and other soil fauna during land regeneration. However, little research exists which investigates CGW interaction with earthworm populations in reclaimed soils. The few available studies suggest that CGW can promote the development of earthworm populations on reclaimed sites, and may help promote soil development and tree growth (Lowe and Butt, 2004). Further research therefore is required to build a body of evidence regarding these interactions and provide more data on tree growth and soil development over time after CGW and earthworm addition. An experiment was therefore set up to address the following objectives:

1. measure the effects of CGW addition and earthworm inoculation on tree growth and survival on a reclaimed landfill site,
2. investigate the effects of CGW application and tree growth on earthworm population and community dynamics on a reclaimed landfill site,
3. investigate the effects of CGW application and earthworm addition on soil carbon and nutrient cycling, and soil physical and chemical quality.

5.2. Materials and methods

5.2.1. Study site

The location of this experiment was Ingrebourne Hill Community Woodland, a 54 ha area of land in Rainham, Essex, UK (Nat. Grid Ref: TQ 52572 83192) (Figure 5.1). This site is former gravel extraction and inert and putrescible waste disposal landfill, which underwent clay capping, followed by placement of screened construction waste materials as soil substrate (see section 3.2.1 for detailed site information).

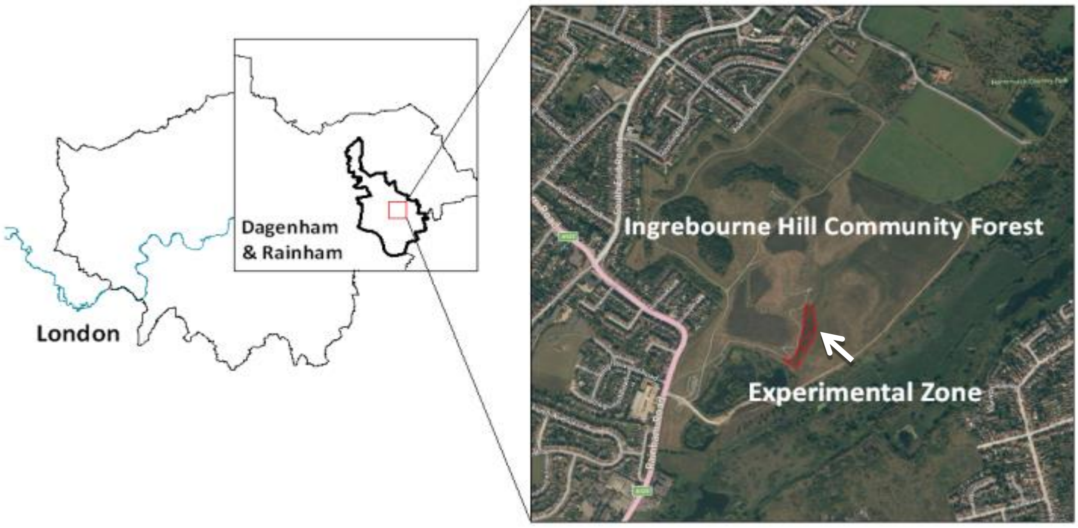


Figure 5.1. Experiment location (marked in red) within Ingrebourne Hill Community Woodland, a regenerated landfill site in Rainham, London (image source: Google maps).

5.2.2. Experimental design

Figure 5.2 shows the layout of the experimental plots. The experimental design consisted of five blocks, each containing a randomised arrangement of four treatment plots (i.e. 20 plots in total). Each 100 m^2 plot contained two sub-plot monoculture planting stands (one per tree species), separated by an inter-plot buffer zone. Prior to tree planting, each plot underwent complete cultivation of soil to 0.5 m depth by digging and mixing the soil with a hydraulic excavator, following guidance by Forest Research (2006), to relieve soil compaction. For plots receiving CGW treatment, cultivation also achieved the incorporation of CGW into the soil, through surface application of CGW followed by thorough mixing into the soil during cultivation, as recommended by Moffat (2006).

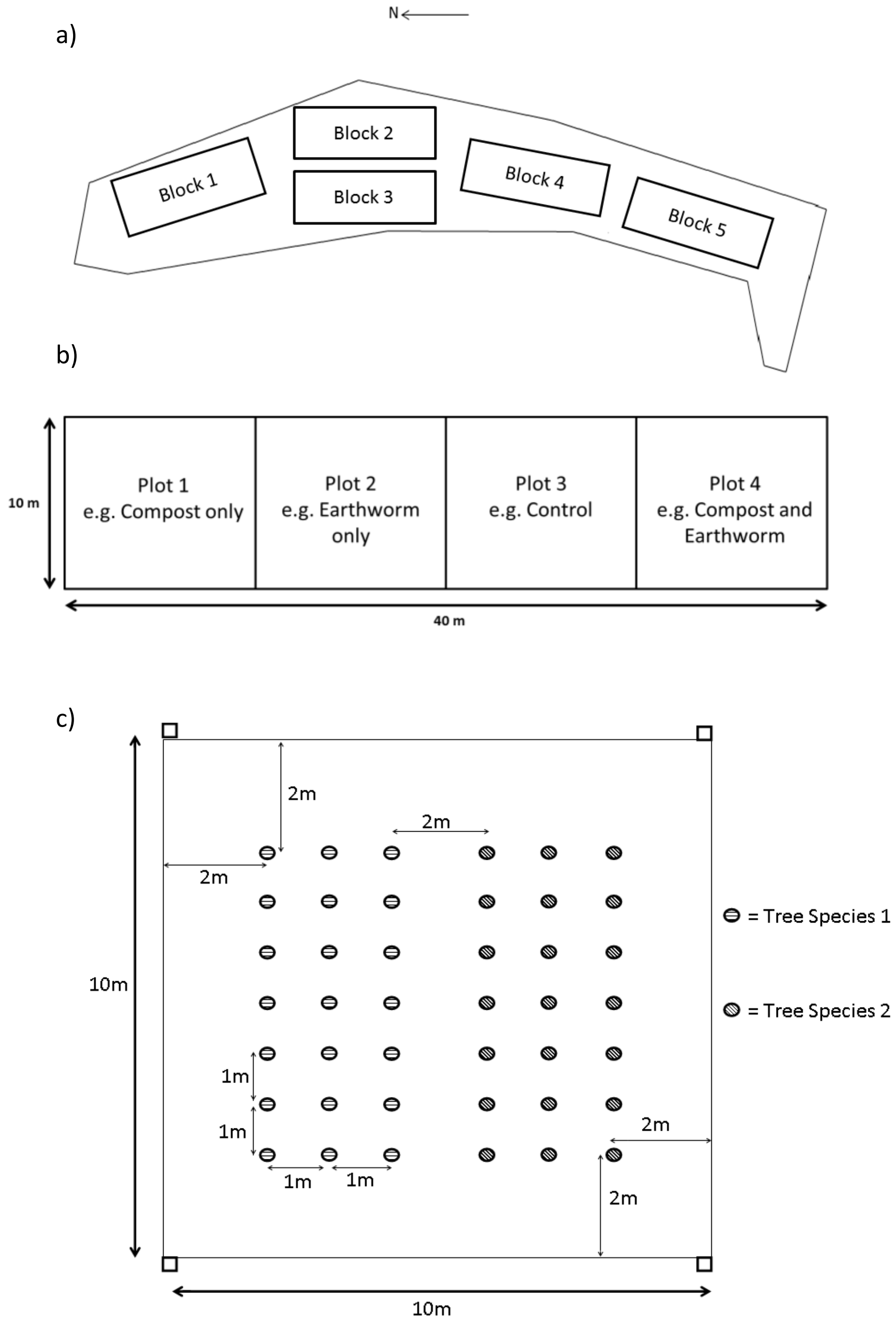


Figure 5.2. Diagram showing the Ingrebourne Hill experiment design; a) arrangement of blocks within the fenced area, b) random layout of treatment plots within an individual block, c) layout of planting sub-plots within a treatment plot.

A physical barrier to earthworm ingress/egress from experiment plots was installed following cultivation of the plots. This consisted of sheets of LDPE damp-proof membrane buried to 0.5 m depth (sufficient to prevent earthworm lateral movement), with 0.2 m above-ground along perimeter of all experimental plots (after Bohlen *et al.*, 1995) (Figure 5.3). The perimeter of the experiment location was surrounded by a fence to prevent damage to trees by the public and browsing animals. Following cultivation, plots were seeded with Masterline Pro-master 'PM 25 R Gro-slow plus' grass seed mix for reclaimed land, to help suppress weed growth. The experiment started in April 2013 and ran to early June 2015 (i.e. just over 24 months). Glyphosate herbicide was applied around the base of each tree during the first year to suppress weed growth within each plot.

5.2.3. Experimental treatments

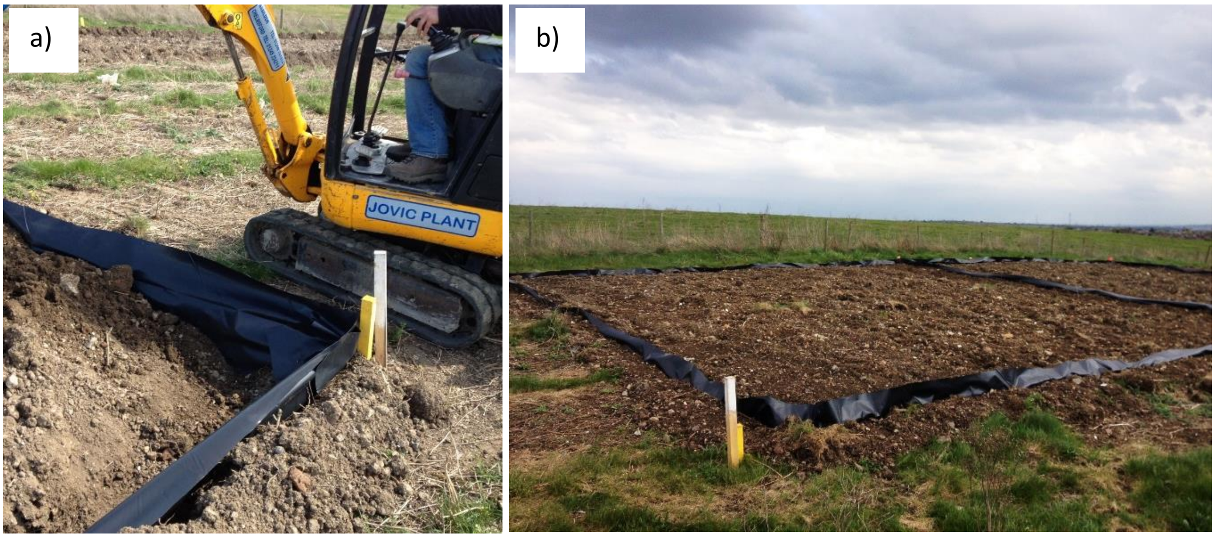


Figure 5.3. a) Installation of and b) completed 100 m^2 plot with LDPE membrane earthworm barrier.

This experiment employed four treatment combinations: no treatment (control), CGW addition only, earthworm inoculation only, and both CGW addition and earthworm inoculation. For CGW-treated plots, soil cultivation included incorporation of screened 0-25mm PAS 100 "Soil Improver" grade CGW (Viridor Ltd) at a rate equivalent to 500 kg Total N ha^{-1} (Following legal limit set by Nitrates Directive for the site which is a Nitrate Vulnerable Zone (NVZ), and in keeping with guidance by Taylor (1991) and Bending *et al.* (1999). The amount of CGW applied per plot was calculated as follows. The Nitrates Directive (91/676/EEC) sets a maximum application rate for PAS 100 CGW at 500 kg total N ha^{-1} yr^{-2} to be applied in the first year (with none applied in the second year). This is equivalent to 5 kg Total N per 100 m^2 plot, and as the CGW is 6.2 kg total N t^{-1} (Table 5.1), then (5 /

6.2) = 0.8 t CGW per 100 m^2 plot. Therefore, each CGW treatment plot within the Ingrebourne Hill field experiment received 0.8 t CGW, incorporated to 0.5 m depth. At 10% N availability, this provided each treatment plot with 0.5 kg available N. Due to difficulty in weighing one tonne of compost in the field, 2 m^3 of compost (one digger bucket full) was added to each plot, which equals approximately 1 tonne (fresh compost weight is roughly 0.5 tonnes m^{-3}).

Figure 5.4. Approximately 2 m^3 of CGW being transported for incorporation to a plot.

Table 5.1. Viridor 0-25 mm PAS 100 Composted Green Waste summary nutrient analysis (source: technical document supplied by Viridor).

Parameter	% Dry Mass	Kg t^{-1} Fresh Weight	Total Kg nutrient t^{-1}	% nutrient availability (in first year)
Nitrogen	1.27	6.20	6.20	10
Phosphate	0.19	0.93	2.12	75
Potash	0.79	3.86	4.65	90
Magnesium	0.26	1.27	2.10	60
Sulphur	0.25	1.22	3.05	30
Organic Matter	60.20	293.78	n/a	n/a

Soils were allowed to settle in the field for one week prior to tree planting. The tree species selected for this experiment were *Alnus cordata* and *Acer platanoides*, based on the rationale provided in section 3.3. One-year-old root-trainer seedlings (the standard age for trees planted in the field) were obtained from the same nursery as used for planting at other areas of the Ingrebourne Hill Community Woodland (Figure 5.5). Trees were left 1 week in the field before earthworm introduction. One-year-old root-trainer seedlings of Norway maple and Italian alder (n = 21 per species) were planted in each plot (20 plots X 21 trees = 420 trees total per species).

Figure 5.5. Bare-root one-year-old Norway maple seedlings planted at the start of the experiment.

This experiment primarily investigated the activity of the earthworm species *A. longa* (anecic), however there was also interest in the species *A. chlorotica* (endogeic). Baseline surveying revealed low numbers of *A. longa* in the experimental plots; however, *A. chlorotica* were more abundant. Therefore, the experimental plots receiving an earthworm treatment were inoculated with *A. longa* to boost the population density of this species; these were collected from surrounding areas of Ingrebourne Hill Community Woodland.

To collect the earthworms for inoculation, mustard suspension vermifuge was used at a concentration of 50 g to 10 litres of water, applied liberally to the soil surface in areas of earthworm casting. Adult *A. longa* were identified in the field during collection, washed to remove vermifuge and briefly stored in trays containing freshly dug soil. In total, 4,200 *A. longa* were collected and transported to the experiment, and randomly assigned to trees at a rate of five per tree. Earthworms were added to 5 cm deep freshly dug holes at the base of each tree, the soil replaced and soaked with fresh water (Figure 5.6).

Figure 5.6. a) Collection and temporary storage of *A. longa* prior to inoculation; b) Inoculation of five *A. longa* at the base of an Italian alder seedling.

5.2.4. Sampling and measurements

Earthworm community density and change were measured to identify the effects of tree species and soil treatment on earthworms. At the start of the experiment, baseline earthworm populations were surveyed within each of the control plots following soil cultivation and tree planting (Figure 5.7a). Across each plot, two ten-metre transect lines were placed, running in a general North-South direction parallel to the side margins of the plot. These transects were separated from each other by three metres, and from the side margins of the plot by 3.5 m. At 1 m, and then 2 m intervals thereafter, a 0.1 m^2 quadrat was placed on the soil surface and the covered area dug to 15 cm, then the topsoil removed and hand-screened for earthworms. A vermifuge solution of 50 g mustard powder to 10 litres tap water was then applied to the pit and left to infiltrate, then re-applied if all vermifuge was absorbed by the soil. The pit was monitored for 5 minutes to allow all worms to emerge. Any earthworms were then collected and placed directly into pre-labelled plastic bottles containing 4% formaldehyde solution, and transported to the laboratory for identification. All adult worms were identified to species level following the identification key of Sims and Gerard (1999). Earthworm sampling was carried out at 30 months (in late October, as this provided more suitable conditions for earthworm sampling than at 24 months, which was in early June), across all plots, to determine the earthworm populations under each treatment.

Tree growth and health were measured at six month intervals, via data on tree survival, height and ground-line diameter (Figure 5.7b). Diameter was measured using callipers at the ground-line of trees, which is defined as the point on the main stem 2 cm above the soil surface (Menes and Mohammed, 1995). To account for unsymmetrical stem growth, diameter was measured twice, at right angles to each other, and mean value reported. The baseline diameter measurements were taken 2 weeks after tree planting, to allow soil at the base of the trees to settle. Tree height was

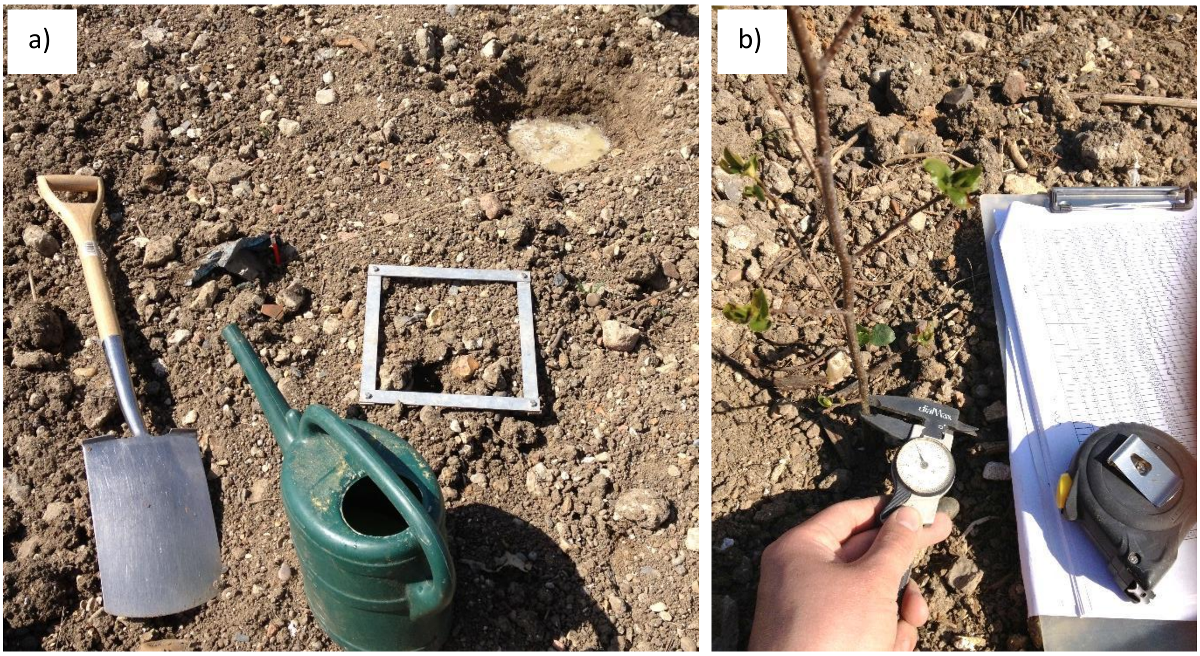


Figure 5.7. a) Sampling for earthworms in control plots at the outset of the experiment; b) Measurement of ground-line diameter using callipers.

measured using a tape measure, in accordance with Forest Research SOP 0232 “Determining tree height assessment points”, which specifies that the total height of a standing tree (in most cases) is the vertical distance from the base of the tree to the uppermost point or tip. If the leader of the tree was not vertical or the stem not straight, then it was carefully straightened as far as practicable. If the tree was heavily leaning, then measurement was taken at the point on the ground where a plumb bob would fall, if suspended from the straightened tip of the tree.

At 0, 6, 12, 18 and 24 months, soil samples were taken from within each experimental plot for chemical analysis. This followed a ‘W-shaped’ sampling approach, whereby in each plot 21 samples were collected from 0 - 15 cm depth using a soil auger, and bulked (Figure 5.8). This method should enable sufficient sampling cover per plot to account for the heterogeneous nature of the soil (Carter and Gregorich, 1993). Bulk soil samples had % total C and N determined using a CN Elemental Analyser (Carlo Erba (THERMO), FLASH EA 1112 Series), and total major elements (P, K, Ca and Mg) analysed after sulphuric acid digestion and inductively coupled plasma-optical emission spectrophotometry (ICP-OES) analysis, soil moisture content analysed by oven drying at 105^{o}C for 24 hours, loss-on-ignition organic matter content determined, and soil pH was measured in water suspension. KCL-extraction was used (MAFF, 1986) on fresh soil for determining levels of inorganic “available” nitrogen, by colorimeter analysis by Rothamstead Research laboratory services.

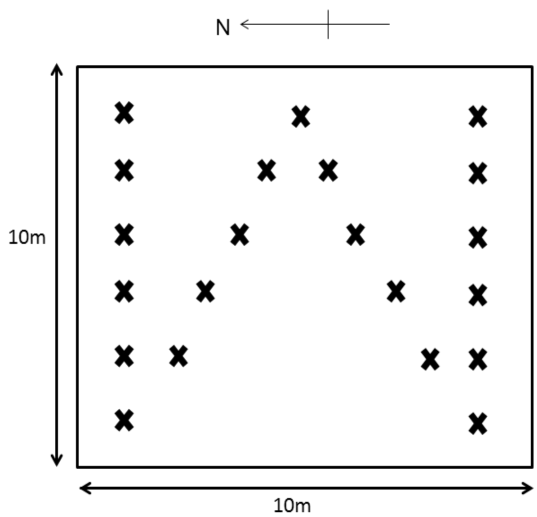


Figure 5.8. The W-shaped soil sampling methodology employed per plot. X = soil sampling

5.2.5. Statistical analysis

Statistical analysis was performed using the statistical software GenStat (Release 16.2). Data were first tested for normality using the Shapiro-Wilk test, which is suited to small sample sizes (in this case n=5). Where data had a normal distribution, they were analysed using one and two-way analysis of variance (ANOVA) with the Tukey-Kramer post-hoc multiple comparison test applied to significant treatment interactions, and time-series data analysed using repeat-measures ANOVA. Where the assumptions of ANOVA were not met, non-parametric Kruskal-Wallis ANOVA or Mann-Whitney U tests were applied, as appropriate. These statistical models were applied to data on tree survival, height and ground-line diameter, earthworm community density and species richness, and soil chemical parameters, as described in section 5.2.4.

5.3. Results

5.3.1. The influence of tree species and compost addition on earthworm populations

Baseline surveying of the control plots showed five earthworm species within the experimental site, all at low mean levels: *Lumbricus festivus* (1 m^{-2}), *Lumbricus castaneus* (10 m^{-2}), *L. terrestris* (0.2 m^{-2}), *A. longa* (1 m^{-2}) and *A. chlorotica* (8 m^{-2}). The abundance of *L. festivus* was found to be significantly different between plots (Kruskall-Wallis non-parametric ANOVA, df = 4, p = 0.014). All other species displayed no significant differences in abundance between blocks. Surveying of control plots at 30 months revealed a number of differences in earthworm densities (Figure 5.9). In particular, the final *A. longa* and *A. chlorotica* densities (mean = 18.5 and 28.5 m^{-2}, respectively) were significantly higher than the baseline (Mann-Whitney U test, df = 4, p = 0.016 and p = 0.032, respectively). This represents an increase of *A. longa* density of 1,750%, and an increase of *A. chlorotica* density of 275%. Comparatively, *L. castaneus* density (mean = 0.5 m^{-2}) was significantly lower after 30 months (Mann-Whitney U test, df = 4, p = 0.016), equivalent to a 95% reduction. Total earthworm densities were not significantly different after 30 months, although the total earthworm density had increased by 45%.

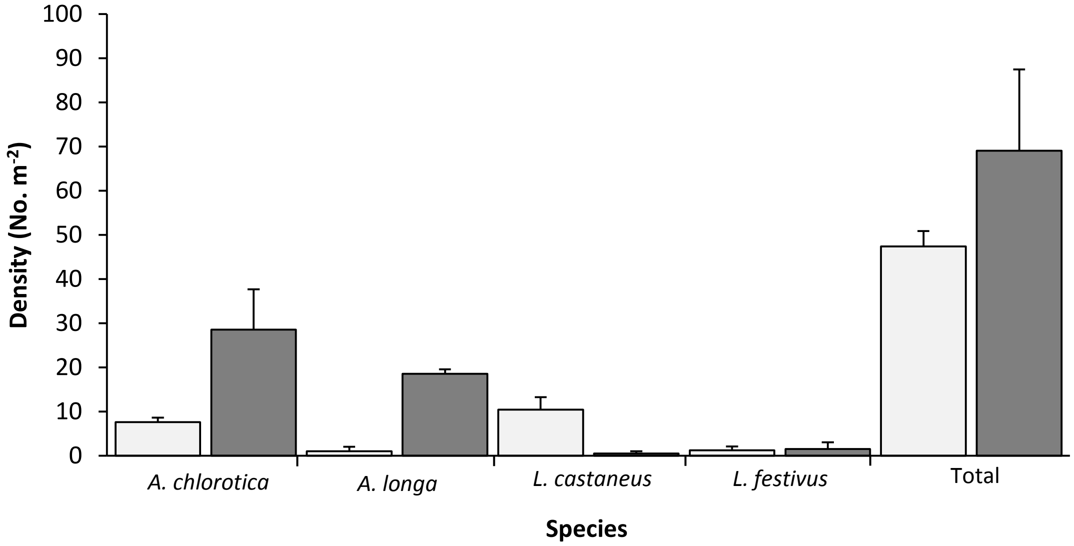


Figure 5.9. Mean earthworm density (+ SE) in control plots at the start (light grey) and termination (dark grey) of the experiment (30 months (n =5).

Following completion of the experiment at 30 months, the control and compost-only plots were found to contain a similar mean number of the experimental earthworm species to the earthworm inoculated plots (Figure 5.10). The highest *A. chlorotica* density was found under the compost-only treatment (mean = 48.5 m^{-2}). Both the highest *A. longa* and total earthworm density was under the combination treatment (19.5 m^{-2} and 90 m^{-2}, respectively) although there was no significant effect of treatment on earthworm density (non-parametric ANOVA, $p > 0.05$).

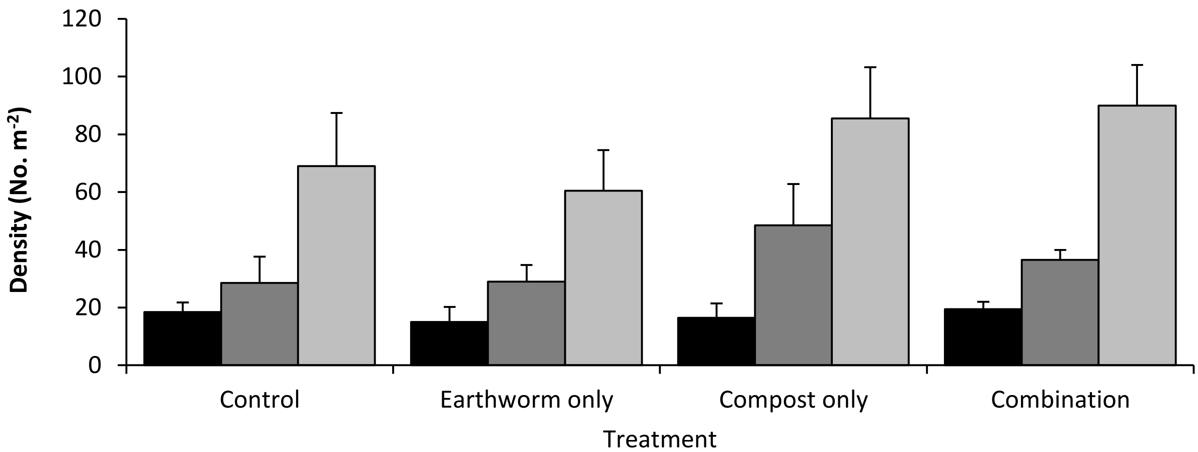


Figure 5.10. Mean density (+ SE) of selected earthworm species per treatment at 30 months (n=5). Earthworm species: *A. longa* (black), *A. chlorotica* (dark grey), total earthworms, including juveniles (light grey).

At 30 months, there was a similar mean number of the experimental earthworm species underneath both tree species and the tree-free control points (Figure 5.11). There was no statistically significant effect of treatment on earthworm distribution under tree species. Highest *A. chlorotica* density was found under Norway maple (mean = 39 m^{-2}), as was highest total earthworm density (mean = 74 m^{-2}). Highest *A. longa* density was under the tree-free control (21 m^{-2}). However, there was no significant effect of tree species or presence on earthworm density within the experiment plots (Kruskall-Wallis non-parametric ANOVA, p >0.05).

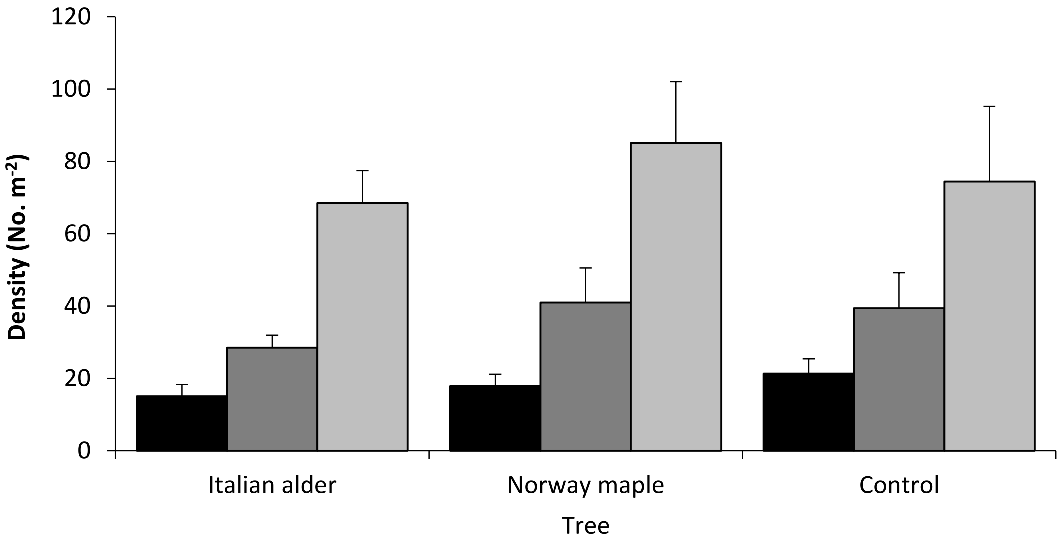


Figure 5.11. Mean density (+ SE) of selected earthworm species at 30 months, according to proximity to experimental tree species or tree-free control (n=5). Earthworm species: *A. longa* (black), *A. chlorotica* (dark grey), total earthworms (light grey).

5.3.2. The effects of earthworm inoculation and compost addition on tree survival and growth

At termination of the experiment, Italian alder demonstrated markedly higher survival rates compared with Norway maple, across all treatments (Table 5.2). Italian alder demonstrated high survival rates (>88%) across all treatments; however highest survival was recorded with presence of compost with or without earthworm addition (95.25% and 93.33%, respectively). Norway maple showed highest survival under the earthworm-only treatment (54.29%). However, no statistically significant treatment effects (p >0.05) were found on Italian alder or Norway maple survival after 24 months.

Table 5.2. Mean tree survival (%) after 12 and 24 months under experimental treatments, ± SE (n =5).

	Italian alder		Norway maple	
Treatment	12 months	24 months	12 months	24 months
Control	93.3 ± 1.9	90.5 ± 2.1	63.8 ± 8.3	45.7 ± 11.3
Earthworm only	90.5 ± 1.5	88.6 ± 2.4	57.1 ± 8.1	54.3 ± 10.3
Compost only	98.1 ± 1.2	93.3 ± 1.2	51.4 ± 9.8	41.9 ± 10.4
Earthworm-compost combination	99.1 ± 1.0	95.2 ± 2.6	59.1 ± 6.8	45.7 ± 6.5

At the start of the experiment, for both tree species there was no significant difference in the mean tree height between treatments (Figure 5.12). At termination of the experiment, a significant treatment effect was found on Italian alder height, under the combination and compost-only treatments (ANOVA, ($F_{(3, 12)} = 13.71$, $p <0.01$)). Throughout the duration of the experiment, there was a significant effect of treatment (repeated measures ANOVA ($F_{(3, 12)} = 10.29$, $p <0.001$)), and significant interaction effect of time and treatment (repeated measures ANOVA ($F_{(12, 64)} = 5.85$, $p <0.001$), under the combination and compost-only treatments. No significant treatment effect was found (ANOVA and repeated measures ANOVA, p = >0.05) on Norway maple height.

At termination of the experiment, Italian alder had outperformed Norway maple in height across all treatments. Following planting, Italian alder mean height increased slowly for the first six months. Thereafter, height increased greatly, with all treatments approaching double the initial mean planting height after 12 months (Figure 5.12). Comparatively, Norway maple showed little change in mean height after 6 months, after which height slowly increased, with no trees reaching double initial planting height after 24 months.

At the start of the experiment, for both tree species there was no significant difference in the mean basal diameter of each tree between treatments (Figure 5.12). At termination of the experiment, a significant treatment effect was found (ANOVA, (F (3, 12) = 7.61, p <0.01) on Italian alder diameter, under the combination and compost-only treatments. During the experiment, there was a significant effect of treatment (repeated measures ANOVA (F (3, 12) = 5.81, p <0.05)), and significant interaction effect of time and treatment (repeated measures ANOVA (F (12, 64) = 3.27, p <0.01)), under the combination and compost-only treatments. Norway maple showed no significant effects of treatment on basal diameter (ANOVA and repeated measures ANOVA, p = >0.05).

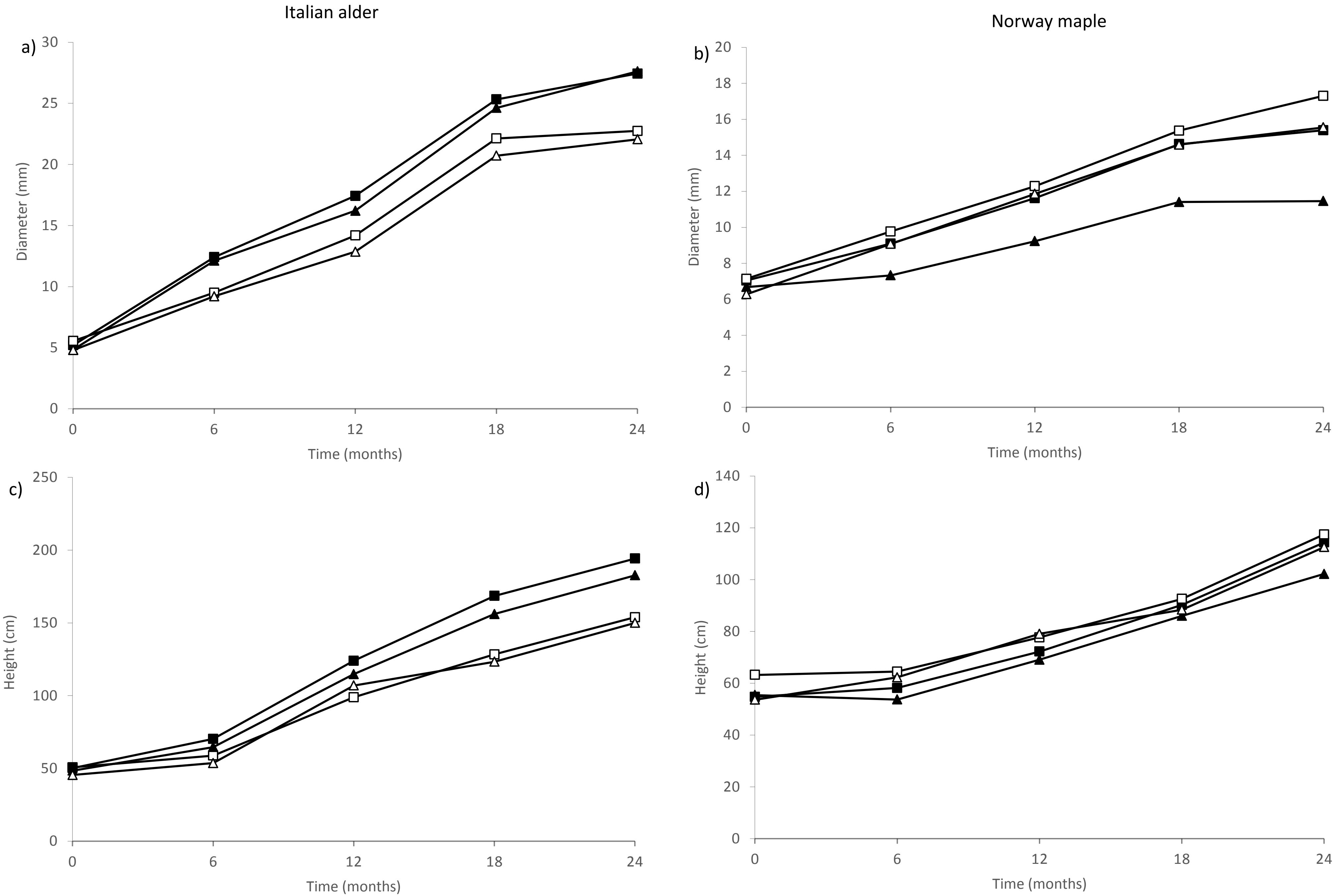


Figure 5.12. Mean tree height (cm) of (a) Italian alder and (b) Norway maple; and mean basal diameter of (c) Italian alder and (d) Norway maple throughout the experiment (n =5). Error bars excluded for clarity. Treatments: ■= combination, ▲= compost only, △= earthworm only, □= control

5.3.3. The effects of compost addition and earthworm activity on soil quality

At termination of the experiment, soil chemistry results showed a number of effects of treatment on soil carbon and plant nutrients. Soil organic carbon (%) and organic matter (%) content increased across all treatments throughout the experiment (Figure 5.13a,b). However, there was significantly greater soil organic carbon and organic matter content (%) in soils receiving the combination treatment (repeated measures ANOVA, treatment effect ($F(3, 12) = 3.82$, $p < 0.05$), and ($F(3, 12) = 3.82$, $p < 0.05$), respectively). Total N (%) remained steady across all treatments until 18 months, after which total N concentration increased for all treatments (Figure 5.13c). Final total N (%) was significantly greater in soils receiving both the combination and compost-only treatments (repeated measures ANOVA, treatment effect ($F(3, 12) = 5.07$, $p < 0.05$). At the start of the experiment, soil total K (mg/kg) levels were higher in the compost and combination treatments, however after 24 months there was similar K levels across all treatments (Figure 5.13d), and this change was statistically significant (treatment effect, repeated measures ANOVA, ($F(3, 12) = 14.07$, $p < 0.001$). Soil Na (mg/kg) levels were initially higher in the compost and combination treatments, however after 24 months the levels had reduced across all treatments to a similar level (data not shown). This change was statistically significant (treatment effect, repeated measures ANOVA, ($F(3, 12) = 9.42$, $p < 0.01$). Soil pH rose slightly across all treatments, with highest initial pH of 8.3 under the combination treatment, and highest final pH of 8.4 under the control treatment (treatment effect, repeated measures ANOVA, ($F(3, 12) = 3.48$, $p < 0.05$).

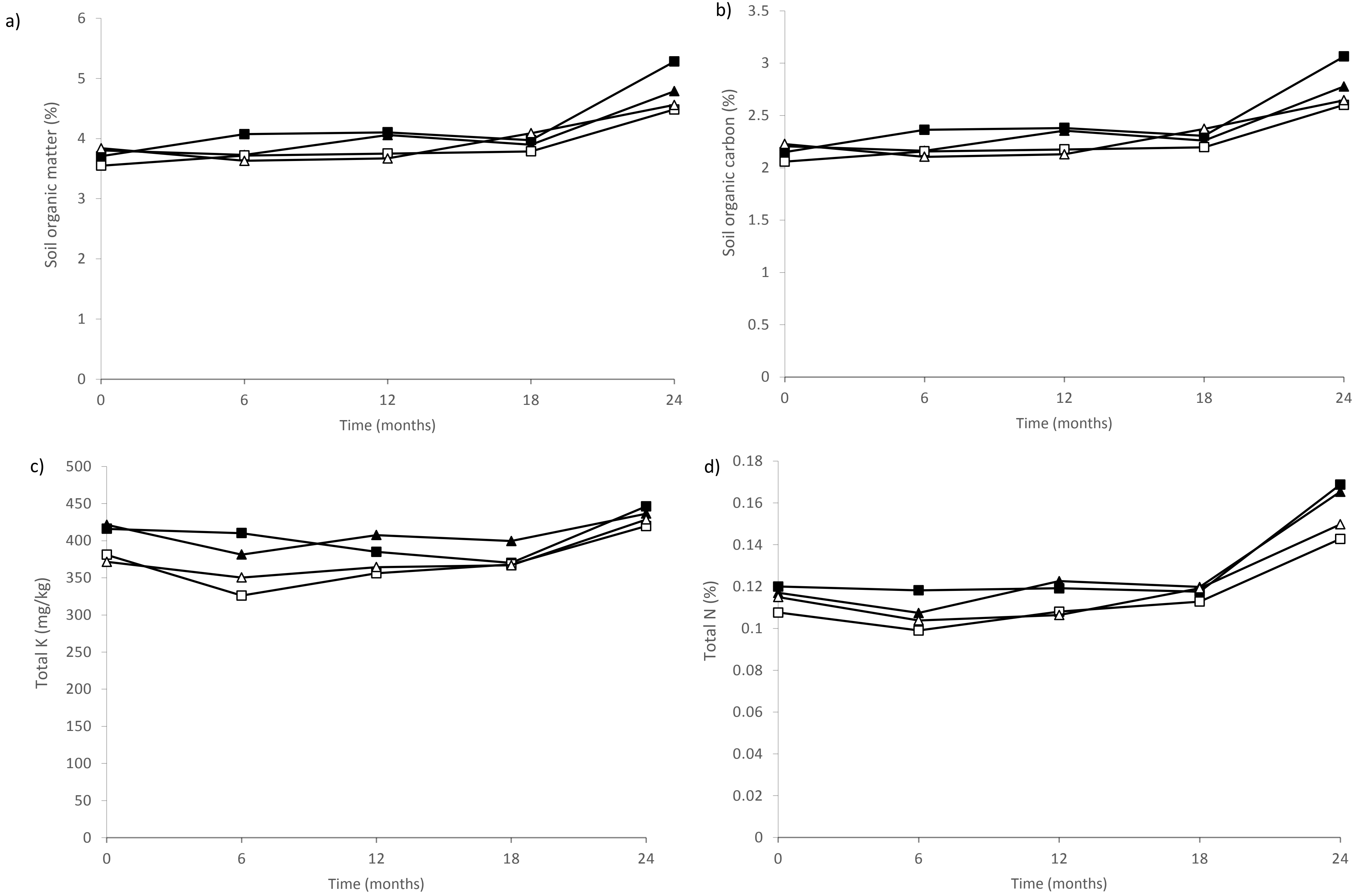


Figure 5.13. Selected mean soil chemical parameters throughout the experiment: (a) Soil organic matter (%), (b) Soil organic carbon, (c) Total K (mg/kg), (d) Total nitrogen (n=5). Error bars excluded for clarity. Treatments: ■= combination, ▲= compost only, △= earthworm only, □= control

5.4. Discussion

5.4.1. The effects of tree species and compost addition on earthworm populations

In this study, final earthworm density and species richness was similar across all treatments, although highest under the compost only and combination treatments. It would appear that inoculation of *A. longa* has not affected the density of this species (an average density increase of 1.4% in inoculated plots), most likely due to a high mortality rate following inoculation. The methodology adopted for this experiment (a modified form of broadcasting – section 5.2.3), whilst suited to the short set-up timeframe for establishment of the experiment, was not as likely to ensure survival of the inoculated earthworms as methods such as the Earthworm Inoculation Unit (EIU), for example (Butt *et al.*, 1995). Mixed successes have been previously achieved in experiments using a broadcasting method for earthworm inoculation. Formalin vermifuge extraction and broadcasting of a mixture of earthworms species (*A. longa*, *A. caliginosa* and *L. terrestris*) onto a 1 ha area of landfill dressed with organic waste showed inconsistent evidence of earthworm establishment 6 years later (Marfleet, 1985; Butt *et al.*, 1993). Earthworm addition to experimental plots by Blair *et al.* (1997) was not found to be an effective way of manipulating population densities compared with methods for reducing populations, however it was still found to increase population density compared with un-manipulated control plots. Blair *et al.* (1997) argue that effects demonstrated by experiments in which earthworm numbers are reduced or increased, rather than completed eliminated, are likely to be more reflective of real-world scenarios, in which earthworm densities naturally fluctuate rather than simply be a case of presence or absence of earthworms.

The success of earthworm establishment following inoculation is principally dependent upon species selection and the soil conditions onsite (Butt, 2011b). Since the inoculated *A. longa* were collected from another part of the Ingrebourne Hill site, with a similar reclamation history, they may be expected to have tolerance to the soil in the experimental plots. However, the location of the field experiment was on a younger area of the site, and as such there may not by then have been sufficient organic matter in the soils to support high numbers of this species following inoculation; as soil organic matter is particularly important for supporting earthworm establishment on hostile landfill caps (Butt *et al.*, 1993).

As the earthworm density in the control plot after 30 months was similar to the other treatments, it is likely that the earthworm densities across all treatments are the result of natural population growth from the low baseline populations recorded per plot. The populations may therefore reflect the carrying capacity of the soil at the time of the experiment. The higher densities recorded in the plots receiving CGW indicate that its application was a more effective method of increasing earthworm density that earthworm inoculation. CGW has been shown to support populations of *A. chlorotica* and *A. longa* in reclaimed soil for at least 2 years following surface application (Lowe and Butt, 2004). However, after four years Lowe and Butt (2004) recorded a sharp decline in the population of *A. longa* in the experimental plots, associated with the complete removal of surface organic matter and subsequent inter-specific competition for limited food resources. This was perhaps exacerbated by a lack of leaf litter input to the soil due to high tree mortality in that experiment. Follow-up surveying of the Ingrebourne Hill experiment presented in this chapter could inform whether the tree species and subsequent litter additions to the soil are capable of supporting earthworm populations following the depletion of the soil CGW nutrients.

A common issue with outdoor mesocosm/macrocosm earthworm experiments is the egress of experimental earthworms from the system, or the ingress of invasive earthworms from the surrounding environment (Lubbers and van Groenigen, 2013). It is possible that the observed increase in earthworm numbers in the plots which originally received no earthworm introduction is the result of earthworm migration between plots, by crossing the plot membrane barriers. These were observed to have lost above-ground rigidity, and as such may not have been an effective barrier to earthworm movement. Similar designs have been successfully employed to control migration between earthworm treatment plots, using PVC walls to 45 cm depth (Bohlen *et al.*, 1995; Blair *et al.*, 1997). However, Blair *et al.* (1997) used strips of metal screen to reinforce the above-ground 15 cm of PVC to prevent earthworm movement between plots, and this could have been used to overcome the issue of barrier instability observed in the experiment presented in this chapter.

There was a similar density of *A. longa* and *A. chlorotica* in both tree species subplots, irrespective of treatment. Butt *et al.* (2004) found a significant effect of alder on earthworm populations compared with those in locations where sycamore had been planted and subsequently failed to establish (mean earthworm density and biomass under alder was 198 m^{-2} and 33.9 g m^{-2}, whilst under former sycamore was 118 m^{-2} and 20.7 g m^{-2}, respectively). Results showed that *A. longa* was significantly affected by tree presence, however *A. chlorotica* was not. *A. chlorotica* dwells within the rhizosphere and forms close associations with plant root systems (Sims and Gerard, 1999). Root-soil interaction may therefore affect this species either directly or indirectly (e.g. through root exudates, or modifying local soil pH) (Dakora and Phillips, 2002; Rajapaksha *et al*., 2014). Nitrogen-fixing plants such as Italian alder are known to release a net excess of protons to the soil (Dakora and Phillips, 2002), however in this experiment, the method of soil sampling adopted did not enable identification of differences in soil pH and other chemical parameters between tree species subplots; as soil samples from each plot were bulked for analysis (Chapter 6 overcomes this issue). Additionally, tree litter has been shown to influence soil faunal populations (Swift *et al.*, 1979), with different tree species influencing soil quality and soil faunal communities differently through the quality and quantity of their leaf litter (Pigott, 1989; Muys *et al.*, 1992; Reich *et al.*, 2005). As noted by Rajapaksha *et al.* (2014), such differences should be expected to primarily influence anecic earthworm species such as *L. terrestris*, or in the case of this study, *A. longa.* The high levels of Norway maple mortality recorded in this field experiment would have likely resulted in less organic matter input from leaf litter in these subplots compared with the alder. However, there was no significant difference in *A. longa* densities between tree species in the experimental plots at Ingrebourne Hill. This study did not measure the quantity or quality of leaf litter inputs within the tree monoculture subplots, and future research could usefully consider the palatability to earthworms of the litter from the tree species used in this field experiment (see Chapter 8).

5.4.2. The effects of earthworm inoculation and compost addition on tree survival and growth

Tree survival data showed that Italian alder was more tolerant of site conditions than Norway maple, irrespective of treatment. Italian alder showed highest survival rates in the combination followed by the compost-only treatments, which also had the two highest final earthworm densities in the same order. This suggests a benefit to Italian alder survival not only from compost but also from a compost-earthworm interaction, although these survival data were not significantly higher. Norway maple survival rates did not appear to benefit from compost or earthworm presence, however this may instead reflect the effect of hostile soil and climatic conditions within 6 months of planting. The summer of 2013 was recorded as drier and hotter than average, with a prolonged heatwave throughout July (Met Office, 2016). Visual assessment of trees and soil during that period indicated severe soil drought conditions and high levels of Norway maple mortality. Italian alder demonstrated greater drought tolerance than Norway maple, with >90% survival after the first year. Initial site and climatic conditions have been demonstrated to have a greater influence than GCW application on tree survival on reclaimed sites, and the first few years following planting are most crucial to the long-term survival of trees (Foot *et al.*, 2003).

Italian alder survival in the compost-only treatment was 93%, which was much higher than the maximum of 74% (minimum of 11%) recorded on reclaimed landfill by Foot *et al.* (2003) for Italian alder under similar treatment and soil conditions. At Ingrebourne Hill, Norway maple under the compost-only treatment performed poorly (41% survival) compared with the 57% to 83% survival demonstrated by sycamore in the Foot *et al.* (2003) study. These results suggest that sycamore may be better suited than Norway maple to the soil conditions on reclaimed landfill. The presence of earthworms in the compost-only plot of the Ingrebourne Hill experiment may explain the comparatively greater survival of Italian alder, although as earthworm populations were not assessed by Foot *et al.* (2003) this cannot be compared. Moffat *et al.* (2008) reported common alder (*Alnus glutinosa*) survival rates of 75% after 10 growing seasons on a clay-capped landfill, following inoculation with the earthworm species *A. longa* and *A. chlorotica*, and one-time surface application of a CGW mulch mat. They reported 50% survival of sycamore maple under the same conditions after 3 growing seasons, and 25% survival of Norway maple on uncultivated (but CGW mulched) soil after 15 growing seasons on the same experimental site (Butt *et al.*, 1999; Moffat *et al.*, 2008). Again, these suggest that Norway maple may be poorly suited to the conditions commonly present on reclaimed landfill, particularly compared to sycamore maple.

Italian alder clearly outperformed Norway maple in height, and demonstrated higher early growth rates. In a comparable field experiment, Foot *et al.* (2003) found that, under a similar application rate of CGW, Italian alder (*Alnus cordata*) significantly outperformed sycamore (*Acer psuedoplatanus*), and likewise showed a much greater early growth rate. Significantly greater Italian alder height was found under the treatments including compost, however this was not the case for Norway maple, which showed no relationship between height and treatment. These findings are in keeping with those of Foot *et al.* (2003), who found that greatest Italian alder height was recorded under incorporation to 0.6 m depth of CGW at the same application rate used in this current study; although unlike in the Ingrebourne Hill experiment they did not find this relationship to be statistically significant. The improvement in alder growth through CGW addition was attributed to the encouragement of an open-structure in the soil, thus enabling deeper root penetration and subsequently greater opportunity for nitrogen fixation (Foot *et al.*, 2003). This was considered to be more likely than the CGW conveying direct nutrient benefits to alder, which are not N-limited, unlike Norway maple. On a capped landfill, Moffat *et al.* (2008) found that inoculation of *A. chlorotica* and *A. longa* led to a mean height of 2.09 m for common alder after ten growing seasons. Although the natural growth rates for the two alder species are likely to differ, it is worth noting that Italian alder under the combination and CGW-only treatments in the Ingrebourne Hill study almost reached this height after only two years.

Although Norway maple showed no height benefit from CGW addition in this study, Foot *et al.* (2003) found sycamore height to be significantly greater with CGW application, and suggested the greater response of this species to CGW was due to nitrogen being a limiting factor for sycamore. The slow growth of Norway maple may, like the low survival rate, be an artefact of wider negative environmental influences including the hostile summer conditions experienced during the first year. It may be the case that the CGW application rate used in this study provided insufficient soil organic matter to retain a soil moisture content sufficient for the newly planted Norway maple trees to survive and grow normally. Moffat *et al.* (2008) recorded a mean Norway maple height of 0.79 m following 15 growing seasons on an uncultivated landfill cap receiving CGW mulch treatment, though the clayey soil onsite was likely devoid of earthworms. Under the same conditions, they recorded a mean sycamore height of 0.96 m and 0.67 m on cultivated and uncultivated soils respectively. The Norway maple in the Ingrebourne Hill experiment exceeded all of these mean heights after 2 years, although it is not possible to state whether this is due to the improved soil conditions at Ingrebourne Hill, increased earthworm activity or a combination of both.

Healthy trees are expected to increase in height with each growth season, however a common occurrence in this type of experiment is dieback of the main shoots in the first year of planting, followed by growth of a new shoot from the main stem in the second year (Foot *et al.*, 2003; Moffat *et al.*, 2008). It was not specified in these two studies whether this was recorded for the maple or alder species, however in the Ingrebourne Hill experiment this occurrence was almost exclusively associated with Norway maple (Figure 5.14). As recognised by Moffat *et al.* (2008), the trees affected by this are less likely to demonstrate normal height increases in the short-term. Foot *et al.* (2003) included the height measurements of affected trees in their calculations of tree height performance according to CGW application rates. However, this resulted in average height reductions in some cases, which may not be a result of the treatment applications but instead individual tree physiological responses to other environmental conditions, e.g. drought stress. In the Ingrebourne Hill experiment these trees were therefore omitted from height analysis in the interest of clearly identifying tree height responses to the treatments employed. It was expected that basal diameter growth data would reflect any effect of treatment on the die-back and re-growth of damaged trees. Whilst not ideal from the perspective of capturing effect of treatment on height data, this tendency to dieback and re-grow from basal sections indicates that Norway maple would be a suitable species for short rotation coppice - one of the interests for this species from site managers at Ingrebourne Hill and other sites. Similarly, the height data from replacement trees in the first year ('beat ups') was not included, as these did not yield comparable temporal data to the original trees in the study.

Figure 5.14. An example of Norway maple dieback, followed by growth of a new shoot from the main stem.

Few studies were found which investigated basal diameter as a measure of tree growth in response to soil treatments (Avendaño-Yáñez *et al.*, 2014; Rajapaksha *et al.*, 2014). In a mesocosm experiment investigating the effect of inoculated *L. terrestris* and *A. chlorotica* on eucalyptus and birch growth, Rajapaksha *et al.* (2014) found that earthworm activity showed no influence on basal stem diameter of both tree species. In the Ingrebourne Hill experiment presented in this chapter, basal diameter data mirrored the trends found in height data, with significantly greater basal diameter of Italian alder in treatments containing CGW. Norway maple displayed no significant effect of treatment on basal diameter, however the data reflected the same growth trends as the height data, indicating that this may be a successful surrogate measure of tree growth in the absence of height data due to stem dieback.

5.4.3. The effects of compost addition and earthworm activity on soil quality

An influence of CGW addition and earthworm activity was found on soil carbon and nutrient levels, with significantly greater soil organic carbon and organic matter content (%) in soils receiving the combination treatment. Since there was slightly higher earthworm density under this treatment, this may be attributed in part to increased accumulation of leaf litter alongside the CGW in these plots through earthworm activity (Lowe and Butt, 2003). Earthworm activity has been previously demonstrated to increase soil carbon content, for example Welke and Parkinson (2003) found that lower horizon mineral soil contained significantly higher organic matter content in the presence of earthworms. In a mesocosm experiment, Rajapaksha *et al.* (2014) identified an effect of earthworm activity on carbon content in bulk soil at 0.2–0.4 m depth under birch. Whilst CGW application to soil forming materials increases soil organic matter (SOM) content, SOM tends to quickly decline after application through utilisation by soil fauna (Gregory and Vickers, 2003; Lowe and Butt, 2004). In the case of the current study, SOM and soil organic carbon both remained relatively constant across all treatments, and then increased after 18 months. This may be due to the experimental trees reaching sufficient age at this point to begin contributing leaf litter input to the soil. CGW has been demonstrated to support earthworm populations for up to four years, however these crashed after this time, possibly due the absence of litter input following low tree survival (Butt *et al.*, 2004). CGW addition may therefore serve as a suitable source of organic matter to sustain soil faunal populations on reclaimed sites in the initial period when trees are still becoming established, which is normally considered to be a 3-5 year period (Dobson and Moffat, 1995; Bending and Moffat, 1997).

Nitrogen is the nutrient most often deficient in reclaimed soils (Bending *et al.*, 1999). Total N followed a similar pattern to soil carbon, remaining steady across all treatments until 24 months, when it began to increase (and was significantly higher in compost-receiving plots). A longer experiment timeframe could help identify whether this was the start of a trend or an anomaly at this time point. This suggests that CGW was the primary source of soil total N until leaf litter inputs to soil increased after this time. Final total N was significantly higher in the two treatments containing CGW, and since earthworm density was similar under these, it cannot be confirmed whether this is due to the compost alone or an interactive CGW-earthworm effect. There was no effect of treatment on inorganic NH_4^+ in this study, however Bohlen and Edwards (1995) found that earthworms increased the amounts of extractable N and NH_4^+ from manure and legume organic waste treatments. The influence of tree species on soil chemistry could not be distinguished in the Ingrebourne Hill experiment, as soil collected from both tree species planting blocks were bulked per plot. It may be expected that soil N levels were raised by the presence of the N-fixing alder species. However, Moffat (2000) observed that it may take up to five years before alder can accumulate sufficient N in soils to improve surrounding tree growth on reclaimed land.

Typically, levels of other essential plant nutrients in reclaimed soils vary widely depending on the nature of the source materials (Bending *et al.*, 1999). Irrespective of treatment, levels of soil K, P and Mg were all higher than their minimum standard ADAS index values required for woodland establishment (Forest Research, 2015). This indicates that these should not have been limiting to plant growth. Compost addition was shown to raise initial soil K levels, however after 24 months there were similar K levels across all treatments. The initially high levels of K may be explained by the tendency for K to be rapidly released during CGW decomposition (Foot *et al*., 2003). However, at the application rate used in this study, Foot *et al*. (2003) found that K was still released in sufficient amounts to support tree growth up to four years after application.

5.4.4. Summary of chapter findings

The findings of this research were as follows, summarised against the original chapter objectives:

1. Italian alder is generally well suited to planting on reclaimed soils, and showed significantly greater growth and highest survival when soil biological and chemical quality was improved through CGW addition in the presence of earthworms. No benefits from earthworm activity and compost addition was observed on Norway maple growth or survival, most likely due to negative impacts of severe soil drought conditions during the first few months after tree planting.
2. Earthworm density and species richness was similar across all treatments, although highest under the compost only and combination treatments. Inoculation of *A. longa* did not significantly affect the density of this species after 30 months, most likely due to a high mortality rate following inoculation. Earthworm densities were also similar between tree species, irrespective of soil treatment.
3. CGW addition and earthworm activity was found to significantly increase soil organic carbon, organic matter and available nutrient levels.

6. A NURSERY INVESTIGATION INTO RECLAIMED SOIL, COMPOSTED GREENWASTE, TREE AND EARTHWORM INTERACTIONS

6.1. Introduction and objectives

Research indicates that on restored sites, the addition of organic matter to soil may be important for establishing sustainable earthworm populations and supporting tree growth and survival (Lowe and Butt, 2002b, 2004; Foot *et al.*, 2003; Moffat *et al.*, 2008). It has been shown that certain earthworm species will actively incorporate and mix organic waste materials into soils, enhancing mineralisation and benefiting soil fertility (Piearce and Boone, 1998). The addition of earthworms may therefore be an effective way of enhancing the benefits for utilisation of organic wastes, such as composted greenwaste (CGW), by vegetation and soil fauna and flora during land regeneration. As identified during the review of literature (Chapter 2), further research was needed to investigate the effects on soil quality and tree growth of organic waste incorporation into the soil profile, for processing in the rhizosphere by soil-dwelling earthworms.

The nursery experiment described in this chapter was designed to complement the field experiment of Chapter 5, to allow a more detailed investigation into the factors affecting tree growth and nutrient uptake, soil nutrient cycling and earthworm population dynamics in the field. The experimental design adopted in this chapter is based upon that of Rajapaksha *et al.* (2014), who set up a field-based mesocosm experiment to investigate the effects of a combination of *Lumbricus terrestris* and *Allolobophora chlorotica* on two short-rotation forestry (SRF) tree species. One-year old birch (*Betula pendula*) and eucalyptus (*Eucalyptus nitens*) seedlings were grown in tubes containing de-faunated Kettering loam soil, with half of the tubes receiving the combined earthworm inoculate, and half as earthworm-free controls. Organic matter was added to the soil surface in all tubes, in the form of leaves of the host plant. Eucalyptus demonstrated a 25% increase in total biomass and a 27% increase in foliar nitrogen concentration in earthworm-containing soils compared with controls, however no significant earthworm effect was found for foliar nutrient content or biomass of birch. Rajapaksha *et al.* (2014) concluded that whilst there was evidence for a beneficial earthworm-tree interaction, further study was required to investigate the interaction effects between different earthworm species and SRF tree species.

Under controlled field conditions, the objectives of this study were to:

1. measure the effect of composted green waste addition and earthworm inoculation, or their interactions, on tree growth and nutrient uptake in reclaimed soil,
2. investigate the effects of composted green waste addition and tree species, or their interactions, on earthworm population density in reclaimed soil,
3. investigate the effects of composted green waste application, tree species, and earthworm addition, or their interactions, on reclaimed soil carbon and plant macro-nutrient status.

6.2. Materials and methods

6.2.1. Study site and experimental design

The location of the experiment was Forest Research's Headley Nursery Enclosure, Hampshire, detailed in section 3.2.3. This study utilised a planting-tube mesocosm technique, similar to that employed by Rajapaksha *et al.* (2014). The experimental planting tubes consisted of 0.25 m diameter, 3 mm thick PVC tubes cut into 0.60 m length pieces. The base of each tube was covered with fine mesh (1 mm, supplied by Amari Plastics) to prevent earthworm ingress/egress. Earthworms were further confined inside the open-top mesocosms through the application of two unbroken strips of adhesive plastic hook ('velcro') tape applied to the inside of the tubes, following the design of Lubbers and van Groenigen (2013). This study relied on this method and did not employ the addition of a mesh to the top of the tubes, as it was desired not to present a physical barrier to tree growth, or to limit litter addition to the soil which would have made this study unrepresentative of field conditions. Tubes were buried in the ground to 0.4 m depth, with 0.2 m protruding above ground level. This technique allows removal of whole soil/root system from the tube at the termination of the experiment and permits detailed examination for each desired soil depth. This methodology has been successfully used for tree root experiments (Bending and Moffat, 1997) and tree growth/earthworm interaction experiments (Rajapaksha *et al.*, 2014). Each tube was filled to 0.4 m depth with a soil treatment, and a single one-year-old root-trainer seedling of either Norway maple or Italian alder (n = 20 per species) was planted in the middle of each tube. Surveying of all tubes was undertaken weekly during autumn, with the number of leaves on the soil surface recorded. The experiment started in mid-June 2014 and ran to early July 2015 (i.e. just over 12 months).

Figure 6.1 shows the layout of tubes within the experimental plot. The setup consisted of five blocks, each containing a randomised placement of 9 planting tubes (4 treatments X 2 tree species, and 1 soil-only control). Each block was separated by a 3 m buffer zone, and within blocks each planting tube was separated by 1.5 m. This arrangement facilitated good experimental design, although the wider experimental plot itself was homogenous and each planting tube was separated from the surrounding soil. As such, each tube acted as an individual experimental unit (e.g. replicate), irrelevant of location on site. The perimeter of the experiment location was surrounded by an electrified rabbit-proof fence to prevent damage to trees by small herbivorous mammals. Following tree planting, a continuous drip irrigation system was applied to each tube to maintain soil moisture level at 25-30% for optimal tree growth.

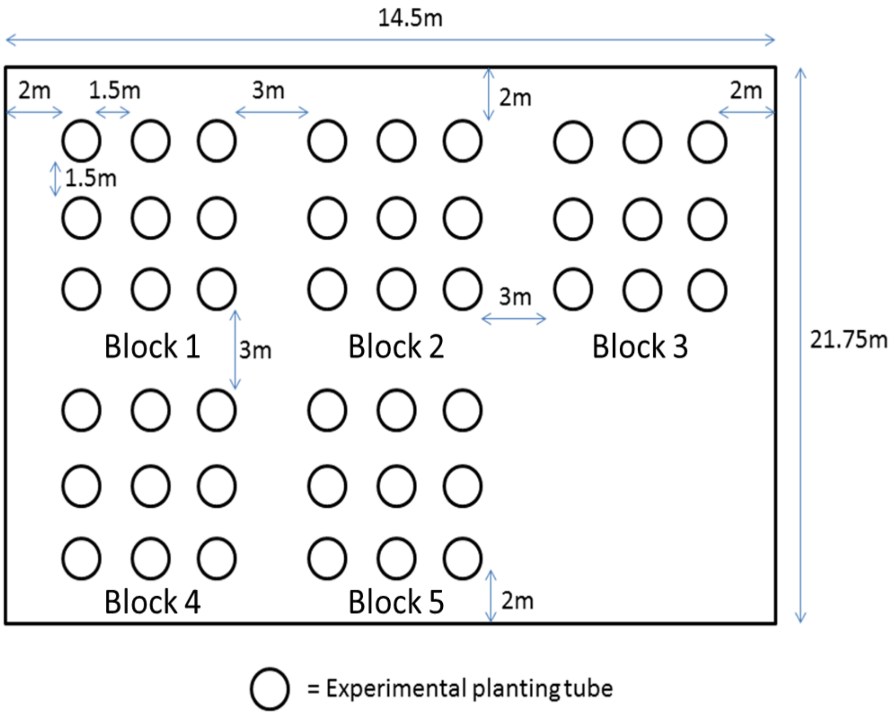


Figure 6.1. Layout of planting tubes within the experiment at Headley Nursery.

A single Eijkelkamp rhizometer (1 mm diameter, 100 mm long), was installed in each tube, within the top 0.1 m of the soil profile, to allow for soil solution samples to be taken (Figure 6.2). These were subsequently found to be unable to remove sufficient soil water samples for chemical analysis, and were replaced with larger Prenart Super Quartz soil water sampler (PTFE suction cup lysimeters, 25 mm diameter, 95 mm length). Despite soil in tubes being kept sufficiently moist through irrigation, repeated attempts to extract soil water from the rhizosphere (vacuuming sample jars to -0.007 kPa pressure, left in place for one week and repeated if necessary), failed to produce adequate water supply for analysis (perhaps due to high clay fraction in this soil leading to high water retention) and this method of sampling was abandoned.

Figure 6.2. Inspection of in-situ soil water samplers. In the foreground the drip-irrigation system is visible, as are the white velcro strips within the experimental tubes to prevent earthworm escape.

6.2.2. Experimental treatments

This experiment used the same treatment selection as the field experiment described in Chapter 5. This meant that half of the experimental tubes contained reclaimed soils blended with composted green waste (CGW); half had no CGW added and remained as controls. Furthermore, half of the tubes received an earthworm inoculation treatment, and half of the tubes were kept as a control. This was repeated for both tree species, meaning that each block contained a representative of both tree species in all four treatment combinations. Each block had a tree-free control tube, which contained de-faunated reclaimed soil only, to account for the effect of tree species alone on soil parameters. Each of the nine tree-treatment combinations had five replicates, and there was a total of 45 tubes in this experiment.

Fresh reclaimed soil was removed from close to the location of the field experiment at Ingrebourne Hill Community Woodland (see Chapter 5), and de-faunated in bulk by placing the soil into 30 litre sealed plastic containers, and stored at -5^{o}C for 7 days to destroy native earthworms and other potential competitors/predators (Butt, 2011b). Soil was then allowed to thaw before being fully homogenised using a cement-mixer (Figure 6.3a). The cement mixer was cleaned thoroughly before use, and some disposable soil run though the mixer first to collect any contaminants. The homogenised soil was then placed into clean tonne soil bags ready for addition to the experimental tubes. The volume of soil added to tubes was measured (figure 6.3b) to replicate the mean bulk density observed at the control plots in the Ingrebourne Hill field experiment (Chapter 5), which was 1.055 g cm^{-3}. Therefore, to achieve the same bulk density in the 19.63 litre volume (to 0.4 m depth for each planting tube):

$$19{,}623\ (\text{cm}^3) \times 1.055\ (\text{g cm}^{-3}) = 20{,}704\ \text{g (dry soil)}$$

Accounting for the moisture content of the de-faunated field soil (12.5 %): 20,704 / 12.5 = 2,588 g

20,704 + 2,588 = <u>23,292 g wet soil (23.3 kg) per 19.62 litres (i.e. per tube)</u>

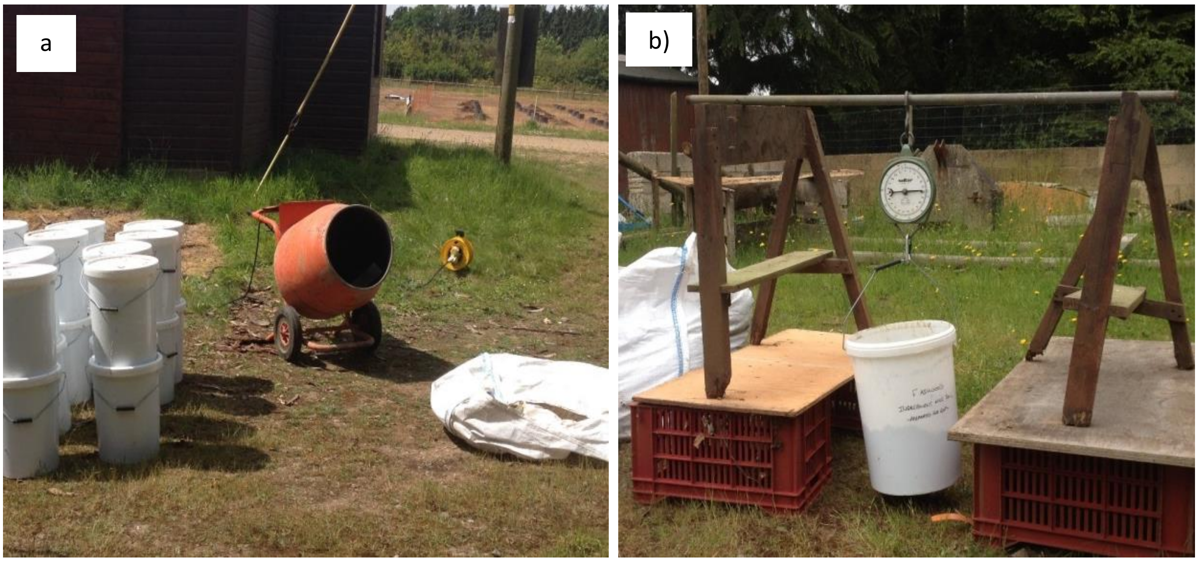


Figure 6.3. Preparation of soils for the experiment tubes; a) Homogenisation of the de-faunated reclaimed soil using a cement mixer, b) mass determination of soil to ensure the target bulk density was achieved per tube.

For CGW-treated tubes, the soil had CGW incorporated manually by loose-tipping into the planting tube during soil placement at an amendment rate equivalent to 500 kg Total N ha^{-1}; in keeping with the amendment rate used at the Ingrebourne Hill experiment, and following guidance by Taylor (1991) and Bending *et al*. (1999). The amount of CGW applied per tube was calculated as follows: in accordance with limits set by the Nitrates Directive (91/676/EEC), each plot within the Ingrebourne Hill field experiment received 0.8 t CGW 50 m^{-3} (0.8 t CGW per 100 m^{-2} plot, incorporated to 0.5 m depth). Therefore, to achieve the same rate of compost addition to the 19.63 litres volume (the tube volume to 0.4 m depth) in each of the planting tubes in this experiment:

$$0.8 \text{ t} = 800000 \text{ g}, 50 \text{ m}^3 = 50000000 \text{ ml}$$

$$800000 / 50000000 = 0.0016 \text{ g CGW per 1 ml of soil}$$

$$0.0016 \times 19{,}630 = \underline{31.4 \text{ g CGW per 19.62 litres (per 0.4 m tube)}}$$

Soils were allowed to settle in the tubes for one week prior to tree planting. The tree species used in this experiment were Norway maple (*Acer platanoides*) and Italian alder (*Alnus cordata*), selected for the criteria provided in section 3.3 and repeating the species selection for the field experiment described in Chapter 5. One-year-old root-trainer seedlings (the standard age for trees planted in the field) were obtained from the same nursery as used for the field experiment at Ingrebourne Hill (Chapter 5). Trees were given 2 weeks to equilibrate in the tubes before earthworm introduction.

This experiment investigated two earthworm species: *A. longa* (anecic) and *A. chlorotica* (endogeic). All earthworms were collected from agricultural pasture at Walton Hall Farm, Preston, UK (Nat. Grid Ref: SD 55050 28100), via digging and hand-sorting of soil, then transferred and stored in fresh soil collected from Ingrebourne Hill, before transport to the Nursery experiment. For tubes receiving an earthworm inoculation treatment (n=20), earthworms were introduced as a mixed culture of *A. longa* (n=5) and *A. chlorotica* (n=10). These numbers were based on recorded field densities at the Ingrebourne Hill field experiment following inoculation with *A. longa* (Chapter 5), and are in keeping with the numbers used in a similar experiment by Rajapaksha *et al*. (2014), of 5 *L. terrestris* and 10 *A.*

chlorotica per tube. One of the primary reasons for selecting *A. chlorotica* alongside *A. longa* was because this species is closely associated with the root systems of plants and is therefore useful for assessing root-earthworm interactions (Rajapaksha *et al.*, 2014). Furthermore, little research has been conducted into identifying potential synergistic effects of inoculations using a combination of earthworm ecological groupings. The *A. chlorotica* used in this experiment were of mixed pink and green morphs (Lowe and Butt, 2008), however all were selected to be of similar biomass, and morph was not considered to be a limiting factor as reproductive output was not one of the measurements.

6.2.3. Experimental sampling

At the termination of the experiment after 12 months, the tubes were carefully removed from the ground, ensuring that the fine mesh was still covering the base and keeping the experimental mesocosm intact. The tubes (still containing soils and trees) were then transported to an on-site workshop, where they were processed. Each tube was cut in half along the vertical axis using a portable circular saw to allow access to the undisturbed soil column inside (Figure 6.4a). The tree height and ground-line diameter was recorded, and then the above-ground section of the tree was removed by severing the main stem at the ground-line. This was then processed into three sub-samples for analysis; these were main stem, branches and leaves. The soil column and plant roots were subsequently divided in two along the horizontal axis, into the upper and lower sections (0 to 0.2 and 0.2 to 0.4 m, respectively, see figure 6.4b). Firstly, earthworms were hand-sorted from the soil in each section and numbers recorded. These were collected in 4% formaldehyde solution and later identified in the laboratory following the key of Sims and Gerard (1999).

Figure 6.4. Destructive sampling of soil columns at termination of the experiment; a) a Norway maple tube, cut with a circular saw to reveal the soil column and tree root system, b) a soil column separated into the upper (0-0.2 m) and lower (0.2 – 0.4 m) sections.

The upper and lower soil sections were then divided into bulk and rhizosphere (root attached) soil. Roots were removed, and one sample of bulk soil was collected from both the upper (0-0.2 m) and lower (0.2-0.4 m) soil sections. Rhizosphere soil was obtained by shaking the roots from each section inside a clean plastic sample bag. Root samples were then divided into the two sub-categories of main root (stump and roots larger than 2 mm diameter), and fine roots (<2 mm diameter) from upper and lower soil sections. Before chemical analysis, all root samples were jet-washed through a fine sieve (0.5 mm) to remove attached soil. Plant and soil samples were processed at Forest Research Laboratory Services at Alice Holt Lodge, Farnham, UK. A random sample of 100 leaves was taken from each tree to have Specific Leaf Area (SLA) ($cm^2\,g^{-1}$) per dry weight calculated, following the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests standard methodology described by Pitman *et al.* (2010). This was conducted using a Delta-T Area Meter (MK2) linked via video camera to a Delta-T Conveyor Belt Unit Area Measurement System (Delta-T devices, Cambridge, England). All plant material was then oven-dried at 70°C for 48 h, then the dry biomass for each plant section recorded, and samples analysed chemically. Plant and soil samples had total C and N determined using a CN Elemental Analyser (Carlo Erba (THERMO), FLASH EA 1112 Series), and major elements (P, K, Ca and Mg) analysed after sulphuric acid digestion and inductively coupled plasma-optical emission spectrophotometry (ICP-OES) analysis, soil moisture content analysed by oven drying at 105°C for 24 hours, and soil pH was measured in water suspension. The 1M KCL-extraction method was used (following the procedure described in MAFF, 1986) on fresh soil to provide filtered samples for determining levels of inorganic “available” nitrogen, e.g. NO_3^- and NH_4^+ by colorimeter analysis by Rothamstead Research laboratory services.

6.2.4. Measurements and statistical analysis

Earthworm species density and population change were measured to identify the effects of tree species and soil treatment on earthworms. The effect of soil treatment and/or earthworm activity on tree growth and health were measured via data on tree survival, tree nutrient status, SLA, ground-line diameter, and tree biomass data above and below-ground. Bulk and rhizosphere soil samples were chemically analysed (total C and N, major elements, soil moisture content, pH) to investigate the effect of tree, CGW addition and earthworm activity on soil quality. Data were first tested for normality using the Shapiro-Wilk test, which is suited to the sample size in this experiment (n=5). As all data for each species and treatment had a normal distribution, the data were analysed using one and two-way analysis of variance (ANOVA), with the Tukey-Kramer post-hoc multiple comparison test applied to significant treatment interactions. Baseline soil analysis were performed using a 2 sample student’s T-test. Statistical analysis was performed using the statistical software GenStat (Release 16.2). These statistical models were applied to data on tree (above and below-ground) biomass, height and ground-line diameter, tree nutrient status, earthworm population density and species richness, and soil chemical parameters, as described above.

6.3. Results

6.3.1. The effects of tree and soil treatments on earthworm populations

At termination of the experiment after 12 months, there was significantly higher *A. chlorotica* density in tubes containing Norway maple with compost treatment than all other treatments and tree species tubes in the experiment (ANOVA, df = 2, p = 0.002). There were no significant differences in *A. longa* density between tree species or treatments (Table 6.1). Under Italian alder, *A. chlorotica* experienced an equal reduction in population density of 78% for both treatments, a reduction of 82% under Norway maple without compost, and a density reduction of 50% under Norway maple with compost. Comparatively, *A. longa* experienced higher survival rates, with an average reduction in final population density of 24% and 8% under Italian alder with and without compost, respectively. Under Norway maple, *A. longa* density was reduced by 36% and 32% under compost and earthworm only treatment, respectively.

Table 6.1. Mean (±SE) earthworm density per tube containing experimental tree species and soil treatments.

Earthworm species	Baseline density	Italian alder		Norway maple	
		Earthworm only	Earthworm and compost	Earthworm only	Earthworm and compost
A. longa	5	4.6 ± 0.25 ^{a}	3.8 ± 0.59 ^{a}	3.4 ± 0.51 ^{a}	3.2 ± 0.59 ^{a}
A. chlorotica	10	2.2 ± 0.73 ^{a}	2.2 ± 0.66 ^{a}	1.8 ± 0.37 ^{a}	5.0 ± 0.63 ^{b*}

Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * p <0.05

At termination of the experiment, some of the earthworm-free control tubes were found to contain low numbers of *A. longa* and *A. chlorotica*. The mean population density of *A. longa* in control tubes was 0.5 (± 0.3) under Italian alder, and 0.7 (± 0.3) under Norway maple. *A. chlorotica* mean density in control tubes was 0.6 (± 0.4) and 0.2 (± 0.1) for Italian alder and Norway maple, respectively. No other earthworm species were found in the earthworm-control tubes in the experiment, and no earthworms were recovered in the tree-free control tubes. A total of 3 individuals of *L. rubellus* were found across two tubes (containing compost plus earthworm treatment) during sampling, representing a mean density (±SE) of 0.03 (± 0.07) per tube.

6.3.2. The effects of earthworm activity and compost addition on tree survival, growth, biomass and nutrient status

Both tree species achieved 100% survival across all treatments. Italian alder demonstrated markedly greater growth compared with Norway maple throughout the experiment, across all treatments. At the start of the experiment there was no significant difference in individual height or diameter of trees between treatments, for both tree species. At the termination of the experiment, no effect was found under the compost or earthworm treatments, or combination of the two, on Italian alder height and ground-line diameter (Figures 6.5 and 6.6). Norway maple displayed significantly greater height (ANOVA, df= 4, p <0.05), but not diameter, under the earthworm plus compost treatment than the control group for this species (Figure 6.6).

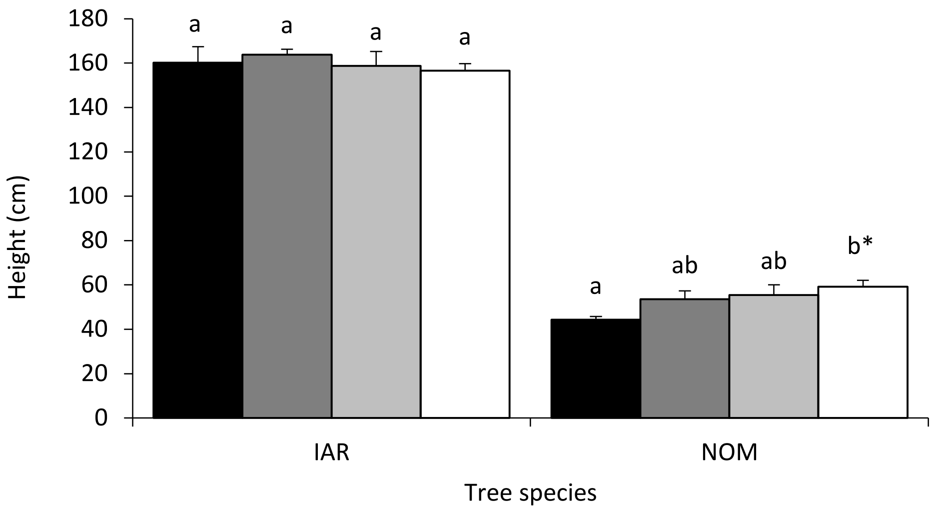


Figure 6.5. Mean (± SE) height (cm) of Italian alder (IAR) and Norway maple (NOM) after 12 months under experimental treatments: Control (■), Earthworm only (■), compost only (□), Compost plus earthworm (□).

Table 6.2. Mean (± SE) initial and final tree height (cm) and percent change after 12 months.

Tree species		Treatment			
		Control	Earthworm only	Compost only	Earthworm and compost
Italian alder	Initial	82.10 ± 10.99	96.60 ± 4.35	90.34 ± 3.59	87.82 ± 3.96
	Final	160.18 ± 7.23	163.82 ± 2.41	158.78 ± 6.50	156.6 ± 3.12
	Change (%)	+48.75	+41.03	+43.10	+43.92
Norway maple	Initial	44.00 ± 1.42	48.80 ± 2.98	53.16 ± 4.33	41.94 ± 1.81
	Final	44.26 ± 1.51[a]	53.54 ± 3.74[ab]	55.44 ± 4.55[ab]	59.20 ± 2.81[b*]
	Change (%)	+0.59	+8.85	+4.11	+29.16

Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * p <0.05

Ground-line diameter was also highest under the earthworm and compost combination treatment for Norway maple, although this was not statistically significant. Full initial and final growth data for both species is provided in Tables 6.2 and 6.3.

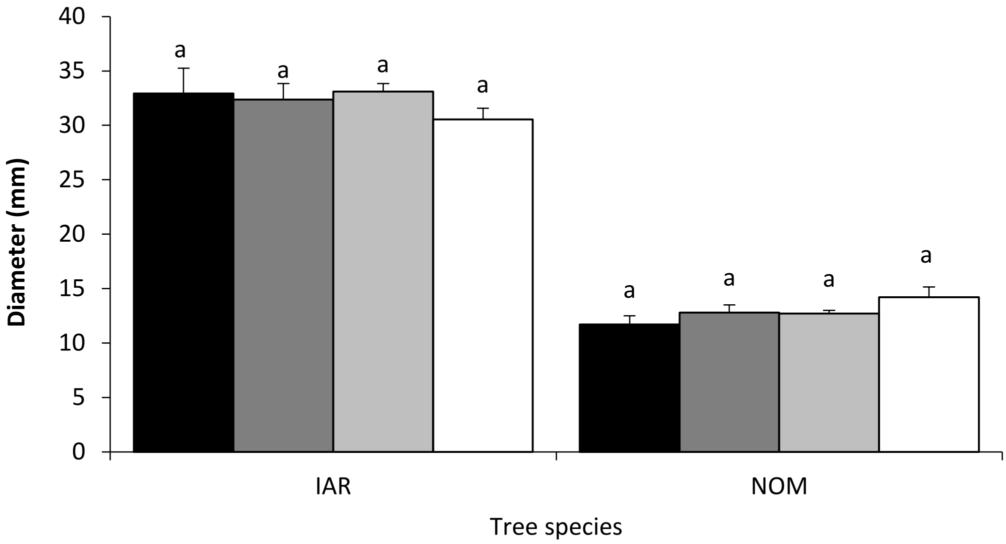


Figure 6.6. Mean (± SE) diameter (mm) of Italian alder (IAR) and Norway maple (NOM) after 12 months under experimental treatments: Control (■), Earthworm only (■), compost only (□), Compost plus earthworm (□)

Table 6.3. Mean (± SE) initial and final tree diameter (mm) and percent change.

Tree species		Treatment			
		Control	Earthworm only	Compost only	Earthworm and compost
Italian alder	Initial	4.18 ± 0.54	5.54 ± 0.61	5.73 ± 0.32	5.14 ± 0.34
	Final	32.92 ± 2.32	32.36 ± 1.49	33.11 ± 0.74	30.55 ± 1.03
	Change (%)	+87.30	+82.88	+82.69	+83.18
Norway maple	Initial	5.43 ± 0.58	5.51 ± 0.26	5.1 ± 0.43	4.74 ± 0.47
	Final	11.7 ± 0.80	12.81 ± 0.69	12.71 ± 0.29	14.22 ± 0.94
	Change (%)	+53.59	+56.99	+59.87	+66.67

No significant effects of treatments were found on biomass for either tree species (Table 6.4). However, Norway maple total above and below ground mean biomass was notably greater under the combined earthworm and compost treatment, compared with all other treatments. Italian alder total biomass was greatest under the compost-only treatment, and lowest under the combined compost and earthworm treatment.

Table 6.4. Mean (± SE) Italian alder and Norway maple above and below-ground biomass (g) after 12 months in different experimental treatments.

	Italian alder				Norway maple			
Tree section	Control	Earthworm only	Compost only	Earthworm and compost	Control	Earthworm only	Compost only	Earthworm and compost
Branch	97.36 ± 9.03	75.24 ± 9.56	75.78 ± 6.89	69.25 ± 6.86	2.59 ± 0.85	2.22 ± 0.22	1.61 ± 0.29	4.08 ± 1.12
Leaves	N/A	N/A	N/A	N/A	6.01 ± 1.18	6.43 ± 0.83	6.32 ± 0.90	9.07 ± 2.18
Stem	112.23 ± 9.76	121.21 ± 7.94	138.9 ± 9.95	109.87 ± 6.36	7.84 ± 1.12	10.17 ± 1.60	10.12 ± 1.18	12.56 ± 2.02
Total above	209.59 ± 17.32	196.45 ± 15.39	214.68 ± 14.28	179.12 ± 11.39	16.44 ± 3.10	18.82 ± 2.34	18.05 ± 2.25	25.72 ± 4.91
Fine root 0.2	8.26 ± 1.13	12.48 ± 2.66	13.03 ± 1.65	8.04 ± 0.34	4.08 ± 1.23	3.91 ± 1.11	4.08 ± 0.58	4.03 ± 0.83
Fine root 0.4	17.30 ± 2.78	17.60 ± 3.13	15.76 ± 2.44	14.55 ± 2.26	4.70 ± 1.41	5.07 ± 1.01	5.20 ± 0.84	5.40 ± 0.71
Main root	68.37 ± 9.61	60.90 ± 5.84	74.56 ± 6.92	57.82 ± 6.97	9.03 ± 1.61	9.65 ± 1.23	10.06 ± 1.43	15.06 ± 4.30
Total below	93.92 ± 11.67	90.98 ± 9.11	103.36 ± 8.04	80.41 ± 7.74	17.82 ± 4.13	18.63 ± 2.53	19.34 ± 2.19	24.49 ± 4.99
Total tree	303.51 ± 28.44	287.43 ± 24.50	318.03 ± 18.49	259.53 ± 13.66	34.25 ± 7.17	37.45 ± 4.84	37.39 ± 4.34	50.21 ± 9.78

Earthworm presence increased the C content of Italian alder leaves in both the earthworm only and earthworm and compost treatment, compared with the earthworm free control group (ANOVA, df = 4, p 0.001) (Table 6.6). Italian alder fine root (0 - 0.2 m soil) Ca levels were significantly higher (ANOVA, df= 4, p <0.05) in the earthworm-only treatment than the compost-only treatment. Earthworm and/or compost treatments showed no other significant effects on the C or macro nutrient content of either tree species. Average C: N ratio for Italian alder and Norway maple leaves was 19.4 and 37.5, respectively. Repeated surveying during autumn revealed a number of leaves in all tubes (Table 6.5), however, no leaves were present from a different tree species to the tube's experimentally assigned tree. There was no treatment effect (p >0.05) on the Specific Leaf Area measurements of either tree species in the experiment, as shown in Table 6.7.

Table 6.5. Mean (± SE) leaf input to experimental tubes (number of leaves and leaf biomass).

Tree species	Parameter	Treatment			
		Control	Earthworm only	Compost only	Earthworm and compost
Italian alder	Number of leaves	24.20 ± 2.29b**	10.80 ± 1.74a	19.00 ± 3.77ab	9.60 ± 2.14a
	Leaf biomass	543.23 ± 51.40b**	242.43 ± 39.06a	426.50 ± 84.63ab	215.50 ± 48.04a
Norway maple	Number of leaves	8.80 ± 1.93	7.20 ± 3.73	5.40 ± 1.60	8.40 ± 3.59
	Leaf biomass	247.21 ± 54.22	202.26 ± 104.78	151.69 ± 44.95	235.97 ± 100.85

Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * p <0.05 **, p <0.01, *** p <0.001.

Table 6.6. Mean (± SE) chemical content (%) of tree sections of Italian alder and Norway maple after 12 months in tubes containing different experimental treatments.

Tree section	Element	Italian alder				Norway maple			
		Control	EW only	Compost only	EW and compost	Control	EW only	Compost only	EW and compost
Branch	N	1.04 ± 0.06	0.96 ± 0.06	1.08 ± 0.07	0.98 ± 0.04	0.43 ± 0.03	0.44 ± 0.02	0.45 ± 0.02	0.46 ± 0.02
	C	50.64 ± 0.23	50.68 ± 0.24	50.94 ± 0.20	50.38 ± 0.22	48.33 ± 0.22	48.15 ± 0.32	47.78 ± 0.25	48.24 ± 0.25
	K	0.57 ± 0.02	0.56 ± 0.04	0.61 ± 0.02	0.60 ± 0.01	0.76 ± 0.07	0.81 ± 0.08	0.90 ± 0.09	0.84 ± 0.05
	Ca	0.76 ± 0.05	0.67 ± 0.04	0.69 ± 0.05	0.64 ± 0.06	1.20 ± 0.15	1.15 ± 0.07	1.16 ± 0.07	1.08 ± 0.08
	Mg	0.08 ± 0.01	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.00	0.11 ± 0.01
	P	0.07 ± 0.00	0.08 ± 0.01	0.08 ± 0.00	0.07 ± 0.00	0.09 ± 0.00	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Stem	N	0.46 ± 0.03	0.53 ± 0.01	0.47 ± 0.02	0.51 ± 0.03	0.28 ± 0.01	0.28 ± 0.01	0.29 ± 0.01	0.28 ± 0.02
	C	49.36 ± 0.01a	50.23 ± 0.12b***	49.87 ± 0.07ab	50.24 ± 0.22b***	48.63 ± 0.16	48.92 ± 0.28	48.76 ± 0.17	48.69 ± 0.16
	K	0.28 ± 0.01	0.30 ± 0.01	0.28 ± 0.02	0.32 ± 0.02	0.35 ± 0.02	0.36 ± 0.03	0.40 ± 0.01	0.40 ± 0.03
	Ca	0.29 ± 0.06	0.41 ± 0.04	0.35 ± 0.03	0.39 ± 0.04	0.36 ± 0.03	0.36 ± 0.01	0.38 ± 0.04	0.34 ± 0.04
	Mg	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
	P	0.04 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
Leaves	N	2.83 ± 0.06	2.88 ± 0.15	2.91 ± 0.07	2.81 ± 0.06	1.20 ± 0.05	1.21 ± 0.07	1.23 ± 0.05	1.31 ± 0.06
	C	53.13 ± 0.22	52.89 ± 0.20	53.01 ± 0.09	52.9 ± 0.13	48.98 ± 0.31	48.66 ± 0.23	49.22 ± 0.30	49.11 ± 0.21
	K	0.66 ± 0.02	0.74 ± 0.06	0.73 ± 0.04	0.70 ± 0.01	0.86 ± 0.03	0.96 ± 0.09	1.03 ± 0.10	0.99 ± 0.07
	Ca	0.94 ± 0.07	0.94 ± 0.07	0.87 ± 0.07	0.99 ± 0.10	1.34 ± 0.04	1.32 ± 0.08	1.05 ± 0.09	1.16 ± 0.10
	Mg	0.13 ± 0.01	0.14 ± 0.00	0.14 ± 0.01	0.13 ± 0.01	0.20 ± 0.01	0.20 ± 0.02	0.17 ± 0.01	0.18 ± 0.02
	P	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.12 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
Fine root 0-0.2 m	N	1.39 ± 0.10	1.22 ± 0.04	1.16 ± 0.12	1.39 ± 0.14	0.73 ± 0.02	0.65 ± 0.07	0.73 ± 0.06	0.84 ± 0.18
	C	44.62 ± 1.51	44.80 ± 0.89	44.73 ± 0.62	45.93 ± 0.62	45.43 ± 0.78	44.34 ± 1.96	43.35 ± 2.85	37.43 ± 3.71
	K	0.43 ± 0.02	0.44 ± 0.02	0.47 ± 0.03	0.45 ± 0.01	0.76 ± 0.03	0.79 ± 0.03	0.77 ± 0.05	0.82 ± 0.06
	Ca	1.45 ± 0.08ab	1.69 ± 0.06b*	1.41 ± 0.08a	1.42 ± 0.05ab	1.64 ± 0.19	1.53 ± 0.08	1.50 ± 0.09	1.88 ± 0.35
	Mg	0.17 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.29 ± 0.07	0.21 ± 0.03	0.21 ± 0.02	0.25 ± 0.01
	P	0.07 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.15 ± 0.01	0.14 ± 0.02	0.15 ± 0.01	0.15 ± 0.02
Fine root 0.2-0.4 m	N	0.94 ± 0.04	1.01 ± 0.04	0.95 ± 0.04	1.03 ± 0.03	0.60 ± 0.03	0.52 ± 0.04	0.54 ± 0.03	0.53 ± 0.04
	C	44.49 ± 1.26	44.47 ± 1.25	43.06 ± 1.56	46.11 ± 0.47	34.6 ± 1.79	34.83 ± 2.73	29.61 ± 1.89	30.48 ± 1.87
	K	0.44 ± 0.02	0.47 ± 0.02	0.45 ± 0.03	0.51 ± 0.02	0.89 ± 0.05	0.87 ± 0.07	0.84 ± 0.06	0.81 ± 0.04
	Ca	1.65 ± 0.14	1.73 ± 0.15	1.65 ± 0.09	1.54 ± 0.10	1.88 ± 0.12	1.81 ± 0.14	1.84 ± 0.08	1.97 ± 0.16
	Mg	0.17 ± 0.02	0.18 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.27 ± 0.02	0.23 ± 0.01	0.27 ± 0.02	0.29 ± 0.02
	P	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.00	0.08 ± 0.00	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.02	0.15 ± 0.02
Main root	N	0.7 ± 0.05	0.80 ± 0.10	0.71 ± 0.05	0.70 ± 0.03	0.35 ± 0.02	0.34 ± 0.02	0.33 ± 0.02	0.37 ± 0.01
	C	48.42 ± 0.16	48.45 ± 0.47	48.05 ± 0.52	48.61 ± 0.24	47.53 ± 0.13	47.16 ± 0.23	47.34 ± 0.07	47.63 ± 0.35
	K	0.39 ± 0.03	0.41 ± 0.02	0.42 ± 0.02	0.39 ± 0.00	0.49 ± 0.02	0.52 ± 0.04	0.47 ± 0.02	0.54 ± 0.03
	Ca	0.71 ± 0.11	0.80 ± 0.15	0.69 ± 0.08	0.64 ± 0.06	0.39 ± 0.04	0.46 ± 0.05	0.39 ± 0.03	0.40 ± 0.03
	Mg	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.00	0.07 ± 0.00
	P	0.05 ± 0.00	0.07 ± 0.02	0.06 ± 0.01	0.05 ± 0.00	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.00	0.08 ± 0.00

Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * $p < 0.05$ **, $p < 0.01$, *** $p < 0.001$.

Table 6.7. Mean (± SE) specific leaf area (cm g^{-1}) of Italian alder and Norway maple under the given experimental treatments.

Tree species	Treatment			
	Control	Earthworm only	Compost only	Earthworm and compost
Italian alder	1.54 ± 0.04	1.43 ± 0.06	1.40 ± 0.06	1.48 ± 0.04
Norway maple	4.83 ± 0.83	3.88 ± 0.35	4.67 ± 0.66	5.75 ± 2.87

6.3.3. Soil responses to earthworm activity, compost addition and tree species

At the start of the experiment, there was little variability (CV = <10%) in soil chemical parameters between the sub-samples of compost-free soil (Table 6.8). Compost addition led to significantly higher pH, total N, C organic C, organic matter (%) and PO_4^{3-} (mg/kg). Addition of compost was associated with a significant reduction in C:N ratio, Ca (%), NO_3^- and SO_4^{2-} (mg/kg) than in baseline soils.

Table 6.8. Mean (± SE) baseline (t=0) chemical parameters of control soil and compost-amended soil.

	Treatment	
Chemical parameter	Control soil	Compost-amended soil
pH	7.93 ± 0.02a	8.02 ± 0.03b*
Cond. (µs/cm)	3043 ± 8	2814 ± 127
Total N (%)	0.11 ± 0.00a	0.14 ± 0.00b***
Total C (%)	2.93 ± 0.05a	3.30 ± 0.05b***
C (Org) (%)	2.00 ± 0.04a	2.36 ± 0.05b***
O.M. (%)	3.44 ± 0.07a	4.07 ± 0.08b***
C:N ratio	28.04 ± 0.38a	24.44 ± 0.36b***
Moisture content (%)	20.58 ± 1.00	31.10 ± 0.60
K (mg/kg)	4029 ± 151	3876 ± 21
Ca (mg/kg)	33593 ± 743a	30802 ± 718b*
Mg (mg/kg)	3652 ± 61	3647 ± 121
Na (mg/kg)	318 ± 13.40	323.90 ± 11.20
P (mg/kg)	705 ± 64.29	729.78 ± 13.40
S (mg/kg)	1717 ± 57a	1203 ± 121b**
[N(NH_4^+)] (mg/kg)	2.75 ± 0.29	2.92 ± 0.17
[N(NO_3^-)] (mg/kg)	11.46 ± 0.09	2.307 ± 1.24b***
S(SO_4^{2-}) (mg/kg)	1310 ± 54a	584 ± 77b***
P(PO_4^{3-}) (mg/kg)	29.51 ± 0.31a	39.38 ± 0.65b***

Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * p <0.05 **, p <0.01, *** p <0.001.

At termination of the experiment, soil chemistry results for bulk soil under trees in the compost-free and earthworm-free controls showed a number of effects of tree species, compared with the tree-free control soils (Table 6.9). Under Italian alder, there was significantly ($p < 0.05$) higher organic carbon (%) and organic matter (%), and significantly lower ($p < 0.001$) soil moisture content (%), and plant nutrients K, Mg, NO_3^- and PO_4^{3-} (mg/kg). Bulk soil under Norway maple was not significantly different to the control soil, except in moisture content (%), which was significantly lower than the control (ANOVA, df = 4, $p < 0.001$), but also significantly higher ($p < 0.001$) than under Italian alder.

Table 6.9. Effects of tree species on mean (± SE) bulk soil chemical parameters after 12 months.

Chemical parameter	Control (no tree)	Tree species	
		Norway maple	Italian alder
pH	9.08 ± 0.44	8.90 ± 0.08	8.78 ± 0.34
Cond. (µs/cm)	828 ± 100	1187 ± 171	1697 ± 335
Total N (%)	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
Total C (%)	2.85 ± 0.06	2.97 ± 0.05	2.97 ± 0.07
C (Org) (%)	1.72 ± 0.03a	1.79 ± 0.04a	1.86 ± 0.05b*
O.M. (%)	2.97 ± 0.06a	3.08 ± 0.06a	3.21 ± 0.08b*
C:N ratio	22.88 ± 1.04	23.19 ± 1.13	23.73 ± 1.02
Moisture content (%)	27.14 ± 0.84a	22.55 ± 1.46b***	17.01 ± 1.18c***
K (mg/kg)	123.93 ± 2.66a	111.05 ± 4.36a	87.85 ± 4.08b***
Ca (mg/kg)	2881 ± 314	2940 ± 204	3059 ± 215
Mg (mg/kg)	66.38 ± 4.97a	66.66 ± 5.27a	59.23 ± 4.46b*
Na (mg/kg)	14.85 ± 0.49	15.34 ± 0.83	16.21 ± 0.73
[N(NH_4^+)] (mg/kg)	1.06 ± 0.05	1.03 ± 0.08	0.70 ± 0.12
[N(NO_2)] (mg/kg)	0.36 ± 0.22	0.57 ± 0.35	0.26 ± 0.15
[N(NO_3^-)] (mg/kg)	0.52 ± 0.06a	0.41 ± 0.03a	0.18 ± 0.02b***
S(SO_4^{2-}) (mg/kg)	87.49 ± 13.53	209.08 ± 57.97	287.06 ± 79.67
P(PO_4^{3-}) (mg/kg)	20.38 ± 0.59a	21.11 ± 0.34a	17.36 ± 1.04b***

Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * $p < 0.05$ **, $p < 0.01$, *** $p < 0.001$.

Appendix I shows the chemical parameters of experimental Italian alder bulk soil from the upper and lower soil sections, and rhizosphere soil from the upper section. The lower soil section in these tubes did not provide sufficient root-attached soil for performance of chemical analysis. Bulk soil in the upper 0.2 m soil section, had a significantly higher (ANOVA, df = 4, $p < 0.05$) total organic carbon (%)

and organic matter (%) content in the compost-earthworm combination treatment than in the tree-free control soil upper section. Total N (%) was significantly higher ($p < 0.001$) in both the compost only and compost and earthworm combination treatments than the controls. Bulk soil C:N ratio was significantly higher in the earthworm only treatments ($p < 0.05$) than the compost-only treatment. Soil K (mg/kg) was higher ($p < 0.01$) in the tree-free control, compared with the other control and the earthworm-only treatments. In the 0.2 m rhizosphere soil, the only significant differences were in the compost-only treatment, which had the lowest C:N ratio ($p < 0.001$) and highest PO_4^{3-} (mg/kg) ($p < 0.05$). In the lower 0.2 m of soil under Italian alder, total C (%), organic C (%), organic matter (%) and total N (%) were significantly higher under compost-only and earthworm and compost combination treatments. Total N (%) was higher under the compost-only treatment ($p < 0.001$) than the earthworm-compost combination. Moisture content (%), soil K (mg/kg) and NO_3^- (mg/kg) were higher under the no-tree control (ANOVA, df = 4, $p < 0.001$). C:N ratio in the lower soil layer was significantly higher ($p < 0.01$) in compost-free tubes.

Appendix II provides the chemical parameters of Norway maple bulk soil in the upper and lower soil sections, and rhizosphere soil in the upper section. As with Italian alder, the lower soil section in these tubes did not provide sufficient root-attached soil for chemical analysis to be performed. Bulk soil in the upper 0.2 m soil section, had a significantly higher total N (%), total organic carbon (%) and organic matter (%) content in both the compost only and compost-earthworm combination treatments than the controls and earthworm-only treatments (ANOVA, df = 4, $p < 0.001$). Under the compost-only treatment, total C (%), K (mg/kg), NH_4^+ (mg/kg) and PO_4^{3-} (mg/kg) were significantly higher than control and earthworm-only tubes. As with Italian alder, the upper section bulk soil had significantly higher C:N ratio in earthworm-only treatment than the tubes receiving compost treatments. In the 0.2 m rhizosphere soil, the only significant differences were in the earthworm-compost combination treatment, which had the lowest C:N ratio ($p < 0.001$). In the lower 0.2 m of soil under Norway maple, total N (%), organic matter (%) and PO_4^{3-} (mg/kg) were significantly higher under the compost-only and earthworm-compost combination treatments. Total C (%) was higher under the compost-only treatment (p <0.01) than tree-free control. Moisture content (%), soil K (mg/kg) and NO_3^- (mg/kg) were higher under the no-tree treatment (ANOVA, df = 4, p <0.001). As with the upper 0.2 m bulk soil, the C:N ratio of the bulk lower soil section was significantly higher in the earthworm-only treatment (p <0.001). The Norway maple control tubes had higher conductivity (μs/cm) and SO_4^{2-}.

Appendix III shows the percent difference in chemical parameters of upper section rhizosphere soil for Italian alder and Norway maple. Distinct differences were observed between rhizosphere and bulk soils for both tree species. For example, rhizosphere soil showed higher moisture content, total N, total C, total organic carbon and organic matter (%), Na, Mg and NH_4^+ (mg/kg) levels than bulk soil across all treatments for both tree species. Conversely, there was a general reduction in pH, conductivity (μs/cm), total K, and anions PO_4^{3-}, NO_2^-, NO_3^- and SO_4^{2-} (mg/kg) in rhizosphere soil compared to bulk soil across all treatments for both tree species. In Norway maple rhizosphere soils, the earthworm and compost combination treatment led to the most dramatic reductions and the least pronounced increases in the chemical parameters described. There was no similar trend for Italian alder rhizosphere soils, which showed similar levels of change against bulk soil, across all treatments.

6.4. Discussion

6.4.1. The effects of tree and soil treatments on earthworm populations

Tree litter has been shown to influence soil faunal populations (Swift *et al.*, 1979), and so different tree species influence soil quality and soil faunal communities differently through the quality and quantity of their leaf litter (Pigott, 1989; Muys *et al.*, 1992; Reich *et al.*, 2005). The chemical composition of litter appears to strongly influence earthworm feeding behaviour, particularly aspects such as C:N ratio and the content of nitrogen, calcium, lignin and polyphenols (Satchell and Lowe, 1967; Hendriksen, 1990; Rajapaksha *et al.*, 2013). Additionally, leaf size and shape has been found to affect earthworm litter consumption (Satchell and Lowe, 1967). In this study, there was higher leaf biomass input to the soil in Italian alder tubes than Norway maple, and Norway maple leaves can be considered lower quality than the alder (with lower N levels, higher C:N ratio and higher specific leaf area). As noted by Rajapaksha *et al.* (2014), such differences should be expected to primarily influence anecic earthworm species such as *L. terrestris*, or in the case of this study, *A. longa.* However, there were no significant differences in *A. longa* density between tree species and treatments. Similar findings were reported for *L. terrestris* densities under birch and eucalyptus, which differed in litter quality but not in quantity (Rajapaksha *et al.*, 2014). *A. chlorotica* density was significantly higher under Norway maple, despite the lower quality of the litter material. This was surprising, since alder litter has been demonstrated to be significantly more palatable to *A. longa* and *A. chlorotica* than the litter of sycamore (another maple species) (Rajapaksha *et al.*, 2013). It was expected that leaf litter quality should influence *A. chlorotica* following incorporation of decomposing litter to the soil through the feeding behaviour of *A. longa*. It may be the case that the experimental duration was insufficient for litter to be a determining factor of earthworm populations, as the CGW blended with soil may have been sufficient to sustain the earthworms in the short term. Lowe and Butt (2004) found that surface application of CGW followed by incorporation into the soil by *A. longa* supported a stable population of *A. chlorotica* on a capped landfill site for at least four years.

Since there was little difference in litter addition between Norway maple tubes, the higher *A. chlorotica* density under this tree species may be a result of CGW addition and influences from soil moisture and from the root chemistry of Norway maple. The reduced growth of Norway maple led to higher soil moisture content through reduced root uptake of water and reduced rainfall interception by tree canopy, which may have benefited this earthworm species. It has been noted that in natural systems *A. chlorotica* dwells within the rhizosphere and forms close associations to the root systems of plants (Sims and Gerard, 1999). Therefore, root chemistry might be expected to affect this species either directly or indirectly e.g. through root exudates, or modifying local soil pH (Dakora and Phillips, 2002; Rajapaksha *et al.*, 2014). Nitrogen-fixing plants are known to release a net excess of H^+ to the soil (Dakora and Phillips, 2002), however, in this experiment the alder rhizosphere soil did not differ in pH from the bulk soil, which may be due to the buffering capacity of the soil, given the timeframe of this experiment. Rhizosphere soil chemistry data from both tree species showed that in the presence of Norway maple there were higher levels (%) of K, Ca, Mg and P than Italian alder, but much lower levels (%) of soil total N, total C and Organic C. It is difficult to determine whether the difference in extractable levels of these chemical parameters are the cause or as a result of greater *A. chlorotica* activity in Norway maple rhizosphere soil.

In this study there was similar density of *A. longa* across all tubes, irrespective of treatment or tree species. *A. longa* population density experienced a mean reduction of 20% during the experiment, indicating that whilst soil conditions were not sufficiently negative to cause high mortality in this species, they may not have provided sufficient conditions for reproduction. Under laboratory conditions, reproduction of this species (cocoon to mature adult) has been demonstrated to take between 170 to 260 days under environmental temperatures relevant to this nursery experiment (Lowe and Butt, 2005). It is therefore possible that the population density of *A. longa* could have increased within the timeframe of this experiment (i.e. one year), although a longer experiment duration undoubtedly would have provided more opportunity for this. *A. chlorotica* experienced a 50-80% reduction in population density, and this species has a shorter reproduction time than *A. longa* (Lowe and Butt, 2005). These results suggest that *A. longa* was better suited than *A. chlorotica* to the soil materials used and CGW as a food source. However, CGW has been shown to support populations of both *A. chlorotica* and *A. longa* in reclaimed soil for at least 2 years following surface application (Lowe and Butt, 2004). After four years, Lowe and Butt (2004) recorded a crash in the population of *A. longa* in the experimental plots, associated with the complete removal of surface organic matter and subsequent inter-specific competition for limited food resources. The one-year duration of the nursery experiment presented in this chapter is unlikely to have been sufficient time for organic matter to have been depleted within experimental mesocosm to the stage where inter-specific competition arose between these two earthworm species. Previous research by Rajapaksha *et al.* (2014) found that in a mesocosm experiment of similar design and duration, but using a standard loam soil recommended for earthworm experiments, both *L. terrestris* and *A. chlorotica* decreased under birch and eucalyptus (20% and 10-60% reduction, respectively). The similar mortality rates observed by Rajapaksha *et al.* (2014) in a higher quality soil suggests that earthworm mortality may have been influenced by inoculation stress, coupled with insufficient experimental duration to enable reproduction to increase population density. The success of earthworm establishment following inoculation is principally dependent upon species selection and the soil conditions onsite, and broadcast inoculation (as used in this experiment) has been demonstrated to lead to inconsistent earthworm establishment on reclaimed land (Marfleet, 1985; Butt *et al.*, 1993; Butt, 2011b).

A common issue with outdoor mesocosm experiments, such as this study, is the egress of experimental earthworms from the system, or the ingress of invasive earthworms from the surrounding environment (Lubbers and van Groenigen, 2013). In a similar mesocosm experiment at the same location as this study, Rajapaksha *et al.* (2014) found an average of one invasive *L. rubellus* per tube, even where a fine (1 mm) plastic mesh over the top and base of the tubes was employed. In the current study, a total of only 3 individuals of *L. rubellus* were found (a mean density (±SE) of 0.03 (± 0.07) per tube). The few *L. rubellus* that entered the mesocosms in this study may have done so through holes in the basal mesh, or by scaling the outside wall of the tubes. Future experiments should investigate the use of strips of hook Velcro tape (Lubbers and van Groenigen, 2013) to prevent earthworm ingress as well as egress, by also applying them to the outside wall of planting tubes.

6.4.2. The effects of earthworm activity and compost addition on tree survival, growth, biomass and nutrient status

The growth rates of the two tree species in this investigation are different and therefore directly comparing growth data between species is nonsensical. However, it is clear that after 12 months

Italian alder outperformed Norway maple, across all treatments. In a comparable field experiment, Foot *et al.* (2003) found that under a similar application rate of CGW to capped landfill sites, Italian alder (*A. cordata*) significantly outperformed sycamore (*A. pseudoplatanus*). This was attributed to greater availability of N to the nitrogen-fixing alder species, through its association with *Frankia* bacterium, and the *A. cordata* in the experiment presented in this chapter also showed development of root nodules associated with *Frankia* bacterium.

In the current study, Norway maple displayed significantly greater height, in addition to greatest final ground-line diameter and percentage diameter increase, under the earthworm and compost combination treatment. This increase in growth was reflected in the tree biomass data, in which the greatest total above and below ground mean biomass for Norway maple was recorded under the combined earthworm and compost treatment. The results for these parameters did not differ between the earthworm-only and compost-only treatments, suggesting there is a synergistic effect of earthworm activity and compost addition on the growth of this tree species. This may be associated with the significantly greater density of *A. chlorotica* found under the earthworm-compost treatment for Norway maple, with this earthworm species forming close associations with plant roots (Edwards, 2004). There was no observed difference in the leaf biomass input to the tubes between treatments for this tree species, so it may be suggested that the differences in patterns of growth observed are due to the addition of compost rather than leaf litter, in combination with the action of the inoculated earthworms.

At termination of the experiment, no significant effect of compost or earthworm treatments was found for Italian alder height, ground-line diameter or biomass. Italian alder total biomass was greatest under the compost-only treatment, and lowest under the combined compost and earthworm treatment. Similar results were found by Foot *et al.* (2003), in which CGW application rate was not found to significantly affect Italian alder height; however a relationship was identified between height and the depth of CGW in the soil. Foot *et al.* (2003) found that the greatest Italian alder height was recorded under deep incorporation of CGW at the same application rate used in the current study. This was attributed to the CGW encouraging an open-structure in the soil and enabling deeper root penetration and subsequently greater opportunity for nitrogen fixation, rather than conveying direct nutrient benefits to the trees. In the present study, CGW was mixed throughout the soil column and Italian alder main course root biomass was highest under the compost-only treatment, which supports the above findings. However, the 12-month timeframe for this experiment was not sufficient for the alder trees to extend roots deep enough to confirm any significant benefit from CGW application. Italian alder above-ground and total biomass was lowest in both treatments containing earthworms. On a capped landfill, Moffat *et al.* (2008) found that inoculation of *A. chlorotica* and *A. longa* led to similar mean height and lower survival rate of Common alder as that recorded in uncultivated, earthworm-free plots. Since these species are not nitrogen-limited because of their N-fixing ability, it is possible that earthworm processing of decomposing organic matter is of less benefit to these trees, and the earthworms may be competing with the tree roots for soil organic matter, reducing soil structural benefits described above. Foot *et al.* (2003) suggest that the greater response of sycamore to the addition of composted green waste in their field experiment was due to nitrogen being a limiting factor for sycamore and not Italian alder.

In this experiment, earthworm and/or compost treatments showed no significant effects on the C or nutrient content of any tree section of Norway maple. However, earthworm presence significantly increased the C content of Italian alder stem, and Ca levels were significantly higher in fine root (0 - 0.2 m) soil under the earthworm-only treatment compared with the compost-only treatment. Wolters and Stickan (1991) found higher C content in stems of Beech (*Fagus sylvatica*) seedlings, when grown in forest soils with an individual *Octolasion lacteum* (an endogeic, geophagous earthworm species), compared with controls. Other studies have found higher N content of leaves in the presence of earthworms (e.g. Haimi *et al.*, 1992; Doube *et al.*, 1997; Rajapaksha *et al.*, 2014), however foliar N and other nutrient levels for the tree species in this experiment were similar between treatments. It has been suggested that earthworm necromass may account in some cases for elevated foliar nutrient levels (Haimi *et al.*, 1992). However, this has been demonstrated to account for negligible amounts of N where small-bodied endogeic species such as *A. chlorotica* are used, compared with the input of leaf litter or organic waste treatments (Rajapaksha *et al.*, 2014), and survival rates were similar for the larger earthworm species *A. longa* across all treatments in this study.

Taylor (1991) provides thresholds by which nitrogen, phosphorus and potassium-deficient and optimum foliar content can be assessed for common British forestry species. Against the criteria set for Italian alder and Sycamore (as a proxy for Norway maple), the leaves of Italian alder in this experiment were optimum for N, but deficient in P and K. Norway maple foliar samples were considered optimum for K, but deficient in N and P. According to the thresholds set for Norway maple by Kopinga and Van den Burg (1995), this species foliar samples were considered low for K and P, and deficient in N. Against the thresholds provided for common alder, Italian alder foliar samples were considered normal for N, and low for P and K (Kopinga and Van den Burg, 1995).

6.4.3. Soil responses to earthworm activity, compost addition and tree species

An influence of CGW addition was found on bulk soil carbon and nutrient levels. Bulk soil in the upper 0.2 m section under both tree species showed significantly higher total organic carbon (%) and organic matter (%) in the compost-earthworm combination treatment. This may be explained by leaf litter accumulation in the soil through earthworm activity. Under Norway maple, these were also significantly higher in the compost-only treatment, as were the levels of total N (%), NH_4^+ (mg/kg) and PO_4^{3-} (mg/kg) for both tree species. Interestingly, this suggests that earthworm activity has not increased the level of extractable NH_4^+ released from CGW beyond natural decomposition rates, however Bohlen and Edwards (1995) found that earthworms increased the amounts of extractable N and NH_4^+ from manure and legume organic waste treatments. Vimmerstedt (1983) found field soils with no earthworms to have significantly greater levels of P than those containing earthworms, however Clements *et al.* (1991) identified an increase in PO_4^{3-} in soils receiving N fertiliser and containing earthworms, compared with earthworm-free fertilised plots. In the current study availability of PO_4^{3-} does not appear to have been increased through earthworm activity on CGW in this study, perhaps due to loss through leaching or tree uptake.

In the lower bulk soil section under both tree species, total C (%), organic C (%), organic matter (%) and total N (%) were significantly higher in treatments containing CGW, both with and without earthworms. Rajapaksha *et al.* (2014) identified a positive influence of earthworm activity on C content in bulk soil at 0.2–0.4 m depth under birch. Under Italian alder, lower section bulk soil N (%) was significantly higher under the compost-only treatment than the earthworm-compost combination*,* suggesting either increased uptake of N by these trees in the presence of earthworms, or excess N in the soil due to this tree species ability to fix nitrogen or the use of N by earthworms and the suspected higher microbial biomass in earthworm-compost treatment than in compost only treatment. Earthworm activity was associated with significantly higher C:N ratio in the upper section bulk soil under both tree species, and the lower bulk soil under Norway maple. Under Italian alder the highest C:N ratio in the lower soil layer was found in soils not containing CGW. This may be due to earthworm activity distributing organic matter from leaf litter into the lower soil level (Lowe and Butt, 2003). For example, Welke and Parkinson (2003) found that lower horizon mineral soil contained significantly higher organic matter content in the presence of the endogeic (shallow burrowing) earthworm species *Aporrectodea trapezoides.*

CGW was found to influence the C:N ratio of rhizosphere soil; which was highest in the compost-only treatment under Italian alder, and the earthworm-compost combination under Norway maple. Lower section bulk soil moisture content (%), soil K (mg/kg) and NO_3^- (mg/kg) were consistently higher under the no-tree control than for all treatments for both tree species. The higher levels of K may be explained by its tendency to be rapidly released during CGW decomposition. At an equivalent CGW application rate to the one used in the current study, Foot *et al.* (2003) found that K was still released in sufficient amounts to support tree growth up to four years after application.

Tree roots of both species showed a positive effect on the levels of C, N, and availability of cations in rhizosphere compared with bulk soil, and a negative effect on pH, availability of K and anions and on soil conductivity. These trends were most pronounced under Norway maple; however, the severity of change was greatly reduced in the presence of earthworms and compost, where pH was lowest. Norway maple input higher levels of C, OM and organic C into the soils than Italian alder. Tree roots increase organic carbon and modify nutrient cycles in rhizosphere soil through root exudation of organic chemicals as well as nutrient uptake and root turnover (Day *et al.*, 2010; Lukac and Godbold, 2011). Furthermore, trees can influence rhizosphere nutrient supply through biological N fixation (e.g. by alder species), and through the extraction of nutrients, especially nitrate; with subsequent effects on pH and the mobility of other chemicals, e.g. anions and cations (Day *et al.*, 2010). Different tree species display differential root uptake of N pools, for example, BassiriRad *et al.* (2008) observed preferential Red maple (*Acer rubrum*) and Sugar maple (*Acer saccharum*) uptake of NH_4^+ over NO_3^-. In the experiment presented in this chapter, both tree species' rhizosphere soils displayed a reduction in available NO_3^- and an increase in available NH_4^+ compared with bulk soils, with an associated reduction in soil pH. This may indicate preferential uptake and assimilation of nitrate over ammonium; however, compared with ammonium nutrition, nitrate nutrition can have different consequences on plant growth and tends to result in greater root uptake of cations (Fernandes and Rossiello, 1995). This supports the findings of the current study, in which cation concentrations in rhizosphere soil were reduced when compared with bulk soils.

Italian alder alone led to significantly higher organic carbon and organic matter content in bulk soils, compared with Norway maple and tree-free controls. The Italian alder bulk soils also had a significantly lower moisture content and levels of macro-nutrients K, Mg, NO_3^- and PO_4^{3-} compared with Norway maple and tree-free soil. The leaves of the Italian alder were considered low in N and deficient in K and P, according to the criteria of Taylor (1991) and Kopinga and Van den Burg (1995), so it is likely that within the short timeframe of this experiment insufficient levels of these macro-nutrients were being provided to the soil system by the CGW and leaf litter. Although the alder in this experiment were found to have established extensive root nodules, the short experiment duration may not have provided sufficient time for alder to fix enough N to support growth, and final soil N levels were not raised by the presence of the N-fixing alder species. It has been observed that it may take up to five years before alder accumulate sufficient N in soils to improve the growth of other trees on reclaimed land (Moffat, 2000). The same study found that *A. cordata* planting alone led to elevated NO_3^- levels over a control soil containing larch.

Future studies would benefit from a longer duration, e.g. minimum of 24 months in order to allow biomass and soil data to reflect any longer-term effects of organic waste application. In this study leaf litter input to the soil was unregulated in the interest of reflecting field conditions and not limiting organic matter availability to CGW addition alone; thus starving the inoculated earthworms. However, identification of the effect of litter addition on soil and tree health measurements would be possible if litter inputs were instead regulated (e.g. Rajapaksha *et al.*, 2014). Additionally, although the CGW application rate used in this study was reflective of realistic legal limits, mesocosm studies investigating different application rates of CGW or other organic waste materials may identify opportunities to improve sustainable woodland and earthworm population establishment on reclaimed land. Further work might also be sought to investigate the interaction effects of tree roots and rhizosphere-dwelling earthworms such as *A. chlorotica*.

6.4.4. Summary of chapter findings

The findings of this research were as follows, summarised against the original chapter objectives:

1. Italian alder performed well in reclaimed soil, irrespective of compost or earthworm addition. Norway maple displayed significantly improved growth in reclaimed soil receiving

the earthworm and compost combination treatment, indicating a synergistic effect of earthworm activity and compost addition on tree growth.

2. a) CGW addition may serve as a suitable source of organic matter to sustain soil faunal populations on reclaimed sites in the initial period when trees are still becoming established. Earthworm survival rates indicated that *A. longa* was better suited than *A. chlorotica* to the soil materials used and to CGW as a food source.

   b) *A. chlorotica* density was significantly higher under Norway maple, despite the lower quality of the litter material. This may be a result of CGW addition and influences from the root chemistry of Norway maple and higher soil moisture content under this tree species.

3. CGW was shown to significantly improve the soil resource through increased levels of organic carbon and essential plant macro-nutrients, with earthworm activity assisting decomposition of both leaf litter and CGW in the soil.

7. EARTHWORM SURVIVAL, GROWTH AND REPRODUCTION IN A RECLAIMED SOIL

7.1. Introduction and objectives

Research indicates that composted greenwaste (CGW) can promote the development of sustainable earthworm populations on reclaimed sites, provided sufficient resource is available, and may help promote soil development and tree growth (section 2.5.3). However, the location of organic waste application within the soil profile can influence the establishment of earthworm populations. Lowe and Butt (2002b) investigated the foraging ability of two anecic earthworm species previously used in reclamation experiments, and found that *A. longa* exhibited greater capacity to forage for organic matter horizontally within the soil profile than *L. terrestris*. These findings may help explain the outcomes of a field experiment in which *A. longa* out-performed *L. terrestris* in reclaimed soils in which Separated Cattle Solids (SCS) had been incorporated within the soil, rather than applied to the surface (Lowe and Butt, 2002b). This suggests that decisions on earthworm species selection and method of organic waste application to the soil cannot be made independently. Importantly, however, there is still little research to enable such informed choices.

Lowe and Butt (2004) suggest that surface application of organic wastes may be beneficial to the establishment of anecic earthworm species. In comparison, the findings of Foot *et al.* (2003) indicated that mixing CGW within the soil profile is preferable for early successful tree establishment, to create an open soil structure and provide sufficient nutrients for plant growth. Earthworm activity may provide an effective method for incorporating and mixing organic waste materials into soils (Piearce and Boone, 1998). However, more research is needed to inform this and investigate the long-term effects of surface application of organic wastes, with incorporation into the soil profile and rhizosphere facilitated by anecic and endogeic earthworms. Laboratory-based research by Lowe and Butt (2002b) suggests that certain anecic species (i.e. *A. longa*) may be more able to forage within the soil profile for organic matter than other anecic species. Therefore, the use of *A. longa* in land reclamation may present an opportunity to facilitate organic waste incorporation, enhance mineralisation and improve soil fertility (Piearce and Boone, 1998).

The two laboratory experiments detailed in this chapter were designed to complement the field experiments presented in Chapters 5 & 6. These laboratory experiments allowed an investigation to be made into the behaviour of earthworms used in the field experiment, and identify the influence of reclaimed soil on the survival and reproductive behaviour of four earthworm species native to the UK. Comparisons were made between CGW and horse manure (a proven food source for earthworms) for supporting earthworms in reclaimed soils. These experiments were designed to identify whether earthworms inoculated into the field experiments at Ingrebourne Hill and Headley Nursery were likely to have survived and reproduced, and the factors which influenced this. Specific objectives of the experiments were to:

1. measure the effect of a reclaimed soil on earthworm growth, survival and reproductive output,
2. measure the effect of CGW as a food source on earthworm growth, survival and reproductive output in reclaimed soil, and compare this to the effects of a known food source (horse manure),
3. identify which earthworm species were most suited to the experimental treatments used in both field experiments, to aid interpretation of the field experiment results, and inform species selection for future experiments,
4. compare treated (de-faunated) and homogenous reclaimed soil with freshly-collected reclaimed soil, to identify whether biotic factors influence earthworm results.

7.2. Materials and methods

7.2.1. Experimental design

To assess earthworm performance, a series of culture vessel experiments were set up under controlled environmental conditions at the University of Central Lancashire. These experiments utilised a culture vessel design (after Butt, 2011) to investigate earthworm performance in different soil treatments. This design allowed for earthworm parameters to be regularly monitored and quantified, for example by removing earthworms from the containers for mass determination (Fründ *et al.*, 2010), and by removing and sieving the soil for cocoons (Butt and Grigoropoulou, 2010). An earthworm culture vessel consisted of an opaque, 0.75 L (depth 0.1 m) plastic container (Lakeland Plastics, UK). Each vessel was filled to a depth of 6 cm (100 cm sq. x 6 cm H = 600 ml) with a soil treatment and a food treatment, as described in section 7.2.3 (Figure 7.1).

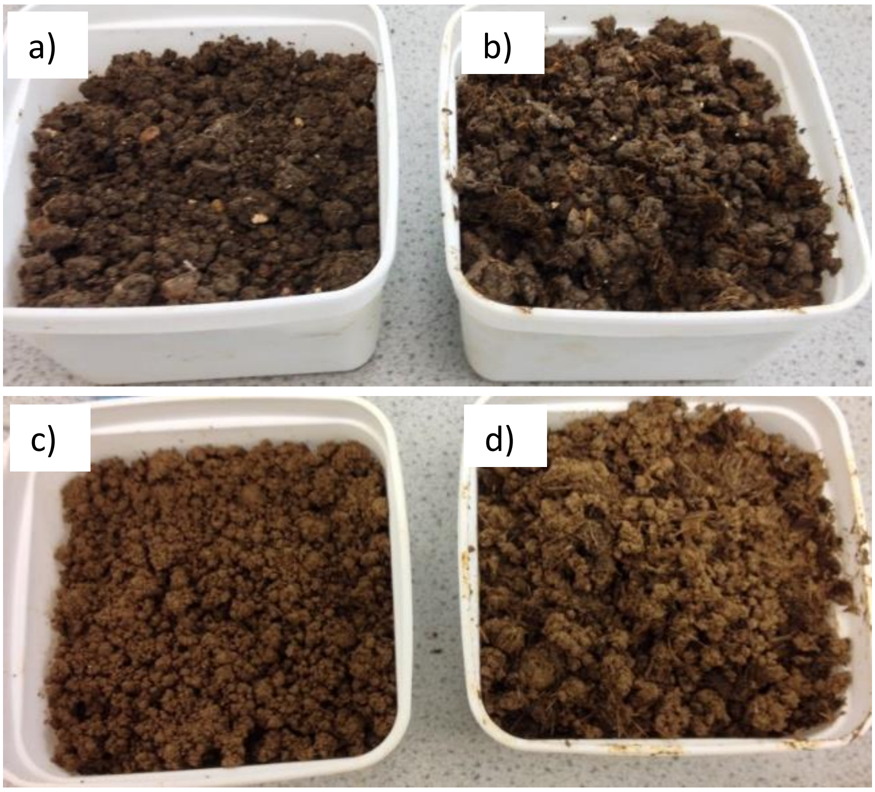


Figure 7.1. Culture vessels containing soil treatments for experiment 1. Treatments were: de-faunated reclaimed soil (a) before and (b) after horse manure addition, Kettering loam (c) before and (d) after horse manure addition.

Prior to experimentation, all earthworms (section 7.2.2) were kept at 15°C in a control soil of Kettering loam and given an excess of re-wetted dried horse manure, for a minimum of 28 days to allow them to equilibrate from field conditions. For the duration of the experiments the culture vessels were kept at 15±1°C in light-proof, temperature controlled incubators (as per Lowe and Butt, 2002, 2005). At the start of each experiment, adult earthworms of each species were randomly assigned to treatments after their masses were determined. At 28 days intervals, earthworms were removed from the vessels, washed with cool distilled water, dried with clean, absorbent tissue paper and had mass recorded by placement into a beaker of cool distilled water on an electronic scale (Fründ *et al.*, 2010). Used vessels were cleaned thoroughly and refilled with freshly prepared soil and food according to each experimental treatment, and earthworms were returned to the vessel and incubator. Experimental soils were wet-sieved through nested soil sieves (1, 2 and 3 mm) to separate out cocoons.

This study comprised two experiments; the conditions for each experiment are summarised in table 7.1. Experiment 1 investigated the performance of four common UK earthworm species in de-faunated reclaimed soil, compared with Kettering loam. This was to measure the effects of a reclaimed soil on earthworm health, survival and reproductive output, and identify which earthworm species may be most suited to the field experiment at Ingrebourne Hill. To control for the influence of organic matter availably to the earthworms, horse manure (a recognised food for earthworms in such studies, e.g. Berry and Jordan, 2001; Butt, 2011) was applied in both treatments. Experiment 2 investigated the performance of three common UK earthworm species in de-faunated reclaimed soil, compared with freshly collected reclaimed soil. To replicate the treatment combinations in the field experiments at Ingrebourne Hill (Chapter 5) and Headley Nursery (Chapter 6), composted greenwaste was either withheld or applied at the rate used in those field experiments.

Table 7.1. Summary of treatment applications for both laboratory experiments. n=5 per species per treatment combination. (X) Treatment combination included in experiment, (-) not included.

Experiment no.	Food treatment	Soil treatment		
		Kettering loam	**De-faunated reclaimed soil**	**Fresh reclaimed soil**
1	Horse manure	X	X	-
2	+ CGW	-	X	X
	No food (control)	-	X	X

7.2.2. Earthworm species

Experiment one investigated the performance of four earthworm species: *A. longa* and *L. terrestris* (anecic); *A. chlorotica* and *A. caliginosa* (endogeic). Experiment two investigated the same species, with the exception of *L. terrestris*, due to an incubator malfunction resulting in the death of the stock of this species prior to the experiment. Each earthworm species was introduced in monoculture to separate culture vessels in the following numbers, according to treatment; *A. longa* (2), *L. terrestris* (2), *A. chlorotica* (4) and *A. caliginosa* (4). These numbers were selected based on mean earthworm biomass, recorded field densities (e.g. Edwards and Bohlen, 1996) and all species being amphimictic (Sims and Gerard, 1999). All earthworms, except *L. terrestris*, were collected from agricultural pasture at Walton Hall Farm, Preston, UK (Nat. Grid Ref: SD 55050 28100), via digging and hand-sorting of soil. Adult *L. terrestris* were collected at Alice Holt forest, Farnham, UK (Nat. Grid Ref: SU 80246 42818), using direct application of mustard vermifuge to middens (Butt and Grigoropoulou, 2010). The *A. chlorotica* used in this experiment were of mixed pink and green morph (Lowe and Butt, 2008), however all were selected to be of similar biomass, and morph was not considered to be a limiting factor in this experiment as cocoon viability was not one of the measurements.

7.2.3. Organic matter treatments

For the vessels in experiment 1 receiving horse manure, this was blended and sieved to <2 mm, then 10 g was wetted (resulting in ~60 g wet material) and placed on the soil surface for anecic earthworm species, or manually incorporated fully into the soil for endogeic species (after Lowe and Butt, 2005). In experiment 2, where CGW was used as a source of organic matter, fresh PAS 100 certified CGW (supplied by Viridor Waste Management, Beddington Lane, Croydon) was blended and sieved to remove >6 mm woody materials. Then 9.6 g of CGW (equivalent to the CGW application rate for the Ingrebourne Hill field experiment) and placed on the soil surface for anecic earthworm species, or incorporated fully into the soil for endogeic species (after Lowe and Butt, 2005). A summary of the chemical composition of both food sources is provided in Table 7.2.

Table 7.2. Summary of chemical analysis of horse manure and Viridor 0-25mm PAS 100 composted green waste (source: Forest Research laboratory services at Alice Holt Lodge, Farnham, and technical document supplied by Viridor).

Parameter	**Horse Manure**	**Composted Green Waste**
Total N (%)	1.10	1.64
Total C (%)	47.60	30.60
C:N	43.20	18.60
K (%)	0.68	1.24
Ca (%)	0.62	2.70
Mg (%)	0.23	0.30
P (%)	0.44	0.23
Na (mg/kg)	4270	717
Organic Matter (%)	N/A	60.20

Each plot within the Ingrebourne Hill field experiment (Chapter 5) received 0.8 t CGW 50 m^{-3} (0.8 t CGW 100 m^{-2} plot, incorporated to 0.5 m depth). Therefore, to achieve the same rate of compost addition to the 600 ml of soil in each of the 750 ml vessel in this experiment:

800000 g (0.8 t) / 50000000 ml (50 m^3) = 0.0016 g CGW per 1 cm^3 of soil

0.16 (g) x 600 (cm^3) = <u>9.6 g CGW per 600 cm^3 soil (i.e. per vessel)</u>

7.2.4. Soil treatments

Fresh reclaimed soil (Figure 7.2) was sampled close to the field experiment at Ingrebourne Hill Community Woodland (Chapter 5), fully homogenised, and large stone materials (>8 mm) removed. Five subsamples of the homogenised materials were tested to determine moisture content, by drying samples in an oven for 24 hours at 105 ^{o}C, and recording the change in mass (mean overall moisture content of 25.3%). This level of moisture was then maintained throughout the experiment at the outset of each 28-day period. Before use, de-faunated reclaimed soil (Figure 7.2) was frozen at -5 ^{o}C for 7 days to kill native earthworms and other potential competitors/predators (Butt, 2011b), then air-dried and sieved to remove materials >8 mm, and re-wetted to 25% moisture content. The control soil used in experiment 1 (Figure 7.1) was sterile Kettering loam (Boughton Loam, Kettering, UK), which is a standard substrate used in general earthworm experiments and choice chamber experiments (Butt *et al.*, 1994b; Rajapaksha *et al.*, 2013), and this was re-wetted to 25% moisture content.

7.2.5. Statistical analysis

Statistical analysis was performed using the freeware statistical software R, version 3.2.2. “Fire Safety” and the R Studio desktop software, version 0.99.486 (R Core Team, 2015; RStudio Team, 2015). Data were first tested for normality using the Shapiro-Wilk test, which is suited to smaller sample sizes (in this case n=5). Two-way repeat-measures ANOVA were run on the complete dataset across all time points, to investigate the influence of experiment duration and treatments on earthworm growth and cocoon production.

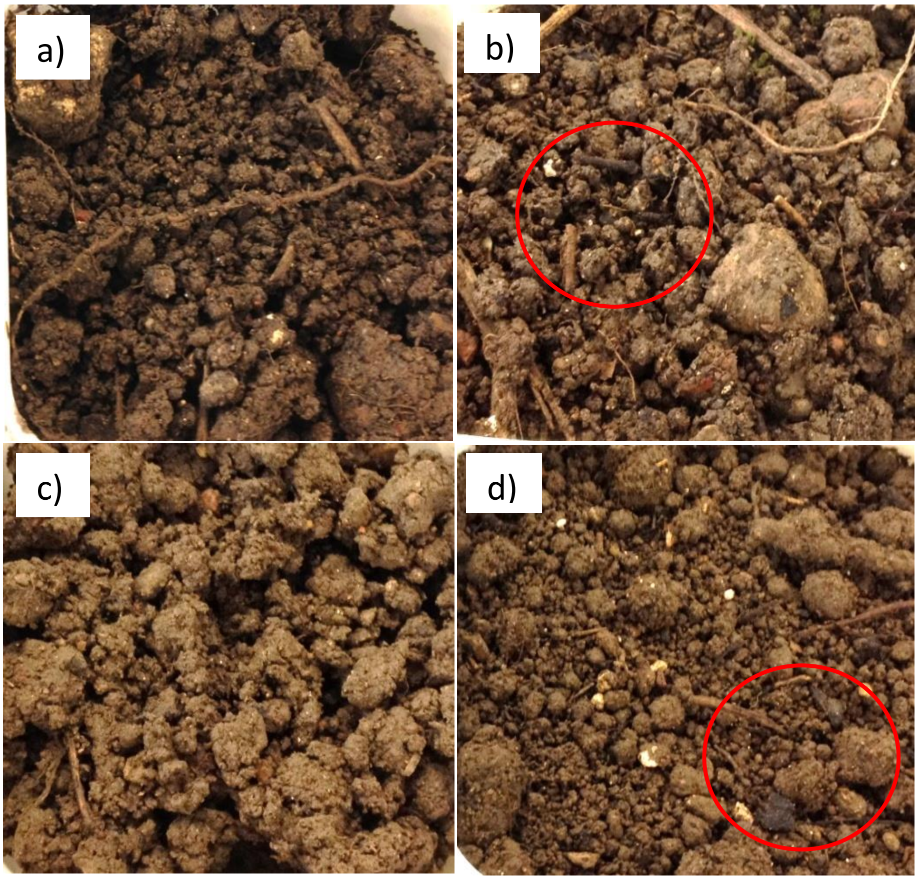


Figure 7.2. Images of the experiment 2 soil treatments. Treatments as follows: (a) Fresh reclaimed soil, (b) fresh reclaimed soil with CGW (circled in red), (c) de-faunated reclaimed soil, and (d) de-faunated reclaimed soil with CGW (circled in red).

7.3. Results

7.3.1. Experiment 1

The culture vessels enabled measurement of earthworm mass and cocoon production throughout the experiment, with generally similar patterns observed for all species across soil treatments. Table 7.3 shows earthworm performance across treatments at the start and termination of the experiment. After 3 months, 100% survival was recorded for all species across all treatments. The largest gains in mass were *L. terrestris* and *A. chlorotica* in loam soil (+3.74% and +3.03%, respectively), whilst the largest losses in mass were *A. chlorotica* and *A. caliginosa* in reclaimed soil (-20.59% and -19.47%, respectively).

Table 7.3. Data for selected earthworm parameters across each treatment at the start and termination of experiment 1 (ind = individual).

Soil	Earthworm Species (treatment)	Number of earthworms (ind tray^{-1})	Initial mean earthworm mass (g ind^{-1})	Final mean earthworm mass (g ind^{-1})	Change in mass (%)	Final cocoon output (ind^{-1})	Survivorship (%)
Loam	*A. longa*	2	2.78	2.65	-4.68	2.3	100
	L. terrestris	2	5.08	5.27	+3.74	0.2	100
	A. chlorotica	4	0.33	0.34	+3.03	1.8	100
	A. caliginosa	4	1.14	0.95	-16.67	3.93	100
Reclaimed	*A. longa*	2	2.83	2.46	-13.07	1.9	100
	L. terrestris	2	4.73	4.86	+2.75	0.2	100
	A. chlorotica	4	0.34	0.27	-20.59	0.95	100
	A. caliginosa	4	1.13	0.91	-19.47	2.73	100

For *A. longa*, there was a statistically significant main effect of experiment duration on earthworm mass, as determined by repeat measures ANOVA ($F (3,8) = 9.09$, $p = 0.002$), but no significant effect of treatment alone or an interaction effect of treatment and time (Figure 7.3). Figure 7.4a shows *A. longa* cocoon production in reclaimed soil and loam supplied with horse manure over a 3-month period. One month into the experiment, mean cocoon production in the loam soil treatment was lower than in the reclaimed soil treatment (0.5 cocoons ind^{-1}, and 1.6 ind^{-1}, respectively). This trend reversed at the 2-month time point, and at termination of the experiment, mean cocoon output in the loam treatment was 2.3 cocoons^{-1} ind^{-1}, compared with 1.9 cocoons^{-1} ind^{-1} for the reclaimed soil treatment. Repeat measures ANOVA revealed that experiment duration significantly affected cocoon production ($F (3,24) = 30.46$, $p <.001$), and there was a significant interaction effect between time and treatment on *A. longa* cocoon production ($F (3,24) = 5.08$, $p = 0.013$).

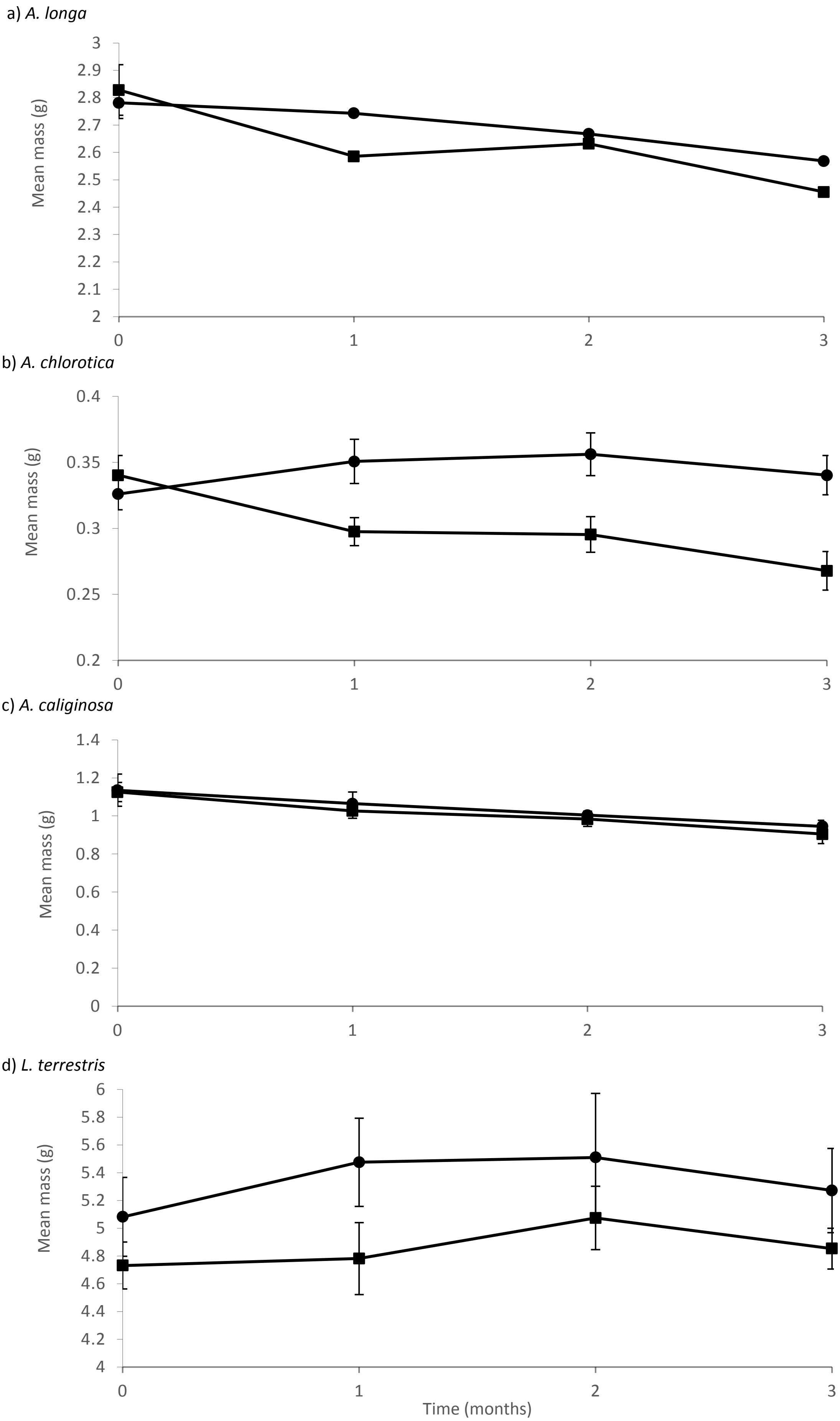


Figure 7.3. Mass changes (g) of four earthworm species in Kettering loam (●) and reclaimed soil (■) with horse manure addition (n=5). Species: (a) *A. longa*; (b) *A. chlorotica*; (c) *A. caliginosa*; (d) *L. terrestris*. Error bars indicate ± 1 SE.

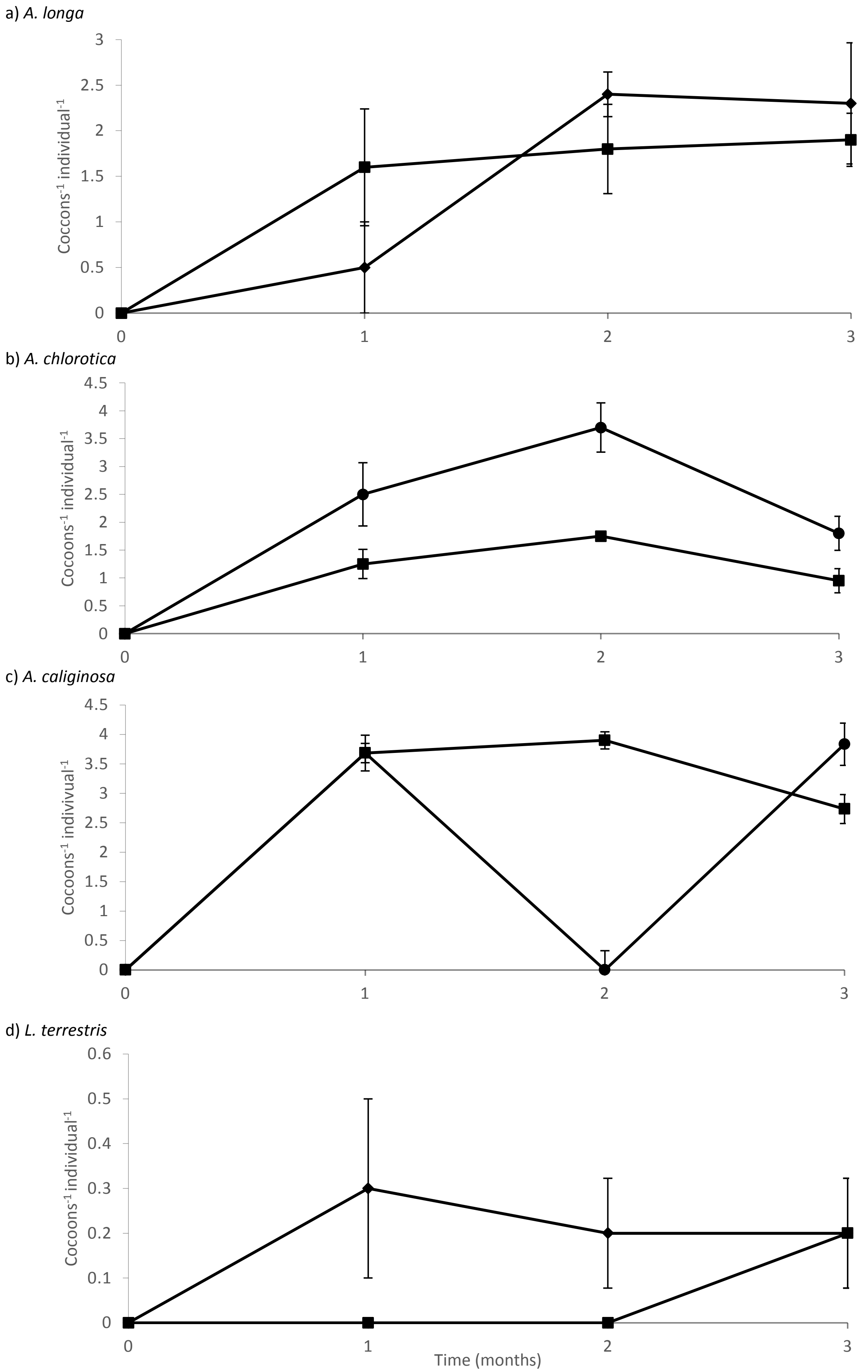


Figure 7.4. Cocoon production of four earthworm species in Kettering loam (●) and reclaimed soil (■) with horse manure addition (n=5). Species: (a) *A. longa*; (b) *A. chlorotica*; (c) *A. caliginosa*; (d) *L. terrestris*. Error bars indicate ± 1 SE.

Over the 3-month experiment, *A. chlorotica* mean mass remained steady in the loam soil treatment and slowly reduced in the reclaimed soil treatment (Figure 7.3). There was a statistically significant main effect of experiment duration on earthworm mass, as determined by repeat measures ANOVA ($F (3,24) = 13.78$, $p < .001$), and also a significant interaction effect between time and treatment ($F (3,24) = 34.55$, $p < .001$). The effect of experiment treatment was suggestive, but not statistically significant towards earthworm mass ($F (1,8) = 5.07$, $p = 0.054$). *A. chlorotica* cocoon production increased in both soil treatments until 2 months, when both began to decline (Figure 7.4). Cocoon production by *A. chlorotica* was higher in loam soil than reclaimed soil throughout, and cocoon production was significantly affected by treatment (repeat measures ANOVA, $F (1,8) = 16.95$, $p = 0.003$), experiment duration ($F (3,24) = 40.46$, $p < .001$) and there was a significant interaction effect of both treatment and time ($F (3,24) = 6.22$, $p = 0.011$) on cocoon production.

A. caliginosa mass steadily decreased in both soil treatments (Figure 7.3), with a final mass change of -16.67 % and -19.47% in loam and reclaimed soil, respectively (Table 7.3). This is reflected by a statistically significant main effect of experiment duration on earthworm mass (repeat measures ANOVA, $F (3,24) = 6.59$, $p = 0.003$), and also a significant interaction effect between time and treatment (repeat measures ANOVA, $F (3,24) = 18.37$, $p < .001$), however the effect of soil type alone was not found to be statistically significant towards *A. caliginosa* mass. Cocoon production by *A. caliginosa* was 28.7% higher in reclaimed soil than in loam (Figure 7.4). Repeat measures ANOVA revealed that cocoon production was significantly affected by the experiment treatments ($F (1,8) = 10.80$, $p = 0.011$), experiment duration ($F (3,24) = 290.84$, $p < .001$) and there was a significant interaction effect of both treatment and time ($F (3,24) = 17.72$, $p < .001$) on cocoon production.

L. terrestris mass remained consistent in both reclaimed soil and loam supplied with horse manure over the 3-month experiment (Figure 7.3), and was the only species to gain weight in the experiment (mass increase of 3.74% in loam and 2.75% in reclaimed soil, see Table 7.3). Repeat measures ANOVA revealed no statistically significant effect on earthworm mass by the experiment treatments or duration. *L. terrestris* exhibited low cocoon output in both treatments, with cocoon production slightly higher but not significant in loam soil than reclaimed soil for the first two months (average of 0.25 cocoons ind^{-1} in loam and zero cocoons ind^{-1}, respectively). At termination of the experiment after 3 months, both soil treatments had a mean cocoon output of 0.2 cocoons ind^{-1}. Repeat measures ANOVA revealed there was no statistically significant effect on cocoon output by the experiment treatments or duration.

7.3.2. Experiment 2

Table 7.4 shows the performance of three earthworm species across the given four treatments, at the start and termination of the experiment. After 5 months, 100% survival was recorded for all species except *A. chlorotica*, which had 95% survival in the two +CGW treatments. All species lost mass across all treatments; in both treatments the smallest loss was exhibited by *A. longa*, and the largest by *A. caliginosa* in de-faunated reclaimed soil with CGW added (-73.91%). At the termination of the experiment, all species had zero cocoon production, with the exception of *A. chlorotica* in de-faunated reclaimed soil with CGW addition (0.5 cocoons individual^{-1}).

Table 7.4. Data for selected earthworm parameters across each treatment at the start and termination of experiment 2 (ind = individual).

Soil treatment	Food treatment	Earthworm Species	Number of earthworms (ind tray^{-1})	Initial mean earthworm mass (g ind^{-1})	Final mean earthworm mass (g ind^{-1})	Change in mass (%)	Survivorship (%)
Fresh Reclaimed	+CGW	*A. longa*	2	2.01	1.38	-31.34	100
		A. chlorotica	4	0.25	0.07	-72.00	95
		A. caliginosa	4	0.90	0.30	-66.67	100
	- CGW	*A. longa*	2	2.37	1.32	-44.30	100
		A. chlorotica	4	0.26	0.10	-61.54	100
		A. caliginosa	4	0.87	0.28	-67.82	100
De-faunated Reclaimed	+CGW	*A. longa*	2	2.21	1.88	-14.93	100
		A. chlorotica	4	0.23	0.16	-30.43	95
		A. caliginosa	4	0.92	0.24	-73.91	100
	- CGW	*A. longa*	2	2.13	1.50	-29.58	100
		A. chlorotica	4	0.23	0.13	-43.48	100
		A. caliginosa	4	0.84	0.49	-41.67	100

Figure 7.5 shows *A. longa* mass in reclaimed soil over a 5-month period, with an initial period of acclimation when mass steadily decreased under all treatments for the first month. After 2 months,

mass plateaued across most treatments, with the exception of de-faunated reclaimed soil with CGW, in which it subsequently increased (Figure 7.5). There was a statistically significant time-treatment interaction effect on mass under the de-faunated soil and CGW treatment compared with mass for fresh reclaimed soil with CGW (repeat measures ANOVA, $F_{(5,40)} = 5.50$, $p = 0.011$). There were no further significant effects of treatment or treatment-time interactions for *A. longa* mass data under the other treatments. After 1 month, cocoon production decreased across all treatments except for de-faunated reclaimed soil with CGW, where it increased for a further month before decreasing as did the other treatments (Figure 7.6). By the termination of the experiment at 5 months, cocoon output had reduced to zero across all treatments (Figure 7.6). However, throughout the experiment there was a significantly lower cocoon production under the de-faunated soil and CGW treatment compared with cocoon production for fresh reclaimed soil with CGW (repeat measures ANOVA, $F_{(1,8)} = 6.54$, $p = 0.034$). There were no further significant effects of treatment or treatment-time interactions for *A. longa* cocoon production data under the other treatments.

Figure 7.5 shows *A. chlorotica* mass in the reclaimed soil treatments over the 5-month experiment duration. *A. chlorotica* also showed an initial period of acclimation where mass steadily decreased under all treatments for two months, with the exception of the fresh soil without CGW treatment (in which mass remained relatively constant). Mass then began a steady decrease across both fresh reclaimed soil treatments, and increased across both de-faunated soil treatments before sharply dropping in the final month. As with *A. longa*, final mass was highest in the de-faunated soil with CGW treatment, and there was a statistically significant time-treatment interaction effect on mass under the de-faunated soil and CGW treatment compared to mass for fresh reclaimed soil with CGW (repeat measures ANOVA, $F_{(5,40)} = 8.15$, $p = 0.009$). *A. chlorotica* mass in fresh soil with CGW was not significantly different to mass in the fresh soil without CGW treatment. There was a significant effect of treatment ($F_{(1,8)} = 7.48$, $p = 0.026$), and a significant time-treatment interaction ($F_{(5,40)} = 35.13$, $p < .001$) on mass between the de-faunated soil and fresh soil without CGW treatments, with the de-faunated soil treatment having higher final mass (0.13 g vs 0.1 g, see Table 7.4).

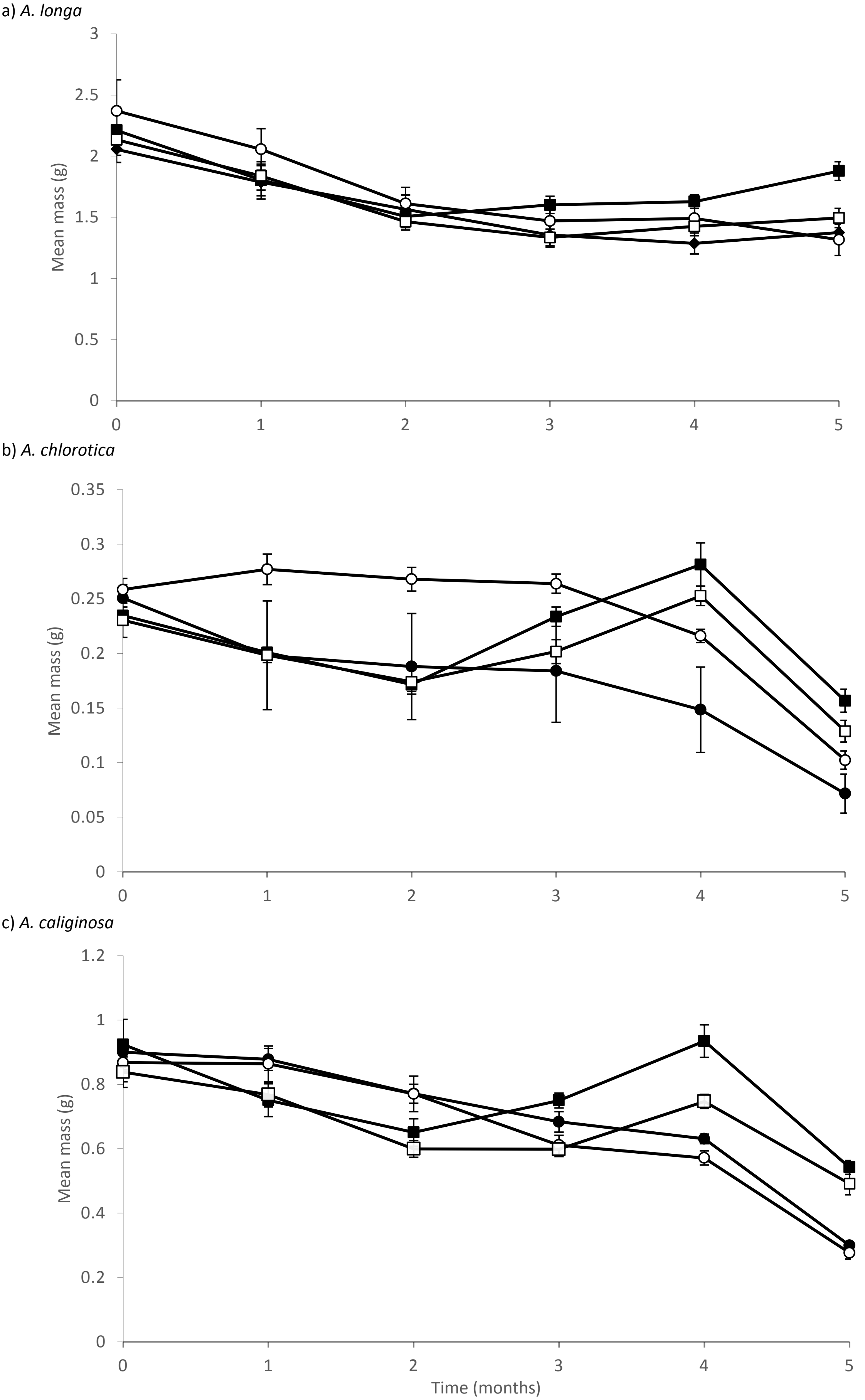


Figure 7.5. Mass changes of three earthworm species in de-faunated and fresh soil with composted greenwaste treatment (n=5). Species: (a) *A. longa*; (b) *A. chlorotica*; (c) *A. caliginosa*. Treatments: Fresh soil with CGW (●), fresh soil only (○), De-faunated soil with CGW (■), de-faunated soil only (□). Error bars indicate ± 1 SE.

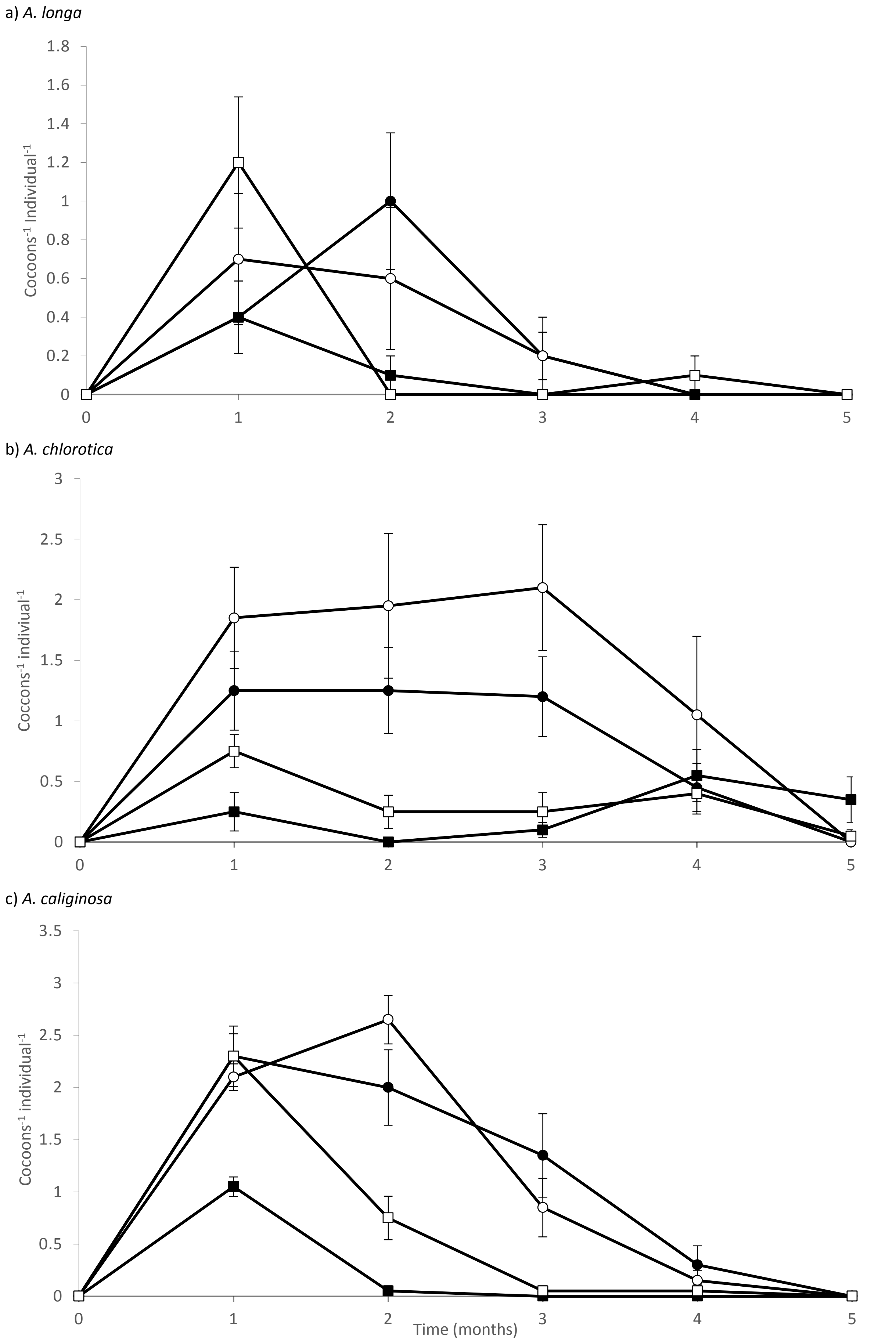


Figure 7.6. Cocoon production of three earthworm species in de-faunated and fresh soil with composted greenwaste treatment (n=5). Species: (a) *A. longa*; (b) *A. chlorotica*; (c) *A. caliginosa*. Treatments: Fresh soil with CGW (●), fresh soil only (○), De-faunated soil with CGW (■), de-faunated soil only (□). Error bars indicate

A. chlorotica cocoon production was higher in the fresh soil treatments throughout the first four months of the experiment (a range of 1 to 2 cocoons per individual), before decreasing to a similar low level as in the de-faunated soil treatments (less than 0.5 cocoons per individual, Figure 7.6). *A. chlorotica* cocoon production in fresh soil with CGW was significantly lower in the fresh soil without CGW treatment (repeat measures ANOVA, $F_{(5,40)} = 8.15$, $p < 0.05$). Average cocoon production was highest in fresh soil without CGW, and this was significantly higher than in fresh soil with CGW (treatment effect, repeat measures ANOVA, $F_{(1,8)} = 5.34$, $p < 0.05$), and significantly higher than in de-faunated soil without CGW (treatment effect, repeat measures ANOVA, $F_{(1,8)} = 55.26$, $p < .001$). Cocoon production was significantly higher in fresh soil with CGW than in de-faunated soil with CGW, affected by treatment (repeat measures ANOVA, $F_{(1,8)} = 6.99$, $p = 0.03$), and time-treatment interaction (repeat measures ANOVA, $F_{(5,40)} = 8.65$, $p < .001$).

A. caliginosa showed a similar pattern of mass change under experimental treatments to *A. chlorotica* (Figure 7.5). As with *A. longa* and *A. chlorotica*, final mass was highest in the de-faunated soil with CGW treatment, and there was a statistically significant time-treatment interaction effect on mass under the de-faunated soil and CGW treatment compared to mass for fresh reclaimed soil with CGW (repeat measures ANOVA, $F_{(5,40)} = 24.25$, $p < .001$). *A. caliginosa* mass in fresh soil with CGW was not significantly different to that in the fresh soil without CGW treatment, however there was a significant treatment-time interactive effect (repeat measures ANOVA, $F_{(5,40)} = 20.06$, $p < .001$) on mass between the de-faunated soil and fresh soil without CGW treatments, with the de-faunated soil treatment having higher final mass (0.49 g vs 0.28 g, see Table 7.4).

A. caliginosa cocoon production decreased across all treatments after the first month of the experiment, except under fresh soil without CGW, in which it increased until the second month, before then decreasing to a similar low level as in the other treatments (Figure 7.6). During this time a number of earthworms were observed to have entered diapause (an inactive state) in the fresh soil treatments. *A. caliginosa* cocoon production in fresh soil with CGW was significantly higher under the fresh soil with CGW treatment than the de-faunated soil with CGW (time-treatment interaction effect, repeat measures ANOVA, $F(5,40) = 24.25$, $p < .001$). However, there was no significant difference between cocoon production in fresh soil with or without CGW addition. Average cocoon production in fresh soil without CGW was significantly higher than in de-faunated soil without CGW (repeat measures ANOVA, treatment effect, $F(1,8) = 23.72$, $p = 0.001$, and time-treatment interaction $F(5,40) = 15.39$, $p < .001$).

7.4. Discussion

7.4.1. Earthworm responses to soil and food treatments

A. longa mass was steady across all reclaimed soil treatments, increasing to a significantly higher final mass in de-faunated reclaimed soil with CGW application at a rate which was equivalent to 80 t ha^{-1} (mixed into soil rather than surface-applied). These results indicate that *A. longa* was likely to be a suitable species for studying in the reclaimed soil at Ingrebourne Hill community woodland (Chapter 5), and at the rate of application or location in the soil profile used, CGW may be a suitable food source for supporting this species in the short-term (with future considerations required for a sustainable population). For example, Lowe and Butt (2004) found that a one-time surface application of CGW at an application rate of 20 t ha^{-1} supported a population of *A. longa* on a reclaimed landfill for a period of 4 years. In the study presented in this chapter, cocoon production by *A. longa* reduced to zero across all treatments by month 5, suggesting that CGW was not the principal factor causing this reduction in cocoon output. This is likely be a function of the field-collected earthworms having a negative response to the experimental soil type, and earthworms collected from a reclaimed site, or given longer to acclimate to the experiment conditions may have exhibited higher cocoon output. A longer experimental duration may have yielded data relevant to long-term *A. longa* population dynamics on reclaimed sites.

A. chlorotica demonstrated significantly higher mass in fresh reclaimed soil with CGW than the other treatments, indicating that *A. chlorotica* was likely to be a suitable species selection for this reclaimed soil and the field experiment at Ingrebourne Hill. Lowe and Butt (2004) found a steady increase in *A. chlorotica* numbers after 4 years following surface application and subsequent incorporation into the soil of CGW at an application rate of 20 t ha^{-1}. This was in stark contrast to the population crash of *A. longa* described above (Lowe and Butt, 2004). In this study, *A. chlorotica* mass was steady across all reclaimed soil treatments for four months, and then began to decrease. Cocoon production by *A. chlorotica* reduced to zero across all treatments by month 5, except in de-faunated soil with CGW added, in which a low but constant rate of cocoon production was shown. *A. chlorotica* has been described as a good medium-long term indicator of reclaimed soil health (Lowe and Butt, 2004), and a longer experiment duration may have provided a clearer picture of this species' long-term response to these soil treatments.

In experiment 1, *A. caliginosa* exhibited higher cocoon production in Kettering loam than reclaimed soil, and higher cocoon production in reclaimed soil with horse manure than in the same soil with CGW. In experiment 2, *A. caliginosa* mass and cocoon output decreased across all treatments, suggesting that CGW was not the principal factor causing these reductions. Additionally, individuals of *A. caliginosa* were observed to have entered diapause in the fresh reclaimed soil treatments, indicating unacceptable soil conditions for this species. Since soil moisture and temperature were maintained at favourable levels, the cause of this diapause must have been related to soil physical or chemical quality. These results indicated that *A. caliginosa* may not have been a suitable species selection for this reclaimed soil. This species has demonstrated low colonisation rates for reclaimed sites (Dunger, 1989; Judd and Mason, 1995), and Lowe and Butt (2004) found poor establishment of this species following inoculation into a reclaimed landfill sites. However, some evidence of this species was found under a plot on the site which received CGW application (2 immature individuals were found), suggesting that CGW may be a suitable foodstuff for this species.

L. terrestris retained a stable mass in Kettering loam and reclaimed soil with horse manure in experiment 1. However, this species exhibited very low cocoon production (final production of 0.2 cocoons $individual^{-1}$ $month^{-1}$) throughout the experiment, with almost no reproductive output in reclaimed soil despite abundance of suitable food source. This is lower than reported cocoon production rates (1-2 cocoon individual^{-1} month^{-1}) for this species in peat and mineral soils (Satchell, 1967; Curry and Bolger, 1984). Unfortunately, an incubator malfunction resulted in the death of the stock of *L. terrestris* prior to the commencement of experiment 2, and the performance of this species under CGW treatments was not investigated. The steady mass recorded for this species in experiment 1 suggests this might have been a suitable species choice for the reclaimed soil at Ingrebourne Hill, however this was likely influenced by the application of horse manure as a feedstock (Lowe and Butt, 2005). This species has not demonstrated positive responses to CGW application on agricultural land (Stroud *et al.*, 2016), and has proven to be an inappropriate species choice for a number of land reclamation-based experiments due to intolerance for soil conditions on early reclaimed sites (e.g. compaction and low availability of organic matter) (Butt, 2008, 2011b).

In experiment 2, all species exhibited higher final mass in the two de-faunated soil treatments than the two fresh soil treatments, suggesting there may be biotic factors in the fresh soil which negatively affected earthworm growth. Biotic factors proposed in the literature include pathogens, predators or other earthworms (and therefore competition for resources) (Butt, 2011b). However, very few non-experimental earthworms were found within the soils at termination of the experiment, so this is unlikely to have been the cause of the biotic influence. Soil moisture content is considered to be one of the most important environmental factors affecting earthworm activity (Lowe and Butt, 2005). It is possible that the different manipulations between de-faunated and fresh reclaimed soil (i.e. air drying, crushing and sieving to remove large stones) changed the soil texture between the two treatments, with subsequent differences in the force with which available water is held by the soil (soil water potential - kPa). This would not have affected the measurable total soil moisture content, but would have undoubtedly influenced earthworm activity, and may explain the performance differences between the two soil treatments (Lowe and Butt, 2005).

Further experiments with a variety of additional earthworm species (preferably in monoculture and in species combinations), in a range of different reclaimed soil types would provide informative results for future restoration projects. Any such experiments should take place over a lengthy timeframe (multiple years) to be fully informative and relevant to field experiments. Investigating a range of CGW application rates (realistic to application in the field) may enable the identification of an optimum rate for supporting sustainable earthworm populations for future land restoration activities.

7.4.2. Summary of chapter findings

The findings of this research were as follows, summarised against the original chapter objectives:

1. Incorporation of CGW into reclaimed soil is sufficient to support populations of *A. longa* and *A. chlorotica*, although repeat CGW addition to the soil surface may be required to ensure that these populations are sustainable.
2. Horse manure application produced greater earthworm growth and cocoon output in reclaimed soil compared with CGW.
3. The earthworm species *A. longa* and *A. chlorotica* were found to be most suited to the experimental conditions at the Ingrebourne Hill site, thus supporting their use in the field experiments previously presented in Chapters 5 and 6.
4. Fresh reclaimed soil may possess factors which negatively affect earthworm growth, e.g. pathogens and competitors, or soil moisture content irregularities due to texture heterogeneity. De-faunation and homogenisation of such soil prior to use in laboratory experiments can improve earthworm performance.

8. ASSESSING LEAF FOLIAR MATERIAL PREFERENCE BY EARTHWORMS IN RECLAIMED SOIL

8.1. Introduction and objectives

In restored woodland, as with natural woodland, the primary form of organic matter added to the soil is leaf litter. Earthworms are capable of incorporating very large quantities of organic matter into the soil, for example in temperate forests, earthworms may have the capacity to incorporate the total annual litter fall (Raw, 1962; Satchell, 1967; Edwards, 2004). Tree health is influenced by soil quality, however the soil materials typically used during landfill restoration contain low levels of plant nutrients and organic matter, which can inhibit the growth of vegetation and the activity of soil organisms (Bending *et al.*, 1999; Dickinson *et al.*, 2005). Research indicates that earthworm-mediated mineralisation of organic matter, improvement in nutrient availability, and subsequent improvements in plant growth, are likely to be greater in nutrient-poor soils (Jana *et al.*, 2010).

Tree leaf litter palatability to soil fauna has been shown to strongly influence soil faunal population development (Swift *et al.*, 1979), and different tree species have been shown to influence soil quality and soil faunal communities through the quality and quantity of their leaf litter (Pigott, 1989; Muys *et al.*, 1992; Reich *et al.*, 2005). It is therefore of value, when planning woodland restoration, to understand whether the tree species planted are likely to provide litter which supports and encourages beneficial soil faunal communities to develop, which in turn will support healthy vegetation growth and provide soil functions and wider ecosystem services (Kibblewhite *et al.*, 2008; Rajapaksha *et al.*, 2013). It has been demonstrated that certain earthworm species can distinguish between, and may show a preference for, specific types of leaf litter. Darwin (1881) observed earthworm preference for leaves and noted that leaf shape influenced their selection behaviour. Satchell and Lowe (1967) conducted a leaf litter choice experiment in a laboratory setting using *Lumbricus terrestris*, which were provided with uniform disks of leaf material from a range of European temperate forest tree species. They found that *L. terrestris* exhibited a preference for common alder (*Alnus glutinosa*), common ash (*Fraxinus excelsior*), common elder (*Sambucus nigra*) and wych elm (*Ulmus glabra*) over European larch (*Larix deciduas*), sessile oak (*Quercus petraea*) and European beech (*Fagus sylvatica*). Furthermore, a preference for weathered (partially decomposed) over unweathered litter material was exhibited by the earthworms. Some researchers have adopted a method of investigating earthworm litter choice in the field by using litterbags. For example, Edwards and Heath (1963) found that, in a litterbag experiment, *L. terrestris* showed preference for oak over beech litter. Using litterbags, Hendriksen (1990) investigated the litter preference of a range of *Lumbricus spp.* across ecological groups in open pasture. Results indicated that geophagous (endogeic) species demonstrated a preference for lime over alder and elm leaves, and detritivorous (anecic and epigeic) species preferred ash, lime and alder litter more than oak and beech.

The earthworm choice-chamber experimental design, as developed by Doube *et al.* (1997), enabled earthworm feeding preferences to be efficiently measured over time in a laboratory setting, by measuring rate of feedstuff removal from detachable microtube food containers. The experiment was used to investigate the feeding preference of *L. terrestris*, *Aporrectodea longa*, *Aporrectodea caliginosa* and *Lumbricus rubellus* for a range of organic waste and soil combinations, alongside sycamore leaf litter. Results showed that all four species of earthworm preferred pure soil and a soil-leaf litter mixture over organic waste mixed with soil, suggesting that soil enhanced litter palatability. However, a number of methodological issues were identified which may have impacted earthworm selection – most notably a lack of soil in the main chamber of the experiment (instead a moist filter paper was used), which may explain why earthworms preferred the soil-based food options (Doube *et al.*, 1997a). Neilson and Boag (2003) and Rief *et al.* (2012) adopted a similar feeding chamber approach to Doube *et al.* (1997), also using moist filter paper in the main chamber of the experimental setup. In these experiments, the researchers did not investigate tree leaf litter as a food source for earthworms, but rather the leaf material of a selection of grasses, herbs and shrubs. Rief *et al.* (2012) found that *L. rubellus* did not distinguish preferentially between plant leaf types, but rejected fresh plant leaves in favour of weathered litter material.

Identifying a lack of investigations into the litter feeding preference of a variety of European earthworm species for both native and non-native tree species, Rajapaksha *et al.* (2013) conducted a series of such litter preference studies. In that study, the researchers built upon the experimental design of Doube *et al.* (1997), by adding Kettering loam to the choice chambers to provide more natural conditions for the earthworms. Unlike the studies described above, Rajapaksha *et al.* (2013) investigated the litter preference of a range of European species of earthworm; *L. terrestris*, *A. longa* (both anecic and surface-feeding), *Allolobophora chlorotica* and *A. caliginosa (*both endogeic and geophagous). They identified the preference of these earthworm species for litter of a variety of native tree species with Short Rotation Forestry potential: common alder (*A. glutinosa*), common ash (*F. excelsior*), silver birch (*Betula pendula*), sweet chestnut (*Castanea sativa*) and sycamore (*Acer pseudoplatanus*), and an exotic Eucalyptus species (*Eucalyptus nitens*). Results indicated that all four earthworm species preferred the leaf litter of *A. glutinosa*, *B. pendula*, and *F. excelsior* over *A. pseudoplatanus*, *C. sativa* and *E. nitens*. However, not all earthworm species performed equally in litter removal. The two anecic earthworm species, *L. terrestris* and *A. longa* showed a rapid and clear pattern of leaf litter removal from choice chambers. The endogeic and geophagous species *A. caliginosa* showed a slow but clear pattern of litter removal, and the endogeic *A. chlorotica* recorded the slowest litter removal from choice chambers. Despite reduced rates of litter removal compared to the anecic species; both endogeic species indicated a similar pattern of litter removal to the anecic earthworms. Rajapaksha *et al.* (2013) observed that the differences in rate of litter removal by different species of earthworms is likely to be associated with differences in feeding behaviour between the species' ecological groupings (i.e. surface feeding versus soil organic matter ingestion) and differences in body size between species.

Previous studies have helped to identify the chemical and physical parameters of litter which influence litter palatability to earthworms. The chemical composition of litter appears to strongly influence earthworm selectivity, in particular aspects such as the C:N ratio and the content of nitrogen, calcium, lignin and polyphenols (Satchell and Lowe, 1967; Hendriksen, 1990). Earthworm preference for weathered/decomposed over unweathered/undecomposed litter material has been linked to bacterial and fungal activity, suggesting that the activity of micro-organisms enhances litter palatability (Satchell and Lowe, 1967; Wright, 1972; Cooke and Luxton, 1980; Cooke, 1983).

With the exception of Rajapaksha *et al.* (2013), the majority of laboratory-based earthworm feeding preference studies have either looked at how earthworm species respond to non-tree leaf material, or how the well-documented earthworm species *L. terrestris* responds to tree litter (Satchell and Lowe, 1967; Doube *et al.*, 1997a; Neilson and Boag, 2003). The findings of Rajapaksha *et al.* (2013) show how four European earthworm species respond to the litter of a set of common temperate tree species, using standard Kettering loam soil as a substrate. However, none of these results may be comparable to the activity of the same earthworms in woodland on reclaimed landfill sites, where alternative tree species and more inhospitable soil materials are likely to be present. Additionally, as identified by Rajapaksha *et al*. (2013), there is as yet no information on how a combination of anecic and endogeic earthworm species perform in choice chamber feeding experiments, which would provide results more comparable to field conditions. Furthermore, most studies have focused on the use of fallen, weathered leaves as feedstock for earthworms, described as 'leaf litter' (e.g. Satchell and Lowe, 1967; Hendriksen, 1990, Rajapaksha *et al.*, 2013). Little information is available on the suitability of green or 'foliar' leaf material as an earthworm food source, particularly in the form of feeding preference studies. In one of the few studies available, Butt (2011) used dried green *B. pendula* leaves as feedstock for *L. terrestris*, and found that switching to green leaves from dried senesced leaves resulted in increased mass and significantly increased cocoon production. This was attributed to the larger nitrate content in green leaves enabling more rapid protein synthesis for growth and reproduction. After 5 months, the feedstock was switched back to dried fallen leaves, resulting in a reversal of this trend. This suggests that for experiments, dried green leaves may be a superior quality food for earthworms than dried, fallen and weathered leaves; however, more data is required from a range of tree and earthworm species.

The choice-chamber experiment presented in this chapter was designed to address the gaps identified above, by including earthworm species combinations, fresh leaf material from previously un-investigated tree species, and which are more suited to a restored woodland setting, and using soil from the studied Ingrebourne Hill reclaimed landfill site (Chapter 5). This enabled the experiment to complement the field experiments at Ingrebourne Hill and Headley Nursery (Chapter 6), by investigating the likely behaviour of the earthworms following inoculation into the soil in these field experiments, and identifying the influence of reclaimed soil on the survival and feeding behaviour of the two earthworm species used. There is currently no information on the palatability of Norway maple (*Acer platanoides*) and Italian alder (*Alnus cordata*) leaf material as a food source for earthworms. By following a similar experimental design and including Kettering loam as a control soil treatment, results from this experiment may be compared to those of Rajapaksha *et al.* (2013).

The specific objectives of this study were to:

1. measure the palatability of *A. platanoides* and *A. cordata* foliar material as a food source for earthworms, and influence on earthworm mass and survival,
2. measure the effect of reclaimed soil on earthworm mass, survival and foliar selection behaviour, compared to a control (Kettering loam) soil,
3. investigate the above for a combination of endogeic and anecic earthworm species, to obtain results relevant to the field experiments conducted in this thesis.

8.2. Materials and methods

To assess the leaf material preference of earthworms, a choice chamber experiment was set up under controlled environmental conditions. This experiment utilised the choice chamber design described by Rajapaksha *et al.* (2013), which is a modified version of the original choice chamber of Doube *et al.* (1997) and Rief *et al.* (2012). This design allows for earthworm food preference to be regularly monitored and quantified by removal of feeding tubes, with minimal disturbance to the central chamber and resident earthworms. The addition of soil to the central chamber rather than moist filter paper (e.g. Doube *et al.*, 1997) provides more natural environmental conditions for endogeic and anecic earthworm species, and in this experiment also allowed for comparison between two soil types. Whilst senesced leaf litter has been used as an experimental food source in previous experiments (e.g. Rajapaksha *et al.*, 2013), this experiment adopted the use of freshly collected tree foliar material. This provided a novel investigation into foliar material as a feedstock for earthworms.

8.2.1. Choice chamber design

The choice chamber design consisted of a circular aluminium foil tray (0.16 m diameter and 0.03 m depth) with standard Eppendorf tubes (0.01 m diameter and 0.04 m depth) embedded into the tray walls as food containers. To enable the tubes to be affixed to the trays and allow earthworm access to tube contents, the caps were removed from the tubes and a hole of approximately 0.01 m diameter was made in each cap (Rajapaksha *et al.*, 2013). An equally-sized hole was then made in the wall of the tray and the caps placed on the inside of the hole, enabling the tubes to be attached on the outside wall of the tray and held in place by the caps (Figure 8.1a). This enables the tubes to be removed from the caps and replaced without disturbing the contents of the main chamber. Tubes were spaced equally around the tray (Figure 8.1b).

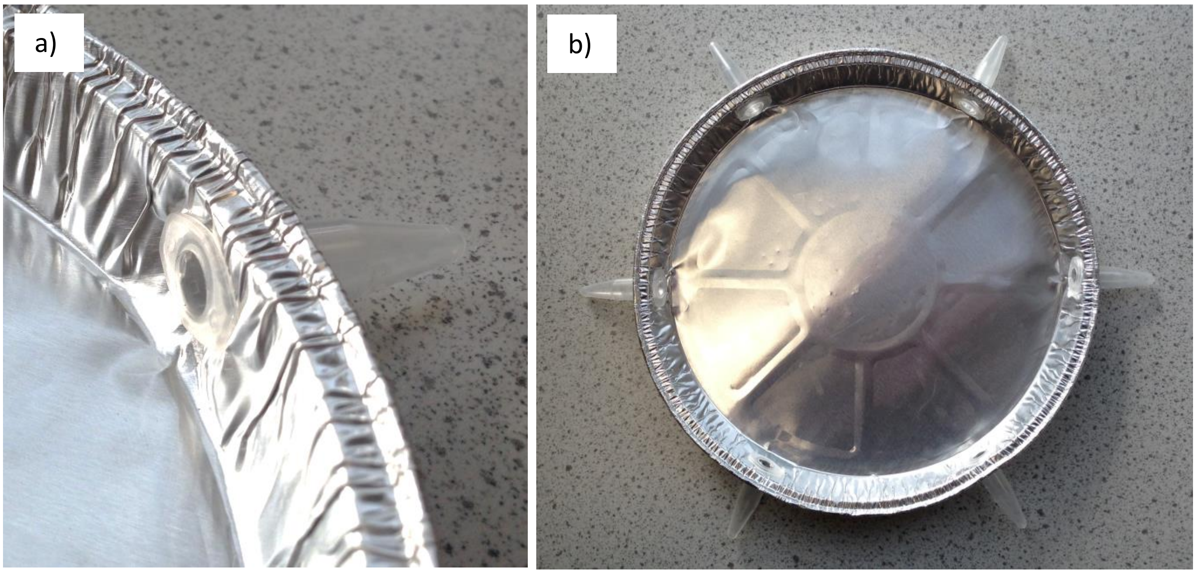


Figure 8.1.a) Empty choice chamber prior to experiment, b) empty Eppendorf tube food vessel fixed to wall of tray via drilled cap.

Prior to the experiment, empty Eppendorf tubes were affixed to the trays, and each tray was filled with soil (details in section 8.2.2) at 25% moisture content. Earthworms were then randomly selected, had mass determined and allocated to the trays according to the species combination treatments, and sprayed with water. To prevent earthworm escape, trays were covered with aluminium foil held in place by an elastic band (Figure 8.2a). Small holes were made in the foil with a mounted needle to allow for air circulation whilst maintaining soil moisture content. All trays were then stored in total darkness in a temperature-controlled incubator at 15^{o}C for a period of 24 hours, to allow earthworms to equilibrate to the experimental conditions (Figure 8.2b).

Figure 8.2 a) Choice chamber with feeding tubes and foil cover attached, b) storage of choice chambers in a temperature and light-controlled incubator at 15°C.

Air-dried leaf materials (originally freshly collected from the field by removing fresh leaves from trees adjacent to experimental plots of the field experiment in Chapter 5) of *A. platanoides* and *A. cordata* were separately ground using a MAGIMIX 4150W food processor, and sieved to obtain leaf particles of 1 - 2 mm size. Particle size has been shown to influence earthworm selection of food material (Lowe and Butt, 2003), and this size range was chosen to prevent this being an issue. A sub-sample of both tree species leaf materials was retained for chemical composition analysis, the results of which are presented in Table 8.1. Fresh Eppendorf tubes were individually labelled, had mass determined and filled with dried and sieved leaf particles of either tree species (between 0.2-0.3 g per tube), and had mass re-determined. The leaf-filled tubes were then topped-up with water and left to soak for 2 hours, and inverted on absorbent paper for 5 minutes to drain excess water away. Tubes then had mass re-determined to obtain the wet starting mass of the leaf materials. These tubes were then assigned to specific choice chambers and used to replace the empty Eppendorf tubes, thus marking the start of the experiment. Throughout the duration of the experiment, as during the initial acclimation period, the choice chambers were maintained in a temperature-controlled incubator at 15°C, in total darkness (Figure 8.2b).

8.2.2. Experimental treatments

Leaf materials from two tree species were selected for use in this experiment; these were Norway maple (*A. platanoides*) and Italian alder (*A. cordata*) (Figure 8.3). Three feeding tubes for each species leaf material were placed in alternating order around each tray, with a total of 6 tubes per tray (figure 8.4). In total, there were five replicate trays per earthworm species treatment, per soil type (n = 30 trays).

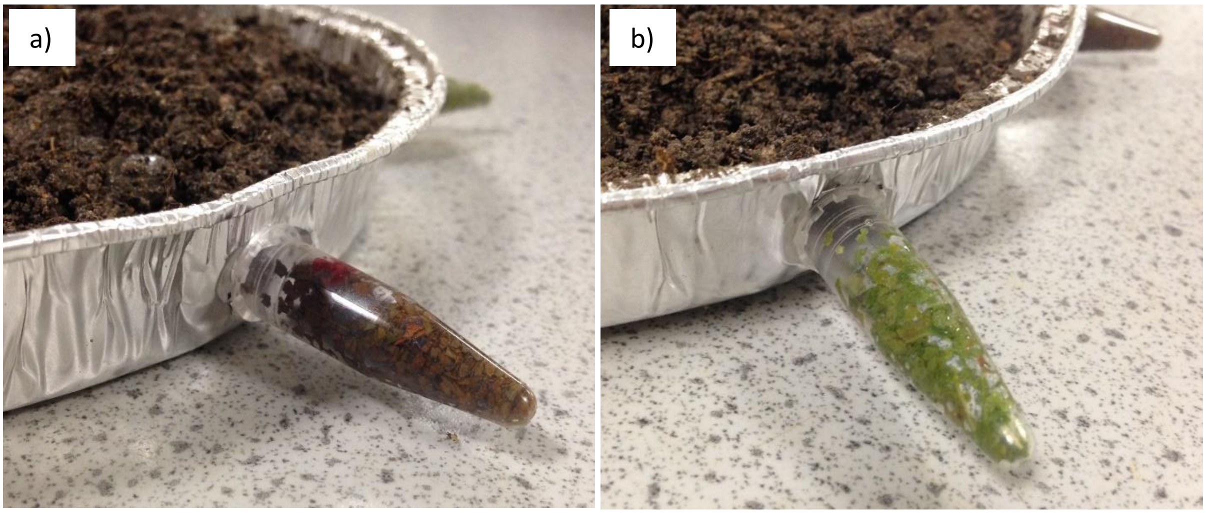


Figure 8.3. Feeding tubes filled with wet leaf material, attached to choice chamber tray units at the beginning of the experiment: a) Italian alder (*A. cordata*), b) Norway maple (*A. platanoides*).

This experiment investigated the leaf material preference of two earthworm species: *A. longa* (anecic) and *A. chlorotica* (endogeic) with initial individual mean masses of 2.30 (± 0.11) and 0.26 (± 0.01) g, respectively. Each earthworm species was introduced to separate choice chambers in the following numbers, according to treatment; monoculture of *A. longa* (4), monoculture of *A. chlorotica* (20), or a mixed culture of *A. longa* and *A. chlorotica* (2 and 10, respectively). These numbers were selected for similar earthworm biomass across trays independent of earthworm treatment, and to ensure a quantifiable rate of leaf material removal within the short timeframe of the experiment. All earthworms were collected from agricultural pasture at Walton Hall Farm, Preston, UK (Nat. Grid Ref: SD 55050 28100), via digging and hand-sorting of soil. The *A. chlorotica* used in this experiment were of mixed pink and green morph (Lowe and Butt, 2008), however all were selected to be of similar biomass, and morph was not considered to be a limiting factor in this experiment as reproductive output was not one of the measurements.

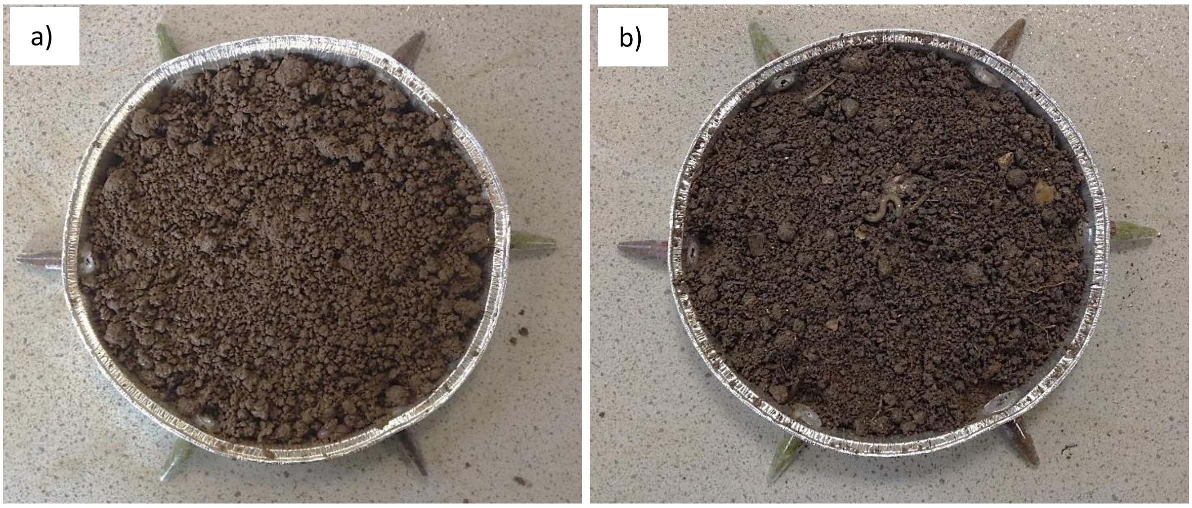


Figure 8.4. Prepared choice chambers at the start of the experiment: a) Kettering loam treatment, b) reclaimed soil treatment (with *A. chlorotica* at soil surface after addition).

The two soil treatments (Figure 8.4) chosen for this experiment were: Kettering loam (Boughton Loam, Kettering, UK), which is a standard substrate for use in general earthworm experiments and choice chamber experiments (Butt *et al.*, 1994b; Rajapaksha *et al.*, 2013), or de-faunated anthropogenic soil materials taken from a field experiment site at Ingrebourne Hill, Rainham, UK (see Chapter 5). The use of Kettering loam as a control substrate enabled direct comparisons to be made between the results of this study and those of the only other existing leaf preference experiment, which also used Kettering loam (Rajapaksha *et al.*, 2013). De-faunated, field-collected reclaimed soils were sieved to remove materials >4 mm, then frozen at -5^{o}C for 7 days to destroy native earthworms and other potential competitors and predators (Butt, 2011b).

At intervals of three days, choice chambers were removed from the incubators to allow the rate of leaf material consumption to be measured. This was achieved by removing the assigned food tubes and measuring the mass loss of each individual tube. Each tube was then re-attached in the same location. During measurement periods, each tray had its foil lid removed and was inspected for signs of any dead earthworms, with any mortalities being recorded. Soil moisture content was maintained in each tray by spraying each with an equal amount of water during measurement. The experiment was terminated after 27 days, or earlier for any tray when all leaf material had been removed from the feeding tubes. At termination of the experiment, earthworm survival and final masses were recorded for each tray.

8.2.3. Statistical analysis

Leaf material removal from feeding tubes was regularly measured by determining the mass of each tube, and subtracting the remaining leaf mass from the original mass per tube, having discounted the mass of the tube itself. Earthworm preference was assumed to be associated with leaf removal, i.e. more material removed (%) indicates greater earthworm preference and vice versa. Statistical analysis was performed using the freeware statistical software R, version 3.2.2. "Fire Safety" and the R Studio desktop software, version 0.99.486 (R Core Team, 2015; RStudio Team, 2015). Data were first tested for normality using the Shapiro-Wilk test, which is suited to smaller sample sizes (in this case n=5). As all leaf removal data for each species and soil treatment had a normal distribution, the data were analysed using one and two-way analysis of variance (ANOVA), and two-way repeated measures ANOVA. One and two-way ANOVA was run on the leaf removal data at the point at which 50% total leaf material (per tree species) was removed from trays, similar to Doube *et al.* (1997) and Rajapaksha *et al.* (2013). Two-way repeated measures ANOVA was run on the complete dataset across all time points, to investigate the influence of experiment duration alongside treatments on earthworm leaf material removal.

8.3. Results

The choice chambers enabled accurate monitoring of earthworm feeding behaviour, with clear visual and gravimetric evidence of leaf foliar material removal throughout the experiment, and a generally similar pattern for all species combinations across soil treatments.

8.3.1. Effect of earthworm species combinations and soil type on consumption of leaf material

Table 8.1 provides the average chemical composition of both soil treatments at the start of the experiment. The reclaimed soil treatment possessed a higher pH, conductivity, total C, organic carbon, organic matter (%), C:N ratio and total K (%) than the Kettering loam treatment. The loam soil had a higher total N (%) and Ca, and both soils had similar levels of Na and Mg.

Table 8.1. Mean selected soil parameters at the start of the experiment, ± SE (n = 3).

Parameter	Soil type	
	Kettering Loam	Reclaimed Soil
pH	7.85 ± 0.03	8.13 ± 0.02
Cond. (µs/cm)	748 ± 31.3	1559 ± 98.0
Total N (%)	0.27 ± 0.00	0.21 ± 0.00
Total C (%)	3.04 ± 0.02	4.56 ± 0.09
C (Org) (%)	2.73 ± 0.03	3.41 ± 0.04
O.M. (%)	4.71 ± 0.05	5.88 ± 0.07
C (org):N ratio	10.01 ± 0.11	16.06 ± 0.12
Total K (mg/kg)	188 ± 1.8	461 ± 1.4
Ca	4324 ± 3	3933 ± 64
Mg	119.8 ± 0.2	121.0 ± 0.7
Na	23.55 ± 0.17	19.65 ± 0.51

Table 8.2 shows earthworm performance across treatments at the start and at termination of the experiment. After 27 days, 100% survival was recorded for *A. longa* across all treatments. *A. chlorotica* had 98-99 % survival in reclaimed soil, while much lower (35-46%) in the loam treatment. *A. chlorotica* lost mass across all treatments (range of -4.0 to -41.0%), *A. longa* lost mass in the monoculture loam treatment combination (-1.89% loss) and gained mass across all other treatment/species combinations (+15.5 to +20.0% gain). The largest gain in mass was *A. longa* in mixed species under loam soil (+20.0%).

Table 8.2. Data for selected earthworm parameters across each treatment at the start and termination of the experiment (after 27 days).

Soil	Earthworm species (treatment)	Number of earthworms (ind tray^{-1})	Initial mean earthworm mass (g ind^{-1})	Final mean earthworm mass (g ind^{-1})	Change in mass (%)	Survivorship (%)
Loam	*A. longa* (mono)	4	2.49	2.45	-1.9	100
	A. chlorotica (mono)	20	0.26	0.15	-41.0	35
	A. longa (mixed)	2	2.48	2.97	+20.0	100
	A. chlorotica (mixed)	10	0.26	0.20	-25.8	46
Reclaimed	*A. longa* (mono)	4	2.09	2.42	+15.5	100
	A. chlorotica (mono)	20	0.23	0.22	-4.0	99
	A. longa (mixed)	2	2.12	2.48	+17.1	100
	A. chlorotica (mixed)	10	0.23	0.22	-6.6	98

Figures 8.5 to 8.8 show the pattern of leaf litter removal from choice chambers by all three earthworm species combinations supplied with *A. cordata* and *A. platanoides* foliar material over 27 days for both soil treatments. The *A. longa* monoculture showed an average litter removal in loam soil of 100% (*A. cordata*) and 92.1% (*A. platanoides*), and 99.4% (*A. cordata*) and 95.6% (*A. platanoides*) under the reclaimed soil treatment (Figure 8.8). After 12 days, *A. longa* had removed 72% and 48% of *A. cordata* foliar material in the loam and reclaimed soil treatments, respectively. At the same time point, only 13% and 18% of *A. platanoides* foliar material was removed (Figure 8.5). After 12 days, the rate of *A. platanoides* leaf material removal by *A. longa* rapidly increased.

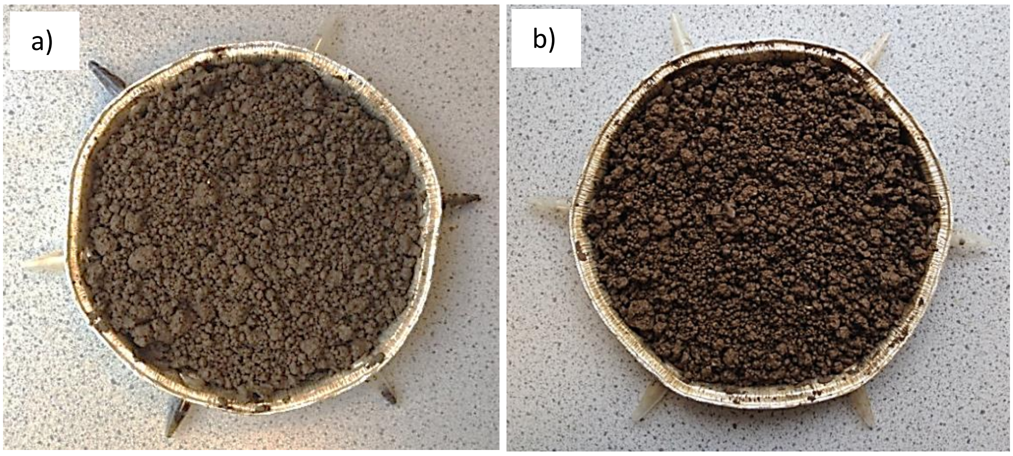


Figure 8.5. Leaf litter removal from food tubes by *A. longa* in the Kettering loam soil treatment. At two stages of the experiment: a) 12 days into the experiment, the three *A. cordata* litter tubes are almost emptied, the three *A. platanoides* tubes are still mostly full, b) after 27 days all food tubes emptied.

The *A. chlorotica* monocultures had an average litter removal in loam soil of 7.6% (*A. cordata*) and 5.9% (*A. platanoides*), and 8.9% (*A. cordata*) and 6.2% (*A. platanoides*) under the reclaimed soil treatment. Despite the large difference in survivorship between *A. chlorotica* in the loam and reclaimed soils (35 and 99%, respectively, Table 8.2), there was little difference in final litter removal between treatments (see also Figure 8.6). Foliar material removal by *A. chlorotica* was linear throughout the experiment, although far reduced compared with *A. longa. A. cordata* was initially preferred over *A. platanoides*, as observed for the other earthworm species combinations.

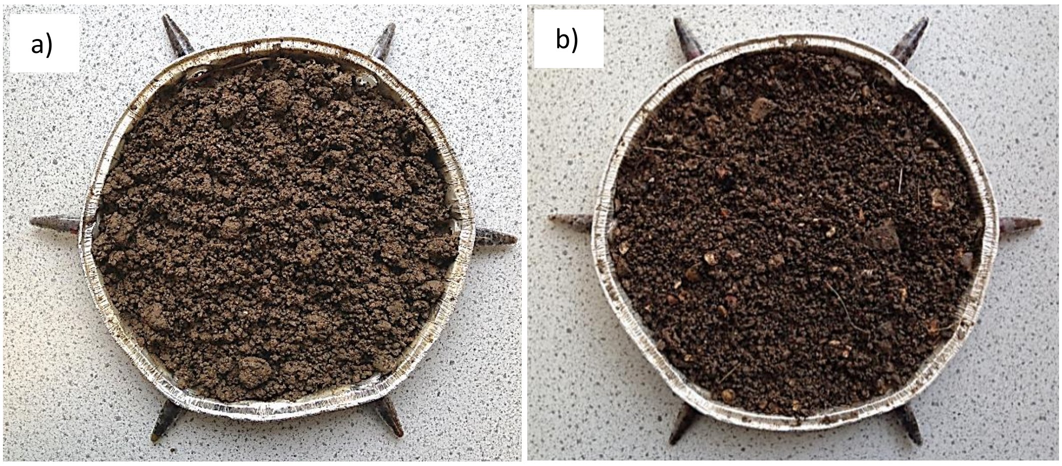


Figure 8.6. Leaf litter removal from food tubes by *A. chlorotica* at the termination of the experiment after 27 days for the two soil treatments: a) loam soil, b) reclaimed soil.

The mixed earthworm species treatment showed an average litter removal in loam soil of 88.8% (*A. cordata*) and 82.3% (*A. platanoides)*, and 91.7% (A. cordata) and 86.7% (*A. platanoides)* under the reclaimed soil treatment. After 12 days, the combined *A. longa* and *A. chlorotica* had removed 43.2% and 33.8% of *A. cordata* foliar material in the loam and reclaimed soil treatments, respectively. At the same time point, only 6.7% and 5.2% of *A. platanoides* foliar material was removed. As observed for the *A. longa* monocultures, after 12 days, the rate of *A. platanoides* leaf material removal by the combined earthworm species rapidly increased under both soil treatments.

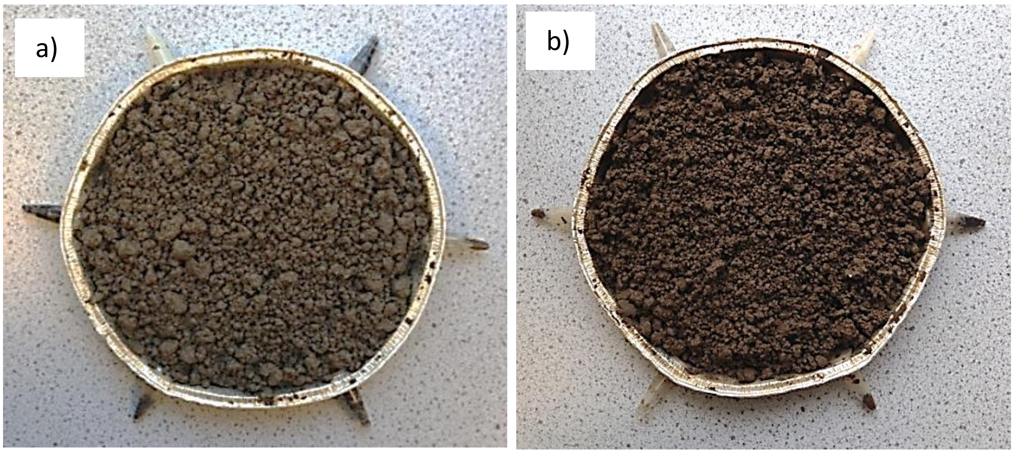


Figure 8.7. Leaf litter removal from food tubes by combined *A. longa* and *A. chlorotica* in the Kettering loam soil treatment. At two stages of the experiment: a) 12 days into the experiment, over half the *A. cordata* foliar material is removed, all three *A. platanoides* tubes are still full; b) after 27 days all food tubes are partially empty.

For *A. longa* in the loam soil treatment, at 15 days (the point of 50% total leaf removal) the remaining leaf material of *A. cordata*, was significantly less than *A. platanoides* (ANOVA, F (1, 8) = 25.66, p < 0.001, see Table 8.4). In the reclaimed soil treatment, *A, longa* displayed a similar pattern of litter removal, which was also statistically significant (ANOVA, F (1, 8) = 9.77, p = 0.014). There was also a significant effect of soil on leaf material removal (two-way repeat measures ANOVA, F (1, 16) = 6.39, p = 0.022). The combined species treatment showed a similar, although less pronounced litter preference result to *A. longa* monocultures and the results were not statistically significant. *A. chlorotica* showed a clear trend of litter removal, although 50% was not reached at termination of the experiment after 27 days. As with the other earthworm species treatments, *A. chlorotica* consumed more *A. cordata* than *A. platanoides* leaf material, in both soil treatments.

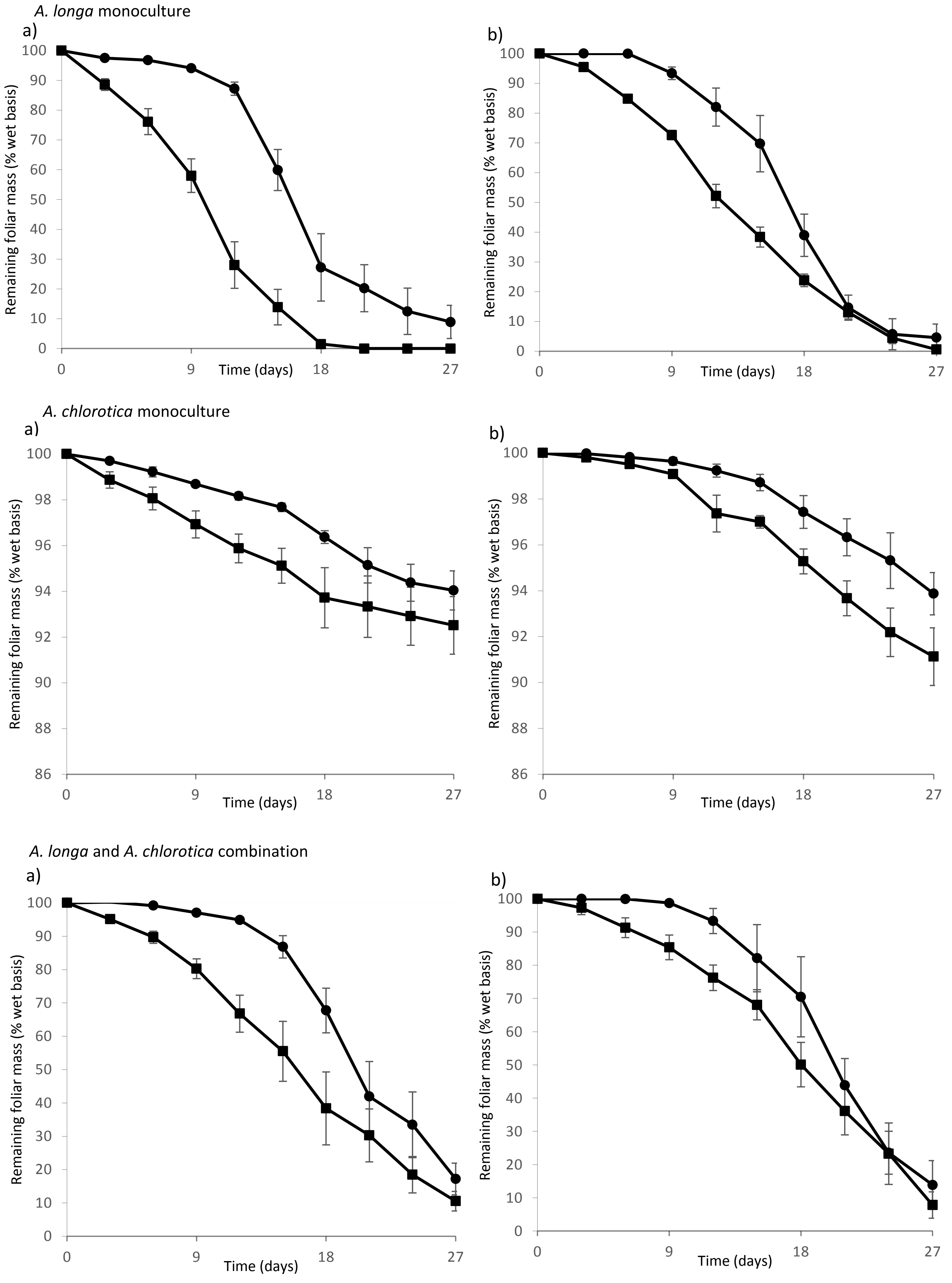


Figure 8.8. Mean (± SE) foliar mass remaining (% wet basis) in choice chamber experiment for the mixed earthworm species treatment. Earthworm combinations as labelled, in (a) loam soil and (b) reclaimed soil. Tree foliar species: *A. platanoides* (●) and *A. cordata* (■).

8.3.2. Effect of leaf palatability on consumption by earthworms

Table 8.3 provides the results of chemical analysis of leaf material at the start and finish of the experiment (bulked material remaining in *A. chlorotica* monoculture tubes after 27 days), and a sample of *A. platanoides* litter from non-experimental trees at Ingrebourne Hill (Chapter 5). Insufficient *A. cordata* leaf litter was available in the field for collection and analysis. Both tree species leaf material showed an increase in total N, total C, P, Ca and Mg (%) at termination of the experiment, and a reduction in C: N ratio and total K (%). At the start of the experiment, *A. cordata* leaf material had higher total N (%) and lower C: N ratio and Ca (%) than *A. platanoides* leaves. The *A. platanoides* foliar material used in this experiment had a two-fold increase in total N (%), but similar total C (%) compared with the field-collected leaf litter for this species, with an associated reduction in C: N ratio of roughly 50%.

Table 8.3. Chemical analysis of leaf foliar material at experiment start and termination (27 days).

Parameter	*A. cordata*		*A. platanoides*		
	Start	End	Start	End	Litter
Total N (%)	2.76	3.62	1.59	2.27	0.79
Total C (%)	52.60	54.90	47.86	48.10	46.96
C:N	19.06	15.15	30.16	21.15	59.68
P (%)	0.13	0.14	0.15	0.17	0.11
Ca (%)	1.16	1.42	1.98	2.38	2.69
K (%)	0.95	0.84	1.21	1.20	0.55
Mg (%)	0.20	0.22	0.22	0.26	0.24

Table 8.4 displays the remaining leaf litter (%) at 50% of total litter removal for each series of choice chambers in the experiment, the point of which varied with earthworm species combinations, but did not vary across soil treatments; *A. longa* (15 days) *A. chlorotica* (50% not removed by experiment termination at 27 days), and mixed species (21 days). At the point of 50% removal, all earthworm species combinations showed a clear preference for *A. cordata* over *A. platanoides*.

Table 8.4. Mean (± SE) remaining leaf foliar material (% from original mass) in choice chambers of different earthworm species and soil substrate at the point of 50% total litter removal.

Soil	Earthworm Species	Days taken to remove 50% total foliar material	Tree species	
			A. cordata	*A. platanoides*
Loam	*A. longa*	15	13.9 ± 5.9 [a]	59.9 ± 6.9 [b ***]
	A. chlorotica	Not achieved	92.5 ± 1.3 [a]	94.0 ± 0.9 [a]
	Mixed Sp.	21	30.3 ± 7.9 [a]	41.9 ± 10.5 [a]
Reclaimed	*A. longa*	15	38.3 ± 3.3 [a]	69.7 ± 9.5 [b*]
	A. chlorotica	Not achieved	91.1 ± 1.3 [a]	93.9 ± 0.9 [a]
	Mixed Sp.	21	36.1 ± 7.2 [a]	43.9 ± 8.0 [a]

Different letters in a row indicate significant differences, ANOVA, n = 5, * p = <0.05, *** p = <0.001.

Using the results for loam soil treatment presented in Table 8.3, the leaf foliar removal data of *A. longa* and *A. chlorotica* can be compared to the litter preference data for these earthworm species presented by Rajapaksha *et al.* (2013). By comparing the remaining leaf material (%) at the point of 50% total removal in loam soil, earthworm preference for *A. cordata* and *A. platanoides* can be used to compare the leaf litter preference list of Rajapaksha *et al.* (2013), see Table 8.5.

Table 8.5. Tree litter and foliar preference list of Rajapaksha *et al.* (2013), compared with the results of this experiment (in bold) as appropriate for Kettering Loam. Tree species: *Alnus glutinosa* (Ad), *Fraxinus excelsior* (Ah), *Betula pendula* (Br), *Eucalyptus nitens* (En), *Castanea sativa* (Sw), *Acer pseudoplatanus* (Sy), *A. cordata* (Ia) and *A. platanoides* (Nm).

Earthworm species	Tree litter preference order
A. longa	Ad, Ah, Br, **Ia** > En, **Nm** > Sy, Sw
A. chlorotica	Ad, Ah, Br > En, Sy > **Ia**, **Nm**, Sw

8.4. Discussion

8.4.1. Earthworm combinations

All three earthworm treatments demonstrated a clear preference for the foliar material of *A. cordata* over that of *A. platanoides*. The anecic species *A. longa*, either as monoculture or part of a species combination, displayed swift removal of foliar material. In woodland habitat this species feeds directly on leaf litter material on the soil surface, pulling the material into vertical burrows in the soil (Satchell, 1983). By comparison, the endogeic earthworm species *A. chlorotica*, which primarily feeds on organic matter within the soil, demonstrated a much slower removal of leaf material; yet this species also showed a clear preference at the outset of the experiment for *A. cordata* over *A. platanoides* foliar material. Similar trends in relative rates of litter removal from choice chambers was observed by Rajapaksha *et al.* (2013) for different earthworm species representatives of the same two ecological groupings: *L. terrestris* (anecic) and *A. caliginosa* (endogeic). This was attributed to the different feeding behaviours and the differences in physical size between the two species.

In the current experiment, there was little total leaf consumption by *A. chlorotica* in both soil types, indicating that this feeding experiment design is not particularly suited to this species and/or ecological group. This is likely due to the geophagous nature of this species, and as such future feeding experiments involving endogeic geophagous species should take this feeding mechanism into account. As also found by Rajapaksha *et al.* (2013), this study suggests that the current choice chamber design was better suited to larger and litter-feeding earthworm species than smaller and soil-feeding earthworms. If future choice-chamber experiments are to use geophagous earthworms, the experimental design may require modification, e.g. using smaller leaf litter particle sizes, mixed with a known mass of soil. For example, Doube *et al.* (1997) found that soil and organic matter mixtures were more preferable to geophagous earthworms than pure organic matter, although these findings may have been biased due to an experimental design issue whereby there was a lack of soil in the main chamber of this experiment. As such, the active selection of soil by earthworms may not have been as a food source, but also as a more comfortable environment in which to live.

Earthworm body size and food particle size may have also influenced leaf foliar material removal. Neilson and Boag (2003) observed a low removal of food by *A. chlorotica* during a choice experiment, and found that for the six earthworm species investigated, the mass of food removed was positively correlated with earthworm body size. Food particle size has been demonstrated to influence intake by earthworms, with reduced particle size generally being of greater benefit to smaller earthworms; however the effects of food size on growth and reproduction may be both species and life-stage specific (Boyle, 1990; Lowe and Butt, 2003). In an experiment conducted by Boyle (1990), food particles of <0.2 mm resulted in a doubled weight of *A. caliginosa* after 150 days, compared with individuals of the same species fed food particles ranging from 0.2 mm to 1.0 mm. The leaf particles used in the experiment presented in this chapter were uniform and ranged in size between 1 and 2 mm, which is the size range used in a comparable experiment by Rajapaksha *et al.* (2013). Whilst this enables current results to be compared to those of that study, food size may have been a negative influence on the intake of leaf material by the smaller-bodied earthworm species *A. chlorotica*.

The addition of an anecic earthworm species might be expected to provide benefits to an endogeic earthworm species, through comminution and incorporation of leaf litter into the soil where it can be more easily consumed (e.g. Lowe and Butt, 2003). Studies identifying mutualistic or competitive effects of combinations of earthworm species are few in number, but have investigated a range of UK native earthworm species across all three ecological groupings (sensu Bouché, 1977) (Edwards and Lofty, 1978; Butt, 1998; Lowe and Butt, 1999). In controlled laboratory experiments, Butt (1998) and Lowe and Butt (1999) investigated the influence of inter- and intra-specific interactions on earthworm growth rates and reproductive output. Results indicated that earthworm mass was generally negatively affected by the presence of other species, however the severity of the negative influence was related to the extent of niche overlap between the species. They suggested that the greatest competitive interaction effects were present between species representing the same ecological group; findings which support observations by Edwards and Lofty (1978) of negative correlations between ecological grouping and the field densities of four UK earthworm species. Lowe and Butt (2002a) grew hatchling *L. terrestris, A. longa, A. chlorotica* and *L. rubellus* in the laboratory, either in isolation or in pairings with adults representing a range of ecological groups. They found that inter- and intra-specific interactions negatively influenced earthworm growth, maturation and fecundity; and this was again directly related to the extent of niche overlap between pairings. A notable exception was found for *A. chlorotica*, which exhibited enhanced growth and cocoon production in the presence of *A. longa*. It was concluded that the results of earthworm species interactions cannot be predicted simply based on ecological groupings (Lowe and Butt, 2002a).

In this experiment, *A. longa* demonstrated greater increase in final mass when in combination with *A. chlorotica*, compared with *A. longa* monoculture, across both soil types. This supports the findings of Lowe and Butt (2002a), whereby mature anecic *L. terrestris* exhibited greatest masses when paired with endogeic earthworm species. However, the mechanism by which endogeic earthworms might have a positive influence on anecic earthworm mass is difficult to identify. It may be the case that the greater *A. longa* final mass change is the result of reduced inter-specific and intra-specific competition between the two species of different ecological groupings for the limited food resources of the close experimental environment (Lowe and Butt, 1999). This would suggest that the density of *A. longa* in the choice chambers was too high and this created intra-specific competition for food resources, as previously observed by Butt *et al.* (1994a) for the anecic earthworm species *L. terrestris*. There was no evidence of a positive inter-specific relationship for *A. chlorotica* when paired with *A. longa.* There was a reduction in *A. chlorotica* final mass loss in the species combination in loam soil, however this may have been an artefact of the poor survival rate of this species in the loam soil treatment. The lack of any clear change in *A. chlorotica* mass between combined species and monoculture suggests that *A. longa* did not facilitate *A. chlorotica* feeding or overcome any potential issue of litter size during the experiment. This does not support the findings of Lowe and Butt (2002a, 2003), who recorded greater growth of *A. chlorotica* when paired with adult *A. longa* and adult *L. terrestris;* both representatives of the anecic ecological group. Lowe and Butt (2002a) identified that juveniles of one ecological group may have a “niche overlap” and subsequent negative interaction with members of another ecological grouping. However, the earthworms used in the choice chamber experiment presented in this chapter were all adults, and as such this cannot explain the inter-specific negative interaction observed. Interestingly, the earthworm combination treatment was almost as effective as the *A. longa* monoculture at consuming leaf litter. This would seem to suggest that *A. chlorotica* were playing some role in leaf removal alongside *A. longa*, however this does not appear to be reflected in the earthworm mass data for this species. The results of earthworm mass and leaf removal rate for the combined earthworm species suggests that these species can co-exist as an inoculum, and were therefore an appropriate species combination for inoculation into the field experiments described in Chapters 5 and 6 of this thesis.

8.4.2. Leaf palatability

The obvious initial preference for *A. cordata* foliar material over that of *A. platanoides* indicates greater palatability of this tree species leaf material to the earthworm species in the experiment. Previous studies have helped to identify the chemical and physical parameters of litter which influence litter palatability to earthworms. The chemical composition of litter appears to strongly influence earthworm selectivity, in particular aspects such as the C:N ratio and the content of nitrogen, calcium, lignin and polyphenols (Satchell and Lowe, 1967; Hendriksen, 1990; Reich *et al.*, 2005; Rajapaksha *et al.*, 2013). Generally, higher N and Ca content and a lower C:N ratio have been associated with increased palatability of leaf litter to earthworms (Reich *et al.*, 2005; Rajapaksha *et al.*, 2013). Current results generally fit this trend; at the start of the experiment, *A. cordata* foliar material had higher total N (%) and lower C:N ratio and Ca (%) content than that of *A. platanoides*. In a similar study, Rajapaksha *et al.* (2013) found that leaf litter from the least preferred tree species, sweet chestnut (*C. sativa*), demonstrated particularly low levels of nitrogen and calcium, and highest C:N ratio of all tree species investigated: alder (*A. glutinosa*), common ash (*F. excelsior*), silver birch (*B. pendula*), sweet chestnut (*C. sativa*), sycamore (*A. pseudoplatanus*), and an exotic eucalyptus species (*E. nitens*). However, in the experiment presented in this chapter, the preferred tree species *A. cordata* had lower calcium content than the less-preferred *A. platanoides*, which suggests that calcium content may be less important for leaf palatability, compared to other parameters such as N or C:N ratio. Additionally, Rajapaksha *et al.* (2013) found that the maple species they investigated, *A. pseudoplatanus*, showed low palatability to earthworms despite possessing similar levels of N and Ca to more preferred species. The maple species investigated in the experiment presented in this chapter showed initial low palatability to earthworms, and had comparable levels of N and a similar C:N ratio to the sycamore litter investigated by Rajapaksha *et al.* (2013). This indicates that other factors may affect leaf palatability to earthworms besides those already discussed, and indeed other chemical factors such as content of lignin and tannins have been identified as contributing to earthworm preference (Hendriksen, 1990). These, however, were not analysed in the present study.

It has been suggested that litter selection by earthworms can be affected by the state of leaf litter decomposition or weathering (Satchell and Lowe, 1967; Hendriksen, 1990). Earthworms have been shown to prefer decomposed litter over fresh litter, and this increased palatability has been linked to fungal and bacterial colonisation and activity on the leaf material (Satchell and Lowe, 1967; Wright, 1972; Cooke and Luxton, 1980; Cooke, 1983; Hendriksen, 1990). It appears that in the experiment presented in this chapter, there was an effect of natural decomposition on leaf foliar chemical composition and palatability to earthworms over the course of the experiment. Both tree species leaf litter showed increase in total N, total C, Ca and Mg (%) at termination of the experiment, and a reduction in C: N ratio and K (%). This represents a positive change in the key chemical parameters which are thought to affect leaf palatability, and likely explains the sudden increase in *A. platanoides* foliar material removal by all earthworm treatments mid-way through the experiment (since there was still *A. cordata* leaf material available at this point, the increased consumption of *A. platanoides* material was unlikely to due lack of other food resources). These results suggest that after around 12 days in the feeding tubes, microbial degradation of the leaves produced material of sufficient palatability to earthworms. Microbial colonisation of decaying leaf litter, and subsequent biomass and activity has been negatively correlated with C:N ratio, lignin and polyphenol concentrations (Swift *et al.*, 1979; Hendriksen, 1990; Rief *et al.*, 2012). This may explain the recorded increase in total C (%) and associated reduction in C:N ratio of leaves at termination of the experiment, and the increased palatability of this material to earthworms.

The use of leaf foliar rather than litter material was shown to successfully support earthworm growth and survival, particularly so for the anecic earthworm species *A. longa*. This supports the findings of Butt (2011a), who used dried green *B. pendula* leaves as feedstock for *L. terrestris*. Butt (2011a) found that switching from dried senesced leaves to green leaves during an experiment resulted in increased *L. terrestris* mass and significantly increased cocoon production. This was attributed to the larger nitrogen content in green leaves enabling more rapid protein synthesis for growth and reproduction. After 5 months the feedstock was switched back to dried fallen leaves, resulting in a reversal of this trend (Butt, 2011a). Our findings lend support to the proposition that increased nitrogen content of green leaves benefits earthworm growth and survival, with the *A. platanoides* foliar material used in our experiment possessing a two-fold increase in total N compared with the field-collected leaf litter for this species; and showing greater palatability than the leaf litter of another *Acer* species in a similar experiment (Rajapaksha *et al.*, 2013).

8.4.3. Soil treatments

Soil type did not appear to influence earthworm leaf species preference, with the same trend of leaf selection observed for both soil types and earthworm species. There was, however, a slower rate of leaf consumption observed in the reclaimed soil treatment for all earthworm species combinations treatments. This may be linked to higher soil organic matter content in the reclaimed soil (5.9%) compared with the loam (4.7%), which may have enabled increased geophagous feeding rather than direct leaf removal in both *A. chlorotica* and *A. longa* (Lowe and Butt, 2002b). Typically, soil materials on newly reclaimed landfill sites are unlikely to have high levels of organic matter content (Bending *et al.*, 1999). The levels observed in the reclaimed soils used in this experiment may represent the accidental inclusion of root and other dead plant material, since the soil was collected from the rhizosphere of a re-vegetated 10-year-old reclaimed landfill site. Whilst informative for similarly reclaimed landfill sites, the findings from this experiment might not necessarily predict the behaviour of these earthworm species if inoculated into radically different or newly reclaimed soil materials.

In this experiment, both *A. longa* and *A. chlorotica* demonstrated high survivorship (100% and 98%, respectively) in reclaimed soil, as well as highest overall changes in mass. The final mean individual mass of *A. chlorotica* (0.22 g) in reclaimed soil was comparable to the mean mass of this species recorded from Calvert landfill site (0.26 g) by Butt *et al.* (1999), however the final mean individual mass of *A. longa* in reclaimed soil was much higher in this experiment than that recorded at Calvert (2.45 g compared to 1.08 g). The improved performance of *A. longa* might be explained by a reduced clay fraction and level of compaction of the reclaimed soil in the current experiment compared with the field soil on Calvert landfill. Both earthworm species displayed tolerance for soil pH of >8.0, which is above that typically recommended for these species, and higher than previous research suggest *A. longa* may tolerate (Baker and Whitby, 2003; Lowe and Butt, 2005). Overall, both earthworm species demonstrated good tolerance of the reclaimed soil used in this experiment, supporting the findings of Butt *et al.* (2004) who recorded sustainable populations of *A. longa* and *A. chlorotica* over a period of ten years following inoculation into reclaimed landfill.

In the Kettering loam treatment, *A. chlorotica* showed low survivorship and a decrease in final individual mass. This was surprising, since this soil material has been widely successfully used and is recommended as a standard soil for earthworm-focussed laboratory experiments (Butt *et al.*, 1994b; Lowe and Butt, 2005; Rajapaksha *et al.*, 2013). Earthworm survival and activity is greatly influenced by abiotic factors, in particular soil temperature and moisture content; however in this experiment these were maintained at optimal levels and are therefore unlikely to explain the *A. chlorotica* mortality observed (Lowe and Butt, 2005). Starvation of this geophagous species is unlikely to be the cause of death, since the soil organic matter content of the loam used in this experiment (4.7%) was only marginally lower than that used in other experiments (5%) (Butt *et al.*, 1994a; Rajapaksha *et al.*, 2013). One proposed explanation for the high rate of *A. chlorotica* mortality is a negative influence of the decomposition of any early mortalities (e.g. from transport stress) upon the survival of surrounding earthworms in a closed microcosm. There is currently no discussion of this potentially antagonistic effect in the literature, likely due to difficulty distinguishing this from other negative environmental conditions triggering mass earthworm mortality.

8.4.4. Summary of chapter findings

The findings of this research were as follows, summarised against the original chapter objectives:

1. Both *A. longa* and *A. chlorotica* preferred the foliar material of *A. cordata* over *A. platanoides*, however the leaves of both tree species are capable of supporting populations of the earthworms investigated. Foliar (green) leaves are a suitable food source for earthworms in choice chamber experiments, although for some tree species, a degree of bacterial or fungal degradation of this material is required before the leaves become palatable to earthworms.
2. *A. longa* and *A. chlorotica* are both suitable species for inoculation to reclaimed soil, having demonstrated highest survival and most positive final mass change in these soils, in addition to consumption of the litter of the tree species commonly planted on such sites.
3. Positive inter-specific interactions may exist between adults of *A. longa* and *A. chlorotica*, although this is influenced by population densities and availability of food resources.

9. DISCUSSION AND CONCLUSIONS

9.1. Introduction

The purpose of this research was to investigate the interactions between earthworms, composted greenwaste (CGW) and trees on reclaimed land; and, in doing so, identify opportunities to improve habitat establishment and associated ecosystem service provision on reclaimed landfill. Whilst earthworms have been the subject of investigation by restoration ecologists for decades, little is still understood about their population dynamics in response to CGW addition, and the subsequent benefits their interactions may provide to soil quality and tree growth. Very little is also known about the physiological responses of tree species relevant to landfill reclamation, under the influence of earthworm activity and CGW addition. Accordingly, a range of field and laboratory-based experiments and surveying was 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, and this final chapter now draws these together to provide a wider discussion according to the overall research aims of this thesis. Implications of this research for land reclamation and restoration ecology are presented, followed by this thesis' contributions to knowledge, the limitations of the research, and, finally, suggestions are made for future research.

9.2. General discussion

9.2.1. The effects of CGW addition and earthworm activity on tree growth and survival on reclaimed landfill

The two field experiments of this thesis investigated the influence of CGW addition and earthworm inoculation on the growth and survival of two tree species commonly planted on reclaimed landfills in the Thames Chase Community Forest in East London. The large-scale field experiment in Chapter 5 demonstrated that Italian alder is generally well suited to planting on reclaimed soils, and this species showed significantly greater growth and highest survival when soil biological and chemical quality was improved through CGW addition in the presence of earthworms. However, no benefit from earthworm activity and compost addition was observed on Norway maple growth or survival, as this species was likely to have been too severely affected by soil drought conditions during the first few months after tree planting. Due to the field setting of this experiment, these findings can be considered representative of tree performance under similar conditions that could be expected on large-scale reclamation projects, although the large scale of the experiment did not allow for the in-depth exploration of the relationships between individual trees and earthworm species. Therefore, the nursery experiment was set up, replicating the Ingrebourne Hill field experiment, but under more controlled environmental conditions. This experiment successfully demonstrated again that Italian alder performs well growing in reclaimed soil, although there was no significant effect of CGW treatment on this species performance under the irrigated and controlled soil conditions of the nursery experiment. The Chapter 5 field experiment indicates that the CGW and earthworm activity might have been benefiting this tree species improved growth under the hostile field conditions at Ingrebourne Hill. In the nursery experiment, the significantly improved Norway maple growth under earthworm and compost combination treatment indicated a synergistic effect of earthworm activity and compost addition on tree growth. The better growth of Norway maple in the nursery compared with the field experiment at Ingrebourne Hill was likely due to the drip-feed irrigation in the tubes preventing negative drought impact. This then enabled the effects of the CGW and earthworm treatments to be clearly detectable. In the nursery experiment, both tree species exhibited low or deficient levels of N, P and K, likely due to insufficient levels of these macro-nutrients provided to the soil system by the CGW and leaf litter in the short timeframe of this experiment.

These studies demonstrated that on reclaimed landfill, earthworm activity and CGW application can have significant benefits to tree growth. The combination of *A. longa* (anecic) and *A. chlorotica* (endogeic) appears to have benefited the provision of soil quality improvements to tree growth. Italian alder was shown to be well suited to planting on reclaimed soils, whilst Norway maple can survive on such soils but experiences significantly improved growth if soil biological, chemical and physical quality is improved through CGW addition and earthworm activity.

9.2.2. The effects of CGW addition, earthworm activity and tree species on reclaimed soil physical and chemical quality

In both the field experiment and the nursery mesocosm experiment (Chapters 5 and 6), CGW significantly improved soil quality. In the field experiment a positive effect of CGW addition and earthworm activity was found on soil carbon, with significantly greater soil organic carbon and organic matter content in soils receiving the combination treatment. Since there was slightly higher earthworm density under this treatment, this might have contributed in part to increased accumulation of both leaf litter and CGW in these plots through the extra earthworm activity. In the nursery experiment, this interactive effect was confirmed, with a combination of CGW addition and earthworm inoculation resulting in significantly higher total organic carbon and organic matter in soil under both tree species. Earthworm activity was also associated with a significantly higher C: N ratio in the upper section of bulk soil compared with lower section bulk soil under both tree species. The effects of earthworm activity are largely attributed to *A. longa*-assisted incorporation and distribution of leaf litter into the soil in addition to the already-present CGW.

With regards to availabilities of macro-nutrients for tree uptake, in both field experiments, CGW addition alone seemed to be responsible for the observed increases in available soil N levels, with little apparent influence of earthworm activity. At Ingrebourne Hill, final soil total N was significantly higher in the two treatments containing CGW, and since earthworm density was similar under these, it could not be confirmed whether this is due to the compost alone or an interactive CGW-earthworm effect. In the nursery experiment, however, levels of total N and NH_4^+ were significantly higher in the compost-only treatment, for both tree species. Interestingly, this suggests that earthworm activity did not increase the rate of release of N or NH_4^+ to the soil from CGW. Compost addition was shown to raise initial soil K levels, however after 24 months there were similar K levels across all treatments. This is likely due to high K availability and mobility, with rapid release and distribution within the soil during CGW decomposition, for plant uptake. This may also indicate that K had been leached out of the soil profile in the nursery mesocosm tubes. Therefore, at the CGW application rate used in both experiments, repeat application may be required within a period of 2 years to provide sufficient K for healthy plant growth, as supported by the deficiency in foliar K reported for both tree species.

The influence of tree species on soil chemistry could not be distinguished in the Ingrebourne Hill experiment due to sampling limitations. However, this was investigated extensively in the Nursery experiment, with the presence of Italian alder associated with significantly higher organic carbon and organic matter content in bulk soils, compared with Norway maple and tree-free controls. The Italian alder bulk soils also had a significantly lower moisture content and levels of macro-nutrients K, Mg, NO_3^- and PO_4^{3-} compared with Norway maple and tree-free soil, although these were clearly sufficient to support the rapid growth rate observed for this species.

9.2.3. Community dynamics of naturally and artificially introduced earthworms on landfill sites, and responses to tree establishment and CGW addition

Of the four native UK earthworm species investigated in the CGW-reclaimed soil laboratory experiment, *A. longa* and *A. chlorotica* were found to be most suitable for inoculation to reclaimed soils; with high survival and positive mass changes. This was corroborated in the leaf-preference experiment, in which the two earthworm species consumed leaf material of both tree species investigated, which are likely to be increasingly planted on reclaimed landfill sites. These results supported the use of these two earthworm species in the field experiments presented in Chapters 5 and 6. The laboratory-based CGW trial experiment showed that CGW addition may serve as a suitable source of organic matter to sustain earthworm populations on reclaimed sites in the initial period when trees are still becoming established and there is no leaf litter input to the soil. This was supported by the findings of the mesocosm experiment, which indicated that incorporation of CGW into soil in the field is sufficient to support populations of *A. longa* and *A. chlorotica*; although regular CGW addition may be required to ensure the populations are sustainable in the long term.

In the field experiment at Ingrebourne Hill, earthworm density and species richness were similar across all treatments, although highest under the compost only and combination treatments. Inoculation of *A. longa* did not significantly affect the density of this species after 30 months, most likely due to a high mortality rate following broadcast inoculation. No relationship between earthworm densities or species richness and tree species were identified in the field; however, in the Nursery experiment *A. chlorotica* density was significantly higher under Norway maple. This was surprising in light of the findings of the choice chamber experiment, whereby alder leaf material was significantly more palatable in the short-term to *A. longa* and *A. chlorotica* than that of Norway maple. It is suspected that the higher *A. chlorotica* density under this tree species may be a result of influences from the root chemistry of Norway maple providing a more suitable environment for this earthworm species, although this requires further investigation. The reduced growth of Norway maple also led to higher soil moisture content through reduced root uptake of water and reduced rainfall interception by tree canopy, which may have benefited *A. chlorotica* survival. In general, the nursery mesocosm earthworm survival rates indicate that *A. longa* was better suited than *A. chlorotica* to the reclaimed soil materials at Ingrebourne Hill, and to CGW as a food source.

The laboratory-based leaf palatability experiment demonstrated that foliar (green) leaves are a suitable food source for earthworms, although for some tree species, a certain amount of microbial activity on this material is required before the leaves become palatable to earthworms. Both *A. longa* and *A. chlorotica* preferred the foliar material of *A. cordata* over *A. platanoides*; however, over 27 days the leaves of both tree species proved capable of sustaining the earthworms investigated. This supports the planting of these two tree species on reclaimed land as providers of leaf litter which can encourage the development of soil earthworm populations. Furthermore, positive inter-specific interactions were identified between adults of *A. longa* and *A. chlorotica*, although this was influenced by population densities and availability of food resources.

The surveying at Little Gerpins (Chapter 4) did not reveal the anticipated natural colonisation wave of earthworms from the surrounding land; instead earthworm re-colonisation appeared to be occurring from within the site itself via earthworms and cocoons in the stockpile of the original soil materials from the site. Significantly higher earthworm densities were found in areas where the original topsoil (from rough pasture land use) was re-applied to the site, versus imported soil from intensive agricultural land. The species present at Little Gerpins reflected those typically found in established pasture (e.g. *A. chlorotica*, *A. caliginosa*, *A. longa*, *A. rosea*, *L. terrestris*), which was the previous land use prior to reclamation activities on the site. The rapid rate of earthworm population increase on the site indicated facilitative interactions between the dispersing species following re-introduction to the site when stockpiled soil was re-applied to the surface of the site. These findings are of interest because typically the survival rates of earthworms in stockpiled soils are low, reducing the effectiveness of re-colonisation of sites following spreading of the soil. This research indicates that earthworm inoculation may not be necessary on sites where soil quality is sufficiently high from the outset to enable earthworm survival, and legacy soil materials are stockpiled and applied following best practice guidance. Under these conditions, natural earthworm colonisation of sites can be rapid and encourage earlier presence of species which may better facilitate the provision of the soil quality and tree growth benefits identified by this thesis' research.

9.3. Implications for land reclamation

The studies in this thesis demonstrate that CGW application provides an effective method of improving soil quality and tree establishment on reclaimed landfill sites, as well as supporting populations of soil fauna. Incorporation of CGW to soil materials during initial soil placement is likely to provide benefits to vegetation most effectively; and after a few years repeat application to the soil surface as mulch will provide organic matter to support establishment of soil fauna, and ensure trees have access to sufficient nutrients during early establishment. This research also showed that the benefits afforded by CGW can be enhanced in the presence of certain earthworm species, with CGW addition therefore providing land reclamation professionals with an opportunity to increase both soil biological quality and vegetation establishment. Earthworm inoculation can be an effective method for improving soil biological, chemical and physical quality, however it may not be necessary to inoculate earthworms at all; as earthworm colonisation can occur rapidly if an appropriate soil resource is provided from the outset, and if legacy soil materials are properly stored onsite. If earthworm inoculation is carried out, it is important that earthworm species are carefully selected for tolerance of on-site conditions and for desired outcomes, such as soil improvement. The method of earthworm inoculation is also of paramount importance, as some methods (e.g. broadcasting) may not provide acceptable levels of earthworm survival as others (e.g. Earthworm Inoculation Units). Selection should consider how the soil conditions of the intended reclaimed site compare to the physicochemical tolerance of the intended earthworm species. In particular, consideration must be made for the application of organic matter to the site in order to provide the inoculated earthworms with a suitable food source until vegetation onsite is of sufficient age to provide adequate organic matter inputs to the soil. This research indicates that the two species *A. longa* and *A. chlorotica* represent a suitable anecic and endogeic selection for inoculation to reclaimed landfill sites, but experimentation must be undertaken using soils from proposed sites to ensure species suitability on a case-by-case basis. Tree species selection is also critically important for establishing sustainable woodland on restored land. This research indicates that certain species may be more tolerant of hostile reclaimed soil conditions (e.g. Italian alder), however where soil quality is given due consideration and raised through the application of organic wastes then alternate species (in the case of this thesis, Norway maple) may show significantly better performance than might be observed in un-amended soil.

9.4. Contributions to knowledge

This research has provided the following main contributions to knowledge:

1. CGW application can significantly improve the soil resource through increased levels of organic carbon and essential plant macro-nutrients, with earthworm activity increasing the accumulation of organic carbon into reclaimed soils, from both leaf litter and CGW.
2. Italian alder is largely tolerant of soil conditions on reclaimed landfill, whilst Norway maple shows poor growth and survival unless soil quality is improved through CGW addition. A synergistic effect of earthworm activity and compost addition was observed on Norway maple growth.
3. *A. longa* and *A. chlorotica* are particularly suitable species for inoculation to reclaimed soil, having demonstrated highest survival and most positive final mass change in these soils, and ready consumption of the leaves of two tree species commonly planted on such sites.
4. At application rates in line with the Nitrates Directive, CGW addition may serve as a suitable source of organic matter to sustain earthworm populations on reclaimed sites in the initial period when trees are still becoming established. Earthworm survival rates indicate that *A. longa* was better suited than *A. chlorotica* to the soil materials used and CGW as a food source.
5. *A. chlorotica* density was significantly higher under Norway maple, despite the lower quality of the leaf material associated with this tree species. This may be a result of influences from the root chemistry of Norway maple or increased soil moisture content due to less extensive root and canopy associated with this tree species.

6. Foliar (green) leaves are a suitable food source for earthworms in choice chamber experiments, although for some tree species (i.e. *A. platanoides*), a certain amount of leaf degradation is required before the leaves become palatable to earthworms. Both *A. longa* and *A. chlorotica* prefer the foliar material of *A. cordata* over *A. platanoides*, but over time the leaves of both tree species are palatable to these earthworms. These tree species may therefore be capable of supporting populations of these earthworm species on reclaimed landfill.
7. Natural colonisation of reclaimed land by earthworms can occur rapidly (within 2 years), where soil quality is given due consideration and legacy soil materials are stockpiled and applied following best practice guidance. Topsoil origin significantly affects earthworm colonisation rate and success.

9.5. Limitations

Whilst this thesis successfully addressed the research aims set out at the beginning of the project, it must be noted that certain limitations were imposed on the scope of the investigations that could be made. For example, time limitations on the laboratory and field experiments meant that long-term trends could not be identified, and this would have yielded results more relevant to identifying sustainable earthworm and tree establishment on reclaimed landfill sites. Due to time limitations and the depth of investigation needed to identify earthworm-CGW-tree interaction effects, only a limited number of tree and earthworm species could be investigated. Availability of resources and land for conducting field experiments dictated that the Thames Chase Community Forest was used as the location for much of the research in this thesis. However, focussing only on this group of reclaimed landfill sites does limit the applicability of this research only to sites which have similar reclamation histories and/or standards as the ones investigated. Furthermore, because of the legal limitations imposed on the area available for the field experiment, only a single CGW application rate could be tested. Whilst the rate adopted was inherently the most applicable to landfill reclamation in the area, investigating a range of CGW application rates would have yielded results informative to reclamation projects where special dispensation may be made for more CGW addition. This may also have enabled the identification of an ‘optimum’ CGW application rate for tree establishment, and provided rationale for increasing the rate of CGW application to reclaimed land for woodland establishment. Finally, usual resource limitations also prevented further investigations being made during the earthworm colonisation survey at Little Gerpins, preventing follow-up earthworm surveying further inside the site to provide the full scope of earthworm distribution, and at areas outside of the site - which may have acted as an historical or contemporary source of earthworms for natural colonisation.

9.6. Further research

Building 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. An economic quantification and cost-benefit analysis of the ecosystem service benefit of using good standard topsoil and restoration practice, versus poor restoration and low soil ecosystem service output for a number of years following restoration.
2. Repeated mesocosm and field experiments over a longer experimental duration, e.g. minimum of 36 months, to allow tree growth, earthworm population and soil quality data to reflect the long-term impact of organic waste application and earthworm inoculation.
3. An investigation into the effect of litter addition on soil and tree health measurements, where litter inputs are regulated (as undertaken by Rajapaksha *et al*., 2014). In the mesocosm study in this thesis, leaf litter input to the soil was unregulated in the interest of reflecting field conditions and not limiting organic matter availability - thus potentially starving the earthworms.
4. An investigation of different application rates of CGW or other organic waste materials to provide additional information on its effects on woodland and earthworm establishment on reclaimed land (although the CGW application rate used in this study was reflective of realistic legal limits).
5. Further experiments with a variety of additional earthworm species (preferably in monoculture and in species combinations), in a range of different reclaimed soil types. This would provide informative results for future restoration projects.

REFERENCES

Andrews, J. and Kinsman, D. (1990). *Gravel Pit Restoration for Wildlife*. Sandy, Befordshire: RSPB.

Armour-Chelu, M. and Andrews, P. (1994). Some effects of bioturbation by earthworms (Oligochaeta) on archaeological sites. *Journal of Archaeological Science*, 21, p.433–443.

Armstrong, M. J. and Bragg, N. C. (1984). Soil physical parameters and earthworm populations associated with opencast coal working and land restoration. *Agriculture, Ecosystems and Environment*, 11 (2), p.131–143.

Arnold, R. E., Hodson, M. E. and Langdon, C. J. (2008). A Cu tolerant population of the earthworm *Dendrodrilus rubidus* (Savigny, 1862) at Coniston Copper Mines, Cumbria, UK. *Environmental Pollution*, 152 (3), p.713–722.

Ashwood, F. E., Doick, K. J., Atkinson, G. E. and Chenoweth, J. (2014). Under-utilisation of organic wastes during brownfield regeneration to community woodland: tackling the barriers. *Waste Management and Research*, 32 (1), p.49–55.

Avendaño-Yáñez, M. L., Ortiz-Ceballos, Á. I., Sánchez-Velásquez, L. R., Pineda-López, M. R. and Meave, J. A. (2014). Synergic effect of *Mucuna pruriens* var. *utilis* (Fabaceae) and *Pontoscolex corethrurus* (Oligochaeta, Glossoscolecidae) on the Growth of *Quercus insignis* (Fagaceae) Seedlings, a Native Species of the Mexican Cloud Forest. *Open Journal of Forestry*, 4 (1), p.1–7.

Bain, S. O., Butt, K. R. and Morris, R. M. (1999). Survival and reproduction of *Lumbricus terrestris* L. in colliery spoil and sewage sludge. *Pedobiologia*, 43, p.729–734.

Baker, G. H., Brown, G., Butt, K., Curry, J. P. and Scullion, J. (2006). Introduced earthworms in agricultural and reclaimed land: their ecology and influences on soil properties, plant production and other soil biota. *Biological Invasions*, 8 (6), p.1301–1316.

Baker, G. H. and Whitby, W. (2003). Soil pH preferences and the influences of soil type and temperature on the survival and growth of *Aporrectodea longa* (Lumbricidae). *Pedobiologia*, 47 (5-6), p.745–753.

Bardgett, R. (2005). *The Biology of Soil: A community and ecosystem approach*. Oxford: Oxford University Press.

Barley, K. P. and Kleinig, C. R. (1964). The occupation of newly irrigated lands by earthworms. *Australian Journal of Science*, 26 (291), p.415–431.

BassiriRad, H., Gutschick, V. and Sehtiya, H. L. (2008). Control of plant nitrogen uptake in native ecosystems by rhizospheric processes. In: Bruulsema, T. (ed.), *Quantifying and Understanding Plant Nitrogen Uptake for Systems Modeling*, Boca Raton, FL: CRC Press, p.71–91.

Bending, N. A. D., McRae, S. G. and Moffat, A. J. (1999). *Soil-forming Materials: Their Use in Land Reclamation*. London: DETR.

Bending, N. A. D. and Moffat, A. J. (1997). *Tree Establishment on Landfill Sites. Research and updated guidance*. Edinburgh: Forestry Commission.

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

Blair, J. M., Parmelee, R. W., Allen, M. F., McCartney, D. A. and Stinner, B. R. (1997). Changes in soil N pools in response to earthworm population manipulations in agroecosystems with different N sources. *Soil Biology and Biochemistry*, 29 (314), p.361–367.

Blouin, M., Hodson, M. E., Delgado, E. A., Baker, G., Brussaard, L., Butt, K. R., Dai, J., Dendooven, L., Peres, G., Tondoh, J. E., Cluzeau, D. and Brun, J.-J. (2013). A review of earthworm impact on soil function and ecosystem services. *European Journal of Soil Science*, 64 (2), p.161–182.

Blouin, M., Lavelle, P. and Laffray, D. (2007). Drought stress in rice (*Oryza sativa* L.) is enhanced in the presence of the compacting earthworm *Millsonia anomala*. *Environmental and Experimental Botany*, 60 (3), p.352–359.

Bohlen, P. J. and Edwards, C. A. (1995). Earthworm effects on N dynamics and soil respiration in microcosms receiving organic and inorganic nutrients. *Soil Biology and Biochemistry*, 27 (3), p.341–348.

Bohlen, P. J., Parmelee, R. W., Blair, J. M., Edwards, C. A. and Stinner, B. R. (1995). Efficacy of methods for manipulating earthworm populations in large-scale field experiments in agroecosystems. *Soil Biology and Biochemistry*, 27 (8), p.993–999.

Bouché, M. B. (1977). Strategies lombriciennes. In: Lohm, U. and Person, T. (eds.), *Organisms as components of ecosystems*, Stockholm: Ecological Bulletin, p.122–132.

Boyer, S. and Wratten, S. D. (2010). The potential of earthworms to restore ecosystem services after opencast mining – A review. *Basic and Applied Ecology*, 11 (3), p.196–203.

Boyer, S., Wratten, S., Pizey, M. and Weber, P. (2011). Impact of soil stockpiling and mining rehabilitation on earthworm communities. *Pedobiologia*, 54, p.S99–S102.

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 Ph. D. thesis, National University of Ireland. Cited in Edwards, CA (ed) Earthworm Ecology. St. Lucie Press, Boca Raton*, p.37–64.

Bradshaw, A. D. and Chadwick, M. J. (1980). *The Restoration of Land - The ecology and reclamation of derelict and degraded land*. Oxford: Blackwell Scientific Publications.

Bridges, E. M. (1961). Aspect and time in soil formation. *Agriculture*, 68 (7), p.360–363.

British Standards Institute. (2011). *PAS 100:2011 Specification for composted materials*.

Broadmeadow, M. S. J., Ray, D. and Samuel, C. J. A. (2005). Climate change and the future for broadleaved tree species in Britain. *Forestry*, 78 (2), p.145–161.

Brockmann, W., Koehler, H. and Schriefer, T. (1980). Recultivation of refuse tips: soil ecological studies. In: Dindal, D. L. (ed.), *Soil Biology as Related to Land-use Practice*, Washington DC: Environmental Protection Agency, p.200–207.

Brown, D. S., Jarman, S. N. and Symondson, W. O. C. (2012). Pyrosequencing of prey DNA in reptile faeces: analysis of earthworm consumption by slow worms. *Molecular Ecology Resources*, 12 (2), p.259–266.

Brown, G. G., Edwards, C. A. and Brussaard, L. (2004). How earthworms affect plant growth: burrowing into the mechanisms. In: Edwards, C. A. (ed.), *Earthworm Ecology*, Boca Raton: CRC Press, p.13–49.

Brun, J. J., Cluzeau, D., Trehen, P. and Bouché, M. B. (1987). Biostimulation: Prospects and limitations of biological improvement of soils by stimulation or introduction of earthworms. *Revue d'Ecologie et de Biologie du Sol*, 24, p.685–701.

Brussaard, L. (1998). Soil fauna, guilds, functional groups and ecosystem processes. *Applied Soil Ecology*, 9, p.123–135.

Bullock, J. M., Aronson, J., Newton, A. C., Pywell, R. F. and Rey-Benayas, J. M. (2011). Restoration of ecosystem services and biodiversity: Conflicts and opportunities. *Trends in Ecology and Evolution*, 26 (10), p.541–549.

Butt, K. R. (1992). Development of an earthworm cultivation and soil-inoculation technique for land restoration. In: *Proceedings of the 3rd British Grassland Society*, Antrim, N. Ireland: , p.19–20.

Butt, K. R. (1993). Utilisation of solid paper-mill sludge and spent brewery yeast as a feed for soil-dwelling earthworms. *Bioresource Technology*, 44 (2), p.105–107.

Butt, K. R. (1997). Reproduction and growth of the earthworm *Allolobophora chlorotica* (Savigny, 1826) in controlled environments. *Pedobiologia*, 41, p.369–374.

Butt, K. R. (1998). Interactions between selected earthworm species: A preliminary, laboratory-based study. *Applied Soil Ecology*, 9 (1-3), p.75–79.

Butt, K. R. (1999a). Effects of thermally dried sewage granules on earthworms and vegetation during pot and field trials. *Bioresource Technology*, 67, p.149–154.

Butt, K. R. (1999b). Inoculation of earthworms into reclaimed soils: the UK experience. *Land Degradation and Development*, 10 (6), p.565–575.

Butt, K. R. (2000). Earthworms of the Malham Tarn Estate (Yorkshire Dales National Park). *Field Studies*, 9, p.701–710.

Butt, K. R. (2008). Earthworms in Soil Restoration: Lessons Learned from United Kingdom Case Studies of Land Reclamation. *Restoration Ecology*, 16, p.637–641.

Butt, K. R. (2011a). Food quality affects production of *Lumbricus terrestris* (L.) under controlled environmental conditions. *Soil Biology and Biochemistry*, 43 (10), p.2169–2175.

Butt, K. R. (2011b). The earthworm inoculation unit technique: development and use in soil improvement over two decades. In: Karaca, A. (ed.), *Biology of Earthworms*, Berlin: Springer-Verlag, p.87–105.

Butt, K. R. and Frederickson, J. (1995). Earthworm cultivation and soil-inoculation: A practical technique for land restoration. *Journal of Practical Ecology and Conservation*, 1 (1), p.14–19.

Butt, K. R., Frederickson, J. and Lowe, C. N. (1999). Colonisation, survival and spread of earthworms on a partially restored landfill site. *Pedobiologia*, 43, p.684–690.

Butt, K. R., Frederickson, J. and Morris, R. M. (1993). Investigations of an earthworm inoculation experiment, London Borough of Hillingdon. *Waste Planning*, 7 (9-12).

Butt, K. R., Frederickson, J. and Morris, R. M. (1994a). Effect of earthworm density on the growth and reproduction of *Lumbricus terrestris* L. (Oligochaeta: Lumbricidae) in culture. *Pedobiologia*, 38, p.254–261.

Butt, K. R., Frederickson, J. and Morris, R. M. (1994b). The life cycle of the earthworm *Lumbricus* *terrestris* L. (Oligochaeta: Lumbricidae) in laboratory culture. *European Journal of Soil Biology*, 30 (2), p.49–54.

Butt, K. R., Frederickson, J. and Morris, R. M. (1995). An earthworm cultivation and soil inoculation technique for land restoration. *Ecological Engineering*, 4 (1), p.1–9.

Butt, K. R., Frederickson, J. and Morris, R. M. (1997). The earthworm inoculation unit technique: an integrated system for cultivation and soil-inoculation of earthworms. *Soil Biology and Biochemistry*, 29 (3/4), p.251–257.

Butt, K. R. and Grigoropoulou, N. (2010). Basic Research Tools for Earthworm Ecology. *Applied and Environmental Soil Science*, p.1–12.

Butt, K. R., Lowe, C. N., Frederickson, J. and Moffat, A. J. (2004). The development of sustainable earthworm populations at Calvert landfill site, UK. *Land Degradation and Development*, 15, p.27–36.

Butt, K. R., Nieminen, M. A., Sirén, T., Ketoja, E. and Nuutinen, V. (2005). Population and behavioural level responses of arable soil earthworms to boardmill sludge application. *Biology and Fertility of Soils*, 42, p.163–167.

Callaham, M. A., González, G., Hale, C. M., Heneghan, L., Lachnicht, S. L. and Zou, X. (2006). Policy and management responses to earthworm invasions in North America. *Biological Invasions Belowground: Earthworms as Invasive Species*, p.117–129.

Cameron, E. K. and Bayne, E. M. (2009). Road age and its importance in earthworm invasion of northern boreal forests. *Journal of Applied Ecology*, 46 (1), p.28–36.

Carter, A., Heinonen, J. and de Vries, J. (1982). Earthworms and water movement. *Pedobiologia*, 23, p.395–397.

Carter, M. R. and Gregorich, E. G. (1993). *Soil sampling and methods of analysis*. Boca Raton: CRC Press.

Clark, A. (2015). *Personal Communication with the director of Ingrebourne Valley Ltd.* (email).

Clements, R. O., Murray, P. J. and Sturdy, R. G. (1991). The impact of 20 years' absence of earthworms and three levels of N fertilizer on a grassland soil environment. *Agriculture, Ecosystems and Environment*, 36 (1-2), p.75–85.

Cooke, A. and Luxton, M. (1980). Effect of microbes on food selection by *Lumbricus terrestris*. *Revue d'Ecologie et de Biologie du Sol*, 17 (3), p.365–370.

Cooke, J. (1983). The effects of fungi on food selection by *Lumbricus terrestris* L. In: Satchell, J. E. (ed.), *Earthworm Ecology*, London: Chapman & Hall, p.365–373.

Council of the European Union. (1991). *Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources*.

Crampton, C. B. (1967). Soil development on tips in South Wales. *Sylva*, 47, p.12–14.

Craven, D. R. J. (1995). The Hallside steelworks project. *Land Contamination and Reclamation*, (3), p.31–38.

Curry, J. P. (1988). The ecology of earthworms in reclaimed soils and their influence on soil fertility. In: Edwards, C. A. and Neuhauser, E. F. (eds.), *Earthworms in Waste and Environmental Management*, The Hague: SPB Academic Publishing, p.251–261.

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. and Bolger, T. (1984). Growth, reproduction and litter and soil consumption by *Lumbricus terrestris* L. in reclaimed peat. *Soil Biology and Biochemistry*, 16 (3), p.253–257.

Curry, J. P. and Boyle, K. E. (1987). Growth rates, establishment, and effects on herbage yield of introduced earthworms in grassland on reclaimed cutover peat. *Biology and Fertility of soils*, 3 (1-2), p.95–98.

Curry, J. P. and Cotton, D. C. F. (1983). Earthworms and land reclamation. In: Satchell, J. E. (ed.), *Earthworm Ecology*, London: Chapman & Hall, p.215–228.

Daily, G. C. (1997). *Nature's services: societal dependence on natural ecosystems*. Washington, DC: Island Press.

Dakora, F. D. and Phillips, D. A. (2002). Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil*, 245 (1), p.35–47.

Darwin, C. (1881). *The Formation of Vegetable Mould, Through the Action of Worms, with Observations on their Habits*. London: John Murray.

Day, S. D., Wiseman, P. E., Dickinson, S. B. and Harris, J. R. (2010). Tree Root Ecology in the Urban Environment and Implications for a Sustainable Rhizosphere. *Arboriculture and Urban Forestry*, 36 (September), p.193–205.

DCLG. (1996). *Minerals Planning Guidance 7: Reclamation of mineral workings*. London: HMSO.

DCLG. (2011). *Planning Policy Guidance Note 3 (PPG3) (Revised June 2011)*. London: HMSO.

DEFRA. (2006). *Defra circular 01/2006 environmental protection act 1990: Part 2A contaminated land*.

DEFRA. (2009). *Safeguarding our Soils: A Strategy for England*. London: DEFRA.

Dickinson, N. M., Hartley, W., Uffindell, L. A., Plumb, A. N., Rawlinson, H. and Putwain, P. (2005). Robust biological descriptors of soil health for use in reclamation of brownfield land. *Land Contamination and Reclamation*, 13 (4), p.317–326.

Dictionary, O. E. (2016). *Oxford English dictionary online*. [Online]. Available at: http://www.oed.com [Accessed: January 2016].

Dobson, M. C. and Moffat, A. J. (1993). *The potential for woodland establishment on landfill sites.* London: HMSO.

Dobson, M. C. and Moffat, A. J. (1995). Site capability assessment for woodland creation on landfills. *Research Information Note 263, Forestry Authority*.

Doick, K. J. and Hutchings, T. (2007). *Greenspace establishment on brownfield land: the site selection and investigation process*. Edinburgh: Forestry Commission.

Doick, K. J., Sellers, G., Castan-Broto, V. and Silverthorne, T. (2009). Understanding success in the context of brownfield greening projects: The requirement for outcome evaluation in urban greenspace success assessment. *Urban Forestry and Urban Greening*, 8 (3), p.163–178.

Doick, K. J. and Willoughby, I. (2011). *Investigation into low tree establishment rates at Ingrebourne*

Hill's phase IIA restoration area. Farnham: Forest Research.

Dominati, E., Patterson, M. and Mackay, A. (2010). A framework for classifying and quantifying the natural capital and ecosystem services of soils. *Ecological Economics*, 69 (9), p.1858–1868.

Don, A., Steinberg, B., Schöning, I., Pritsch, K., Joschko, M., Gleixner, G. and Schulze, E.-D. (2008). Organic carbon sequestration in earthworm burrows. *Soil Biology and Biochemistry*, 40 (7), p.1803–1812.

Doube, B. M., Schmidt, O., Killham, K. and Correll, R. (1997a). Influence of mineral soil on the palatability of organic matter for lumbricid earthworms: a simple food preference study. *Soil Biology and Biochemistry*, 29 (3/4), p.569–575.

Doube, B. M., Stephens, P. M., Davoren, C. W. and Ryder, M. H. (1994). Interactions between earthworms, beneficial soil microorganisms and root pathogens. *Applied Soil Ecology*, 1 (1), p.3–10.

Doube, B. M., Williams, P. M. L. and Willmott, P. J. (1997b). The influence of two species of earthworm (*Aporrectodea trapezoides* and *Aporrectoedea rosea*) on the growth of wheat, barley and faba beans in three soil types in the greenhouse. *Soil Biology and Biochemistry*, 29 (3-4), p.503–509.

Down, C. G. (1975). Soil development on colliery waste tips in relation to age. I. Introduction and physical factors. *Journal of applied ecology*, p.613–622.

Dunger, W. (1989). The return of soil fauna to coal mined areas in the German Democratic Republic. In: Majer, J. D. (ed.), *Animals in Primary Succession: The Role of Fauna in Reclaimed Lands*, Cambridge: Cambridge University Press, p.307–338.

Edwards, C. A. (2004). *Earthworm ecology*. Boca Raton, FL: CRC Press.

Edwards, C. A. and Bohlen, P. J. (1996). *Biology and Ecology of Earthworms*. 3rd ed. London: Chapman & Hall.

Edwards, C. A., Bohlen, P. J., Linden, D. R. and Subler, S. (1995). Earthworms in agroecosystems. In: Hendrix, P. F. (ed.), *Earthworm ecology and biogeography in North America*, Boca Raton, FL: Lewis Publishing, p.185–215.

Edwards, C. A. and Burrows, I. (1988). The potential of earthworm composts as plant growth media. In: Edwards, C. A. and Neuhauser, E. F. (eds.), *Earthworms in waste and environmental management*, The Hague: SPB Academic Publishing.

Edwards, C. A. and Fletcher, K. E. (1988). Interactions between earthworms and microorganisms in organic-matter breakdown. *Agriculture, ecosystems and environment*, 24, p.235–247.

Edwards, C. A. and Heath, G. W. (1963). The role of soil animals in breakdown of leaf material. In: Docksen, J. and van der Drift, J. (eds.), *Soil organisms*, Amsterdam: North-Holland, p.76–84.

Edwards, C. A. and Lofty, J. R. (1977). *Biology of earthworms*. London: Chapmann and Hall.

Edwards, C. A. and Lofty, J. R. (1978). The influence of arthropods and earthworms upon root growth of direct drilled cereals. *Journal of Applied Ecology*, 15 (3), p.789–795.

Eggleton, P., Inward, K., Smith, J., Jones, D. T. and Sherlock, E. (2009). A six year study of earthworm (Lumbricidae) populations in pasture woodland in southern England shows their responses to soil temperature and soil moisture. *Soil Biology and Biochemistry*, 41 (9), p.1857–1865.

Eichinger, E., Bruckner, A. and Stemmer, M. (2007). Earthworm expulsion by formalin has severe and lasting side effects on soil biota and plants. *Ecotoxicology and Environmental Safety*, 67 (2), p.260–266.

Eijsackers, H. (2011). Earthworms as colonizers of natural and cultivated soil environments. *Applied Soil Ecology*, 50, p.1–13.

Eisenhauer, N., Straube, D. and Scheu, S. (2008). Efficiency of two widespread non-destructive extraction methods under dry soil conditions for different ecological earthworm groups. *European Journal of Soil Biology*, 44 (1), p.141–145.

European Union Projects. (2002). *Regeneration of European Sites in Cities and Urban Environments (RESCUE)*.

Evans, A. C. and Guild, W. J. (1947). Studies on the relationships between earthworms and soil fertility: I. Biological studies in the field. *Annals of Applied Biology*, 34 (3), p.307–330.

Fernandes, M. S. and Rossiello, R. O. P. (1995). Mineral nitrogen in plant physiology and plant nutrition. *Critical Reviews in Plant Sciences*, 14 (2), p.111–148.

Foot, K. J., Hislop, M. and McNeilly, S. (2003). The effect of composted green waste on tree establishment on landfill. In: Moore, H. M., Fox, H. R. and Elliot, S. (eds.), *Land Reclamation - Extending the Boundaries*, Lisse: AA Balkema, p.213–221.

Forest Research. (2011). *Thames Beat – Brownfield Project Little Gerpins Species Selection*. Farnham: Forest Research.

Forest Research. (2015). *Best Practice Guidance for Land Regeneration*. Farnham: Forest Research.

Forestry Commission England. (n.d.). *Little Gerpins: Brownfield Land Regeneration in the Thames Chase Community Forest*. London: Forestry Commission England.

Fresquez, P. R. and Lindemann, W. C. (1982). Soil and rhizosphere microorganisms in amended coal mine spoils. *Soil Science Society of America Journal*, 46 (4), p.751–755.

Fründ, H. C., Butt, K. R., Capowiez, Y., Eisenhauer, N., Emmerling, C., Ernst, G., Potthoff, M., Schädler, M. and Schrader, S. (2010). Using earthworms as model organisms in the laboratory: Recommendations for experimental implementations. *Pedobiologia*, 53 (2), p.119–125.

Gavrilov, K. (1963). Earthworms, producers of biologically active substances. *Journal of General Biology*, 24, p.149–154.

Gregory, A. S. and Vickers, A. W. (2003). The effects of a composted organic amendment on soil biological properties in a clay soil-forming material used as a landfill restoration cap. *Land Contamination and Reclamation*, 11 (3), p.315–321.

Guggenberger, G., Thomas, R. J. and Zech, W. (1996). Soil organic matter within earthworm casts of an anecic-endogeic tropical pasture community, Colombia. *Applied Soil Ecology*, 3 (3), p.263–274.

Haimi, J., Huhta, V. and Boucelham, M. (1992). Growth increase of birch seedlings under the influence of earthworms - A laboratory study. *Soil Biology and Biochemistry*, 24 (12), p.1525–1528.

Hamblyn, C. I. and Dingwall, A. R. (1945). Earthworms. *New Zealand Journal of Agriculture*, 71, p.55–58.

Harris, J. A., Birch, P. and Palmer, J. (1996). *Land Restoration and Reclamation*. Harlow: Addison Wesley Longman Ltd.

Harris, J. A., Birch, P. and Short, K. C. (1989). Changes in the microbial community and physico-chemical characteristics of topsoils stockpiled during opencast mining. *Soil Use and Management*, 5 (4), p.161–168.

Haygarth, P. M. and Ritz, K. (2009). The future of soils and land use in the UK: Soil systems for the provision of land-based ecosystem services. *Land Use Policy*, 26, p.S187–S197.

Heaven, F. W. and Richardson, G. R. (2007). *Soil Investigation at Ingrebourne Valley, Phase IIA, Rainham*. Derby: Land Research Associates.

Hendriksen, N. B. (1990). Leaf litter selection by detritivore and geophagous earthworms. *Biology and Fertility of Soils*, 10 (1), p.17–21.

Hibberd, B. G. (1986). *Forestry Commission Handbook 5: Urban Forestry Practice*. London: HMSO.

Holmstrup, M. (1994). Physiology of cold hardiness in cocoons of five earthworm taxa (Lumbricidae: Oligochaeta). *Journal of Comparative Physiology B*, 164 (3), p.222–228.

Hoogerkamp, M., Rogaar, H. and Eijsackers, H. J. P. (1983). Effect of earthworms on grassland on recently reclaimed polder soils in the Netherlands. In: Satchell, J. E. (ed.), *Earthworm Ecology*, London: Chapman & Hall, p.85–105.

Hutchings, T. R. (2002). *The opportunities for woodland on contaminated land*. Edinburgh: Forestry Commission.

International Standards Organisation. (2006). *ISO 23611-1:2006: Soil quality - Sampling of soil invertebrates - Part 1: Hand-sorting and formalin extraction of earthworms*.

Jana, U., Barot, S., Blouin, M., Lavelle, P., Laffray, D. and Repellin, A. (2010). Earthworms influence the production of above-and belowground biomass and the expression of genes involved in cell proliferation and stress responses in *Arabidopsis thaliana*. *Soil Biology and Biochemistry*, 42 (2), p.244–252.

Jarvis, M. G., Allen, R. H., Fordham, S. J., Hazleden, J., Moffat, A. J. and Sturdy, R. G. (1983). Soils of England and Wales. Sheet 6. South East England. *Ordenance Survey, Southampton*.

Jones, C. G., Lawton, J. H. and Shachak, M. (1994). Organisms as ecosystem engineers. *Oikos*, p.373–386.

Jordan, W. R. and Gilpin, M. E. (1987). *Restoration ecology: a synthetic approach to ecological research*. Cambridge: Cambridge University Press.

Judd, K. W. and Mason, C. F. (1995). Earthworm populations of a restored landfill site. *Pedobiologia*, 39, p.107–115.

Kibblewhite, M. G., Ritz, K. and 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.

Kilian, J. V., Klauda, R. J., Widman, S., Kashiwagi, M., Bourquin, R., Weglein, S. and Schuster, J. (2012). An assessment of a bait industry and angler behavior as a vector of invasive species. *Biological Invasions*, 14 (7), p.1469–1481.

Kopinga, J. and Van den Burg, J. (1995). Using soil and foliar analysis to diagnose the nutritional status of urban trees. *Journal of Arboriculture*, 21, p.17.

Kremen, C. and Ostfeld, R. S. (2005). A call to ecologists: Measuring, analyzing, and managing ecosystem services. *Frontiers in Ecology and the Environment*, 3 (10), p.540–548.

Kruuk, H. and Parish, T. (1985). Food, food availability and weight of badgers (*Meles meles*) in relation to agricultural changes. *Journal of Applied Ecology*, p.705–715.

Kubiëna, W. L. (1953). *Bestimmungsbuch und systematik der böden Europas*. Stuttgart: Ferdinand Enke Verlag.

Land Research Associates. (2007). *Soil Investigation at Ingrebourne Hill phase IIa*.

Laossi, K.-R., Decaëns, T., Jouquet, P. and Barot, S. (2010). Can we predict how earthworm effects on plant growth vary with soil properties? *Applied and Environmental Soil Science*, 2010 (1), p.1–6.

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. and Hendrix, P. (1999). *Earthworm management in tropical agroecosystems.* Wallingford: CABI Publishing.

Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., Margerie, P., Mora, P. and Rossi, J.-P. (2006). Soil invertebrates and ecosystem services. *European Journal of Soil Biology*, 42, p.S3–S15.

Lavelle, P., Lattaud, C., Trigo, D. and Barois, I. (1995). Mutualism and biodiversity in soils. *Plant and Soil*, 170, p.23–33.

Lavelle, P. and Spain, A. V. (2001). *Soil ecology*. Dordrecht: Springer.

Lawrence, A. P. and Bowers, M. A. (2002). A test of the 'hot'mustard extraction method of sampling earthworms. *Soil Biology and Biochemistry*, 34, p.549–552.

Lee, K. E. (1985). *Earthworms: Their ecology and relationships with soils and land use.* Sydney: Academic Press Inc.

Lowe, C. N. and Butt, K. R. (1999). Interspecific interactions between earthworms: A laboratory-based investigation. *Pedobiologia*, 43, p.808–817.

Lowe, C. N. and Butt, K. R. (2002a). Growth of hatchling earthworms in the presence of adults: interactions in laboratory culture. *Biology and Fertility of Soils*, 35 (3), p.204–209.

Lowe, C. N. and Butt, K. R. (2002b). Influence of organic matter on earthworm production and behaviour: a laboratory-based approach with applications for soil restoration. *European Journal of Soil Biology*, 38 (2), p.173–176.

Lowe, C. N. and Butt, K. R. (2003). Influence of food particle size on inter- and intra-specific interactions of *Allolobophora chlorotica* (Savigny) and *Lumbricus terrestris*. *Pedobiologia*, 47 (5-6), p.574–577.

Lowe, C. N. and Butt, K. R. (2004). Use of Composted Green Waste (CGW) to improve landfill cap quality through the agency of earthworms. *Proceedings of Waste 2004: Integrated Waste Management and Pollution Control: Policy and Practice, Research and Solutions*, p.853–862.

Lowe, C. N. and Butt, K. R. (2005). Culture techniques for soil dwelling earthworms: A review. *Pedobiologia*, 49 (5), p.401–413.

Lowe, C. N. and Butt, K. R. (2008). *Allolobophora chlorotica* (Savigny, 1826): Evidence for classification as two separate species. *Pedobiologia*, 52 (2), p.81–84.

Lubbers, I. M. and van Groenigen, J. W. (2013). A simple and effective method to keep earthworms confined to open-top mesocosms. *Applied Soil Ecology*, 64, p.190–193.

Lubbers, I. M., van Groenigen, K. J., Fonte, S. J., Six, J., Brussaard, L. and van Groenigen, J. W. (2013). Greenhouse-gas emissions from soils increased by earthworms. *Nature Climate Change*, 3 (3), p.187–194.

Lukac, M. and Godbold, D. L. (2011). *Soil ecology in northern forests: a belowground view of a changing world*. Cambridge: Cambridge University Press.

Ma, Y., Dickinson, N. M. and Wong, M. H. (2003). Interactions between earthworms, trees, soil nutrition and metal mobility in amended Pb/Zn mine tailings from Guangdong, China. *Soil Biology and Biochemistry*, 35 (10), p.1369–1379.

Ma, Y., Dickinson, N. M. and Wong, M. H. (2006). Beneficial effects of earthworms and arbuscular mycorrhizal fungi on establishment of leguminous trees on Pb/Zn mine tailings. *Soil Biology and Biochemistry*, 38 (6), p.1403–1412.

Macdonald, D. W. (1983). Predation on earthworms by terrestrial vertebrates. In: Satchell, J. E. (ed.), *Earthworm ecology*, London: Chapman & Hall, p.393–414.

Marashi, A. R. A. and Scullion, J. (2003). Earthworm casts form stable aggregates in physically degraded soils. *Biology and fertility of soils*, 37, p.375–380.

Marfleet, B. (1985). GLC restoration experiment with earthworms. *Mineral Planning*, p.9–12.

Marinissen, J. C. Y. and van den Bosch, F. (1992). Colonization of new habitats by earthworms. *Oecologia*, 91 (3), p.371–376.

Marshall, V. G. (1971). Effects of soil arthropods and earthworms on the growth of Black Spruce. *Annals of Zoology and Ecology*, 7, p.109–118.

Maslen, S. D., Chambers, B. J., Hadden, S. W. and Royle, S. M. (2003). Making soil from waste material. In: H. M. Moore H.R. & Elliot S., F. (ed.), *Land Reclamation - Extending the Boundaries*, Lisse: Swets & Zeitlinger, p.207–210.

McColl, H. P., Hart, P. B. S. and Cook, F. J. (1982). Influence of earthworms on some soil chemical and physical properties, and the growth of ryegrass on a soil after topsoil stripping: a pot experiment. *New Zealand Journal of Agricultural Research*, 25 (2), p.239–243.

McKay, H. M., Jinks, R. L. and McEvoy, C. (1999). The effect of desiccation and rough-handling on the survival and early growth of ash, beech, birch and oak seedlings. *Annals of Forest Science*, 56 (5), p.391–402.

Menes, P. A. and Mohammed, G. H. (1995). Identifying the root collar on forest seedlings. *The Forestry Chronicle*, 71 (3), p.304–310.

Met Office. (2015a). *Odiham Climate Information - 1981-2010*. [Online]. Available at: http://www.metoffice.gov.uk/public/weather/climate/gcp76x8dg [Accessed: October 2015].

Met Office. (2015b). *Rainham climate*. [Online]. Available at: http://www.metoffice.gov.uk/public/weather/climate/u10jh6s24 [Accessed: October 2015].

Met Office. (2016). *Summer 2013 UK weather summary*. [Online]. Available at: http://www.metoffice.gov.uk/climate/uk/summaries/2013/summer [Accessed: February 2016].

Millennium Ecosystem Assessment. (2005). *Ecosystems and human well-being*. Washington, DC: Island Press.

Mittelbach, G. G., Steiner, C. F., Scheiner, S. M., Gross, K. L., Reynolds, H. L., Waide, R. B., Willig, M. R., Dodson, S. I. and Gough, L. (2001). What is the observed relationship between species richness and productivity? *Ecology*, 82 (9), p.2381–2396.

Moffat, A. J. (2000). Effects of inoculation with *Frankia* on the growth and nutrition of alder species and interplanted Japanese larch on restored mineral workings. *Forestry*, 73 (3), p.215–223.

Moffat, A. J. (2003). Indicators of soil quality for UK forestry. *Forestry*, 76 (5), p.547–568.

Moffat, A. J. (2006). Use of Sewage Sludges and Composts in Forestry. *Forestry Commission Information Note 79*, p.1–12.

Moffat, A. J., Armstrong, A. T. and Ockleston, J. (2001). The optimization of sewage sludge and effluent disposal on energy crops of short rotation hybrid poplar. *Biomass and Bioenergy*, 20, p.161–169.

Moffat, A. J. and Bending, N. A. D. (1992). *Physical site evaluation for community woodland establishment*. Forestry Commission.

Moffat, A. J., Hutchings, T. R., Tubby, I., Butt, K. R. and Lowe, C. N. (2008). Experimental woodland establishment on brick clays in southern England. *Land Contamination and Reclamation*, 16 (3), p.181–190.

Moffat, A. J. and McNeill, J. (1994). *Reclaiming Disturbed Land for Forestry: Bulletin 110*. Edinburgh: Forestry Commission.

Montecchio, L., Scattolin, L., Squartini, A. and Butt, K. R. (2015). Potential spread of forest soil-borne fungi through earthworm consumption and casting. *iForest-Biogeosciences and Forestry*, 8 (3), p.295-301.

Muscolo, A., Bovalo, F., Gionfriddo, F. and Nardi, S. (1999). Earthworm humic matter produces auxin-like effects on *Daucus carota* cell growth and nitrate metabolism. *Soil Biology and Biochemistry*, 31 (9), p.1303–1311.

Muys, B., Beckers, G., Nachtergale, L., Lust, N., Merckx, R. and Granval, P. (2003). Medium-term evaluation of a forest soil restoration trial combining tree species change, fertilisation and earthworm introduction. *Pedobiologia*, 47, p.772–783.

Muys, B., Lust, N. and Granval, P. (1992). Effects of grassland afforestation with different tree species on earthworm communities, litter decomposition and nutrient status. *Soil Biology and Biochemistry*, 24 (12), p.1459–1466.

Nason, M., Williamson, J., Tandy, S., Christou, M., Jones, D. and Healey, J. (2007). *Using organic wastes and composts to remediate and restore land: best practice manual*. Bangor: School of the Environment and Natural Resources, Bangor University.

Neilson, R. and Boag, B. (2003). Feeding preferences of some earthworm species common to upland pastures in Scotland. *Pedobiologia*, 47 (1), p.1–8.

Nielson, R. L. (1951). Earthworms and soil fertility. In: *Proceedings of the 13th Conference of the New*

Zealand Grassland Association, New Plymouth, 1951, p.158–167.

Nielson, R. L. (1965). Presence of plant growth substances in earthworms demonstrated by paper chromatography and the Went pea test. *Nature*, 208, p.1113–1114.

Noble, J. C., Gordon, W. T. and Kleinig, C. R. (1970). The influence of earthworms on the development of mats of organic matter under irrigated pastures in southern Australia. In: *Proceedings of the 11th International Grassland Congress*, 1970, p.465–468.

Nortcliff, S. (2002). Standardisation of soil quality attributes. *Agriculture, Ecosystems and Environment*, 88, p.161–168.

Pelosi, C., Bertrand, M., Capowiez, Y., Boizard, H. and Roger-Estrade, J. (2009). Earthworm collection from agricultural fields: Comparisons of selected expellants in presence/absence of hand-sorting. *European Journal of Soil Biology*, 45 (2), p.176–183.

Perrow, M. R. and Davy, A. J. (2002). *Handbook of ecological restoration, vol.1. Principles of restoration.* Cambridge: Cambridge University Press.

Piearce, T. G. and Boone, G. C. (1998). Responses of invertebrates to paper sludge application to soil. *Applied Soil Ecology*, 9, p.393–397.

Pigott, C. D. (1989). The growth of lime (*Tilia cordata*) in an experimental plantation and its influence on soil development and vegetation. *Quarterly Journal of Forestry*, 83 (1), p.14–24.

Pitman, R., Bastrup-Birk, A., Breda, N. and Rautio, P. (2010). Sampling and Analysis of Litterfall. In: *Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests*, Hamburg: UNECE ICP Forests Programme Co-ordinating Centre, p.16.

Pizl, V. (2001). Earthworm succession in afforested colliery spoil heaps in the Sokolov region, Czech Republic. *Restoration Ecology*, 9 (4), p.359–364.

Pulleman, M., Creamer, R., Hamer, U., Helder, J., Pelosi, C., Pérès, G. and 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.

R Core Team. (2015). *R: A language and environment for statistical computing*.

Rajapaksha, N. S. S., Butt, K. R., Vanguelova, E. I. and Moffat, A. J. (2013). Earthworm selection of Short Rotation Forestry leaf litter assessed through preference testing and direct observation. *Soil Biology and Biochemistry*, 67, p.12–19.

Rajapaksha, N. S. S., Butt, K. R., Vanguelova, E. I. and Moffat, A. J. (2014). Short rotation forestry--Earthworm interactions: A field based mesocosm experiment. *Applied Soil Ecology*, 76, p.52–59.

Raw, F. (1962). Studies of earthworm populations in orchards. *Annals of Applied Biology*, 50 (3), p.389–404.

Reddell, P. and Spain, A. V. (1991). Earthworms as vectors of viable propagules of mycorrhizal fungi. *Soil Biology and Biochemistry*, 23 (8), p.767–774.

Reich, P. B., Oleksyn, J., Modrzynski, J., Mrozinski, P., Hobbie, S. E., Eissenstat, D. M., Chorover, J., Chadwick, O. A., Hale, C. M. and Tjoelker, M. G. (2005). Linking litter calcium, earthworms and soil properties: A common garden test with 14 tree species. *Ecology Letters*, 8 (8), p.811–818.

van Rhee, J. A. (1969a). Development of earthworm populations in polder soils. *Pedobiologia*, 9, p.133–140.

van Rhee, J. A. (1969b). Inoculation of earthworms in a newly drained polder. *Pedobiologia*, 9, p.128–132.

van Rhee, J. A. (1977). Study of the effect of earthworms on orchard productivity. *Pedobiologia*, 17, p.107–114.

Rief, A., Knapp, B. A. and Seeber, J. (2012). Palatability of selected Alpine plant litters for the decomposer *Lumbricus rubellus* (Lumbricidae). *PLoS ONE*, 7 (9), p.1–7.

Rizhiya, E., Bertora, C., van Vliet, P. C. J., Kuikman, P. J., Faber, J. H. and van Groenigen, J. W. (2007). Earthworm activity as a determinant for N_2O emission from crop residue. *Soil Biology and Biochemistry*, 39 (8), p.2058–2069.

Rogaar, H. and Boswinkel, L. A. (1978). Some soil morphological effects of earthworm activity; Held data and X-ray radiography. *Netherlands Journal of Agricultural Science*, 26, p.145–160.

Rouelle, J. (1983). Introduction of amoebae and *Rhizobium japonicum* into the gut of *Eisenia fetida* (Sav.) and *Lumbricus terrestris* L. In: Satchell, J. E. (ed.), *Earthworm Ecology*, London: Chapman & Hall, p.375–381.

RStudio Team. (2015). *RStudio: Integrated Development for R*.

Rushton, S. P. (1986). Development of earthworm populations on pasture land reclaimed from open-cast coal mining. *Pedobiologia*, 29 (1), p.27–32.

Satchell, J. E. (1967). Lumbricidae. In: Burgess, A. and Raw, F. (eds.), *Soil biology*, London: Academic Press, p.259–322.

Satchell, J. E. (1983). Earthworm ecology in forest soils. In: Satchell, J. E. (ed.), *Earthworm Ecology*, London: Chapman & Hall, p.161–170.

Satchell, J. E. and Lowe, D. G. (1967). Selection of leaf litter by *Lumbricus terrestris*. *Progress in Soil Biology*, p.102–119.

Scheu, S. (2003). Effects of earthworms on plant growth: Patterns and perspectives. *Pedobiologia*, 47, p.846–856.

Scullion, J. (1992). Re-establishing life in restored topsoils. *Land Degradation and Development*, 3, p.161–168.

Scullion, J. (1994). Earthworms and the Rehabilitation of Disturbed Land. *University of Wales Review of Science and Technology*, 9.

Scullion, J. (2007). Making Soil – The Role of Earthworms. *CIWM Briefing Session H*.

Scullion, J. and Malik, A. (2000). Earthworm activity affecting organic matter, aggregation and microbial activity in soils restored after opencast mining for coal. *Soil Biology and Biochemistry*, 32, p.119–126.

Scullion, J., Mohammed, A. R. A. and Ramshaw, G. A. (1988). Changes in earthworm populations following cultivation of undisturbed and former opencast coal-mining land. *Agriculture, Ecosystems and Environment*, 20 (4), p.289–302.

Shipitalo, M. J. and Le Bayon, R. C. (2004). Quantifying the effects of earthworms on soil aggregation and porosity. In: Edwards, C. A. (ed.), *Earthworm Ecology*, Boca Raton: CRC Press, p.183–200.

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.

SNIFFER. (2010). *Code of Practice for the use of sludge, compost and other organic materials for land reclamation*.

Society for Ecological Restoration. (2004). *SER International Primer on Ecological Restoration*. Washington DC: Society for Ecological Restoration.

Sopper, W. E. (1993). *Municipal sludge use in land reclamation*. Boca Raton: CRC Press.

Springett, J. A., Gray, R. A. J., Barker, D. J., Lambert, M. G., Mackay, A. D. and Thomas, V. J. (1998). Population density and distribution of the New Zealand indigenous earthworm *Octochaetus multiporus* (Megascolecidae : Oligochaeta) in hill pastures. *New Zealand Journal of Ecology*, 22 (1), p.87–93.

Springett, J. A. and Syers, J. K. (1980). Effect of earthworm casts on ryegrass seedlings. In: *Proceedings of the 2nd Australian Conference on Grassland Invertebrate Ecology*, 1980.

Stein, J. K. (1983). Earthworm activity: A source of potential disturbance of archaeological sediments. *American Antiquity*, p.277–289.

Stephens, P. M., Davoren, C. W., Doube, B. M. and Ryder, M. H. (1994). Ability of the lumbricid earthworms *Aporrectodea rosea* and *Aporrectodea trapezoides* to reduce the severity of take-all under greenhouse and field conditions. *Soil Biology and Biochemistry*, 26 (10), p.1291–1297.

Stockdill, S. M. J. (1959). Earthworms improve pasture growth. *New Zealand Journal of Agriculture*, 98 (1), p.227–231.

Stockdill, S. M. J. (1966). The effect of earthworms on pastures. *Proceedings (New Zealand Ecological Society)*, 13, p.68–75.

Stockdill, S. M. J. (1982). Effects of introduced earthworms on the productivity of New Zealand pastures. *Pedobiologia*, 24 (1), p.29–35.

Stroud, J. L., Irons, D., Watts, C. W. and Whitmore, A. P. (2016). *Lumbricus terrestris* abundance is not enhanced after three years of compost amendments on a reduced tillage wheat cultivation conversion. *Applied Soil Ecology*, 98, p.282–284.

Swift, M. J., Heal, O. W. and Anderson, J. M. (1979). *Decomposition in terrestrial ecosystems*. Oakland, California: University of California Press.

Swift, M. J., Izac, A. and van Noordwijk, M. (2004). Biodiversity and ecosystem services in agricultural landscapes—are we asking the right questions? *Agriculture, Ecosystems and Environment*, 104 (1), p.113–134.

Symondson, W. O. C., Glen, D. M., Erickson, M. L., Liddell, J. E. and Langdon, C. J. (2000). Do earthworms help to sustain the slug predator *Pterostichus melanarius* (Coleoptera: Carabidae) within crops? Investigations using monoclonal antibodies. *Molecular Ecology*, 9 (9), p.1279–1292.

Taylor, C. M. A. (1991). Forest Fertilisation in Britain. In: *Forestry Commission Bulletin 95*, London: HMSO, p.45.

Thielemann, U. (1986). Elektrischer Regenwurmfang mit der Oktett-Methode. *Pedobiologia*, 29, p.295–302.

Tiwari, S. C. and Mishra, R. R. (1993). Fungal abundance and diversity in earthworm casts and in uningested soil. *Biology and Fertility of Soils*, 16 (2), p.131–134.

Tomati, U., Grappelli, A. and Galli, E. (1983). Fertility factors in earthworm humus. In: *Proceedings of the International Symposium on Agriculture and Environment: Prospects in Earthworm Farming*, 1983, p.49–56.

Vimmerstedt, J. P. (1983). Earthworm ecology in reclaimed opencast coal mining sites in Ohio. In: Satchell, J. E. (ed.), *Earthworm Ecology*, London: Chapman & Hall, p.229–240.

Wall, D. H., Bardgett, R. D., Behan-Pelletier, V., Herrick, J. E., Jones, T. H., Ritz, K. and Six, J. (2012). *Soil ecology and ecosystem services*. Oxford: Oxford University Press.

Wanner, M. and Dunger, W. (2002). Primary immigration and succession of soil organisms on reclaimed opencast coal mining areas in eastern Germany. *European Journal of Soil Biology*, 38 (2), p.137–143.

WD Environmental. (n.d.). *Soil Suitability Report for Little Gerpins*.

Welke, S. E. and Parkinson, D. (2003). Effect of *Aporrectodea trapezoides* activity on seedling growth of *Pseudotsuga menziesii*, nutrient dynamics and microbial activity in different forest soils. *Forest Ecology and Management*, 173, p.169–186.

Wolstenholme, R., Dutch, J., Moffat, A. J., Bayes, C. D. and Taylor, C. M. A. (1992). *A manual of good practice for the use of sewage sludge in forestry.* London: HMSO.

Wolters, V. and Stickan, W. (1991). Resource allocation of beech seedlings (*Fagus sylvatica* L.)—relationship to earthworm activity and soil conditions. *Oecologia*, 88 (1), p.125–131.

Wood, W. R. and Johnson, D. L. (1978). A survey of disturbance processes in archaeological site formation. *Advances in Archaeological Method and Theory*, 1, p.315–381.

Wright, M. A. (1972). Factors governing ingestion by the earthworm *Lumbricus terrestris* (L.), with special reference to apple leaves. *Annals of Applied Biology*, 70 (2), p.175–188.

Yeates, G. W. and der Meulen, H. (1995). Burial of soil-surface artifacts in the presence of lumbricid earthworms. *Biology and Fertility of Soils*, 19 (1), p.73–74.

Zaborski, E. R. (2003). Allyl isothiocyanate: An alternative chemical expellant for sampling earthworms. *Applied Soil Ecology*, 22 (1), p.87–95.

Zhang, H. and Schrader, S. (1993). Earthworm effects on selected physical and chemical properties of soil aggregates. *Biology and Fertility of Soils*, 15, p.229–234.

Appendix I. Mean (± SE) effects of experimental treatments on soil chemical parameters after 12 months in mesocosm tubes containing Italian alder (n=5).

			Italian alder			
Soil Type	Chemical parameter	No tree	Control	Earthworm only	Compost only	Earthworm and compost
	pH	8.52 ± 0.04	8.70 ± 0.08	8.66 ± 0.08	8.48 ± 0.09	8.50 ± 0.03
0 - 0.2 m bulk Soil	Cond. (µs/cm)	1356.98 ± 293.85	1008.68 ± 101.58	1098.1 ± 112.15	1143.1 ± 242.04	867.6 ± 50.83
	Total N (%)	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00ab	0.10 ± 0.00b***	0.10 ± 0.00b***
	Total C (%)	2.92 ± 0.09	2.94 ± 0.11	3.02 ± 0.17	2.96 ± 0.06	3.11 ± 0.08
	C(Org) (%)	1.72 ± 0.03a	1.79 ± 0.07ab	1.94 ± 0.13ab	2.03 ± 0.04ab	2.07 ± 0.10b*
	O.M. (%)	2.96 ± 0.05a	3.08 ± 0.12ab	3.35 ± 0.22ab	3.50 ± 0.07ab	3.56 ± 0.17b*
	C:N ratio	22.54 ± 0.53ab	23.01 ± 0.8ab	23.25 ± 0.78b*	20.59 ± 0.24a	21.30 ± 0.27ab
	moisture content (%)	23.14 ± 0.53b***	16.65 ± 0.98a	16.63 ± 1.34a	14.93 ± 0.69a	16.56 ± 0.450a
	K (mg/kg)	132.27 ± 3.11b**	100.78 ± 6.14a	96.97 ± 3.54a	115.21 ± 3.98ab	113.09 ± 8.74ab
	Ca (mg/kg)	2691.05 ± 113.35	2787.3 ± 58.04	2815.46 ± 82.78	2963.35 ± 76.94	2661.3 ± 43.17
	Mg (mg/kg)	72.36 ± 5.74	61.45 ± 3.94	61.77 ± 5.30	75.97 ± 4.09	73.68 ± 4.17
	Na (mg/kg)	14.86 ± 0.63	14.65 ± 0.38	16.62 ± 0.69	16.73 ± 0.58	15.01 ± 0.54
	[N(NH_4^+)] (mg/kg)	1.13 ± 0.06ab	1.20 ± 0.10b	0.65 ± 0.19a	1.31 ± 0.06b*	1.00 ± 0.153ab
	[N(NO_2^-)] (mg/kg)	0.52 ± 0.33	0.29 ± 0.17	0.10 ± 0.04	0.12 ± 0.02	0.09 ± 0.03
	[N(NO_3^-)] (mg/kg)	0.45 ± 0.06	0.29 ± 0.08	0.21 ± 0.06	0.32 ± 0.04	0.27 ± 0.08
	S(SO_4^{2-}) (mg/kg)	190.33 ± 45.29	124.19 ± 14.95	159.58 ± 25.8	202.1 ± 90.15	86.17 ± 9.46
	P(PO_4^{3-}) (mg/kg)	21.82 ± 1.04b***	18.69 ± 0.7a	18.65 ± 0.37a	22.12 ± 0.53b***	23.48 ± 0.63b***
0 - 0.2 m rhizosphere soil	pH	N/A	8.48 ± 0.11	8.62 ± 0.08	8.40 ± 0.09	8.58 ± 0.07
	Cond. (µs/cm)	N/A	1039.5 ± 146.65	1073.0 ± 146.68	752.1 ± 32.41	881.0 ± 13.68
	Total N (%)	N/A	0.12 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
	Total C (%)	N/A	3.69 ± 0.28	3.22 ± 0.12	3.31 ± 0.11	3.43 ± 0.13
	C(Org) (%)	N/A	2.72 ± 0.24	2.25 ± 0.10	2.42 ± 0.11	2.58 ± 0.14
	O.M. (%)	N/A	4.68 ± 0.41	3.89 ± 0.17	4.16 ± 0.19	4.46 ± 0.24
	C:N ratio	N/A	23.13 ± 0.48b	22.5 ± 0.44b	19.83 ± 0.44a***	21.91 ± 0.61b
	moisture content (%)	N/A	22.65 ± 1.62	18.20 ± 1.89	17.18 ± 1.26	19.19 ± 1.17
	K (mg/kg)	N/A	91.09 ± 3.9	92.73 ± 3.76	96.9 ± 6.77	100.75 ± 2.75
	Ca (mg/kg)	N/A	3159.25 ± 171.27	2934.24 ± 131.38	2994.6 ± 154.01	3120.36 ± 56.64
	Mg (mg/kg)	N/A	101.73 ± 6.93	91.97 ± 5.96	99.52 ± 6.15	103.16 ± 7.66
	Na (mg/kg)	N/A	21.97 ± 0.96	21.68 ± 1.09	21.79 ± 1.61	21.12 ± 1.46
	[N(NH_4^+)] (mg/kg)	N/A	2.12 ± 0.28b*	1.04 ± 0.14a	1.90 ± 0.35ab	1.22 ± 0.31ab
	[N(NO_2^-)] (mg/kg)	N/A	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.02	0.08 ± 0.03
	[N(NO_3^-)] (mg/kg)	N/A	0.23 ± 0.05	0.14 ± 0.01	0.23 ± 0.06	0.22 ± 0.05
	S(SO_4^{2-}) (mg/kg)	N/A	77.84 ± 23.5	85.95 ± 21.65	47.54 ± 8.10	50.76 ± 10.29
	P(PO_4^{3-}) (mg/kg)	N/A	19.48 ± 1.18ab	18.65 ± 0.56a	23.11 ± 1.48b*	22.95 ± 1.52ab
0.2 - 0. 4 m bulk soil	pH	9.08 ± 0.44	8.78 ± 0.34	8.62 ± 0.07	8.44 ± 0.09	8.62 ± 0.07
	Cond. (µs/cm)	827.80 ± 100.41a	1691.6 ± 334.68b*	936.3 ± 127.12ab	877.7 ± 148.49a	841.1 ± 71.56a
	Total N (%)	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00a	0.12 ± 0.00b***	0.1 ± 0.00c***
	Total C (%)	2.85 ± 0.06a	2.97 ± 0.07ab	2.91 ± 0.10a	3.28 ± 0.09b**	3.29 ± 0.11b**
	C(Org) (%)	1.72 ± 0.04a	1.86 ± 0.05a	1.83 ± 0.06a	2.23 ± 0.04b***	2.16 ± 0.07b***
	O.M. (%)	2.97 ± 0.06a	3.21 ± 0.08a	3.15 ± 0.10a	3.84 ± 0.07b***	3.72 ± 0.12b***
	C:N ratio	22.88 ± 1.04b**	23.73 ± 1.02b**	22.54 ± 0.52b**	19.31 ± 0.30a	21.71 ± 0.51ab
	moisture content (%)	27.14 ± 0.84b***	17.01 ± 1.18a	18.19 ± 1.68a	16.06 ± 1.01a	17.97 ± 0.96a
	K (mg/kg)	123.93 ± 2.66b***	87.85 ± 4.08a	83.06 ± 2.89a	96.89 ± 5.82a	99.49 ± 5.13a
	Ca (mg/kg)	2880.70 ± 313.66	3059.31 ± 214.48	2710.74 ± 75.05	2867.89 ± 106.31	2917.29 ± 56.77
	Mg (mg/kg)	66.38 ± 4.97	59.23 ± 4.46	66.01 ± 3.81	71.74 ± 5.41	71.66 ± 4.40
	Na (mg/kg)	14.85 ± 0.49	16.21 ± 0.73	18.11 ± 0.58	17.78 ± 1.35	17.44 ± 0.91
	[N(NH_4^+)] (mg/kg)	1.06 ± 0.05	0.70 ± 0.12	0.40 ± 0.18	0.91 ± 0.20	0.90 ± 0.23
	[N(NO_2^-)] (mg/kg)	0.36 ± 0.23	0.26 ± 0.15	0.05 ± 0.02	0.11 ± 0.01	0.14 ± 0.05
	[N(NO_3^-)] (mg/kg)	0.53 ± 0.06b***	0.18 ± 0.02a	0.16 ± 0.05a	0.22 ± 0.06a	0.29 ± 0.06a
	S(SO_4^{2-}) (mg/kg)	87.49 ± 13.53a	287.07 ± 79.67b*	151.68 ± 54.98ab	91.02 ± 16.81a	95.88 ± 15.39a

Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * $p < 0.05$ **, $p < 0.01$, *** $p < 0.001$.

Appendix II. Mean (± SE) effects of experimental treatments on soil chemical parameters after 12 months in mesocosm tubes containing Norway maple (n=5).

			Norway maple			
Soil Type	Chemical parameter	No tree	Control	Earthworm only	Compost only	Earthworm and compost
0 - 0.2 m Bulk Soil	pH	8.52 ± 0.04	8.72 ± 0.05	8.62 ± 0.13	8.92 ± 0.11	8.72 ± 0.10
	Cond. (µs/cm)	1356.98 ± 293.85a	761.6 ± 62.52ab	949.7 ± 75.48ab	757.9 ± 57.54ab	684.6 ± 98.89b*
	Total N (%)	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00a	0.1 ± 0.00b***	0.09 ± 0.00b***
	Total C (%)	2.92 ± 0.09ab	2.74 ± 0.05a	2.87 ± 0.08ab	3.18 ± 0.13b*	2.97 ± 0.06ab
	C(Org) (%)	1.72 ± 0.03a	1.69 ± 0.02a	1.75 ± 0.05a	2.09 ± 0.05c***	1.93 ± 0.02b***
	O.M. (%)	2.96 ± 0.05a	2.91 ± 0.03a	3.02 ± 0.08a	3.6 ± 0.09b***	3.33 ± 0.03c***
	C:N ratio	22.54 ± 0.53ab	22.25 ± 0.55ab	23.38 ± 0.50b*	20.85 ± 0.66a	20.87 ± 0.78a
	moisture content (%)	23.14 ± 0.58	21.24 ± 0.71	20.69 ± 0.58	19.36 ± 1.78	20.10 ± 0.68
	K (mg/kg)	132.27 ± 3.11a	129.05 ± 2.86ab	125.16 ± 4.68ab	150.65 ± 8.05b*	140.58 ± 7.59ab
	Ca (mg/kg)	2691.05 ± 113.35	2777.75 ± 54.43	2745.83 ± 36.9	2972.11 ± 90.24	2779.5 ± 135.63
	Mg (mg/kg)	72.36 ± 5.74	89.62 ± 8.36	75.82 ± 6.53	87.56 ± 4.55	88.95 ± 4.88
	Na (mg/kg)	14.86 ± 0.63	15.98 ± 0.63	15.93 ± 0.67	16.56 ± 0.64	15.36 ± 0.64
	[N(NH_4^+)] (mg/kg)	1.13 ± 0.06ab	1.31± 0.07ab	0.93 ± 0.20a	1.49 ± 0.11b*	1.41 ± 0.08ab
	[N(NO_2^-)] (mg/kg)	0.52 ± 0.33	0.14 ± 0.03	0.17 ± 0.06	0.12 ± 0.05	0.44 ± 0.16
	[N(NO_3^-)] (mg/kg)	0.45 ± 0.06	0.39 ± 0.05	0.35 ± 0.10	0.58 ± 0.27	0.56 ± 0.09
	S(SO_4^{2-}) (mg/kg)	190.33 ± 45.29	103.47 ± 12.68	166.93 ± 55.68	79.8 ± 19.58	92.6 ± 31.03
	P(PO_4^{3-}) (mg/kg)	21.82 ± 1.04a	21.41 ± 1.01a	21.14 ± 0.51a	26.86 ± 1.58b**	25.87 ± 1.42ab
	pH	N/A	8.46 ± 0.09	8.52 ± 0.06	8.32 ± 0.09	6.82 ± 1.71
0 - 0.2 m Rhizo soil	Cond. (µs/cm)	N/A	845.7 ± 277.84	565.3 ± 409.5	314.6 ± 193.4	446.9 ± 195.37
	Total N (%)	N/A	0.12 ± 0.01	0.11 ± 0.01	0.15 ± 0.02	0.10 ± 0.03
	Total C (%)	N/A	4.34 ± 0.39	3.9 ± 0.48	5.04 ± 0.66	3.04 ± 0.81
	C(Org) (%)	N/A	3.35 ± 0.42	3.02 ± 0.45	4.22 ± 0.68	2.35 ± 0.64
	O.M. (%)	N/A	5.77 ± 0.72	5.21 ± 0.78	7.27 ± 1.16	4.05 ± 1.11
	C:N ratio	N/A	27.74 ± 1.73a	27.82 ± 2.31a	28.06 ± 1.13a	17.97 ± 4.54b*
	moisture content (%)	N/A	34.36 ± 2.73	30.93 ± 3.93	29.58 ± 3.30	25.95 ± 2.21
	K (mg/kg)	N/A	114.34 ± 4.32	136.49 ± 5.1	131.43 ± 8.78	111.37 ± 27.92
	Ca (mg/kg)	N/A	3465.21 ± 280.22	3377.16 ± 97.96	3656.88 ± 284.05	2663.15 ± 699.1
	Mg (mg/kg)	N/A	148.54 ± 14.86	147.29 ± 7.56	151.93 ± 20.17	115.13 ± 31.56
	Na (mg/kg)	N/A	21.88 ± 1.83	23.96 ± 2.11	22.59 ± 0.80	17.64 ± 4.68
	[N(NH_4^+)] (mg/kg)	N/A	2.30 ± 0.44	1.64 ± 0.23	3.01 ± 0.53	2.37 ± 0.21
	[N(NO_2^-)] (mg/kg)	N/A	0.08 ± 0.01	0.08 ± 0.02	0.09 ± 0.01	0.11 ± 0.04
	[N(NO_3^-)] (mg/kg)	N/A	0.38 ± 0.08	0.61 ± 0.09	0.43 ± 0.20	0.47 ± 0.07
	S(SO_4^{2-}) (mg/kg)	N/A	99.81 ± 47.15	114.86 ± 47.69	36.33 ± 5.05	31.3 ± 9.14
	P(PO_4^{3-}) (mg/kg)	N/A	22.35 ± 1.41	26.13 ± 1.81	25.12 ± 2.27	23.3 ± 5.93
0.2 - 0.4 m Bulk soil	pH	9.08 ± 0.44	8.9 ± 0.08	8.62 ± 0.09	8.68 ± 0.14	8.50 ± 0.06
	Cond. (µs/cm)	827.8 ± 100.41ab	1186.5 ± 171.09b**	757.6 ± 86.12a	694.8 ± 17.1a	595 ± 33.41a
	Total N (%)	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00a	0.10 ± 0.01b***	0.11 ± 0.00b***
	Total C (%)	2.85 ± 0.06a	2.97 ± 0.05ab	2.91 ± 0.07ab	3.28 ± 0.06c**	3.16 ± 0.11bc
	C(Org) (%)	1.72 ± 0.04	1.79 ± 0.04	1.81 ± 0.05	2.10 ± 0.05	2.16 ± 0.07
	O.M. (%)	2.97 ± 0.06a	3.08 ± 0.06a	3.13 ± 0.09a	3.62 ± 0.08b***	3.73 ± 0.12b***
	C:N ratio	22.88 ± 1.04ab	23.19 ± 1.13ab	24.15 ± 1.28b**	20.31 ± 0.59ab	19.43 ± 0.52a
	moisture content (%)	27.14 ± 0.84a	22.55 ± 1.46	24.01 ± 0.41	21.02 ± 2.01	22.80 ± 0.52
	K (mg/kg)	123.93 ± 2.66	111.05 ± 4.36	117.62 ± 5.93	128.33 ± 5.77	125.26 ± 4.60
	Ca (mg/kg)	2880.7 ± 313.66	2939.5 ± 203.53	2643.66 ± 107.06	2958.92 ± 194.68	2718.28 ± 83.27
	Mg (mg/kg)	66.38 ± 4.97a	66.66 ± 5.27a	77.71 ± 4.71ab	78.06 ± 6.38ab	90.82 ± 3.32b*
	Na (mg/kg)	14.85 ± 0.49	15.34 ± 0.83	16.21 ± 0.75	17.08 ± 1.20	17.27 ± 0.66
	[N(NH_4^+)] (mg/kg)	1.06 ± 0.05ab	1.03 ± 0.08	0.75 ± 0.13	1.43 ± 0.16	1.21 ± 0.10
	[N(NO_2^-)] (mg/kg)	0.36 ± 0.23	0.36 ± 0.35	0.58 ± 0.08	0.13 ± 0.05	0.15 ± 0.04
	[N(NO_3^-)] (mg/kg)	0.53 ± 0.06	0.53 ± 0.063	0.78 ± 0.35	0.29 ± 0.13	0.37 ± 0.30
	S(SO_4^{2-}) (mg/kg)	87.49 ± 13.53ab	209.08 ± 57.97b*	99.66 ± 27.74ab	110.52 ± 32.61ab	48.42 ± 8.02a
	P(PO_4^{3-}) (mg/kg)	20.38 ± 0.59a	21.11 ± 0.34a	20.55 ± 0.49a	24.64 ± 1.86b*	24.93 ± 1.33b*

Different letters in a row indicate significant differences, ANOVA followed by Tukey-Kramer post-hoc test, n = 5, * p <0.05 **, p <0.01, *** p <0.001.

Appendix III. Mean (%) change of selected chemical parameters of rhizosphere soil, compared with bulk soil, under experimental treatments after 12 months in mesocosm tubes containing Italian alder or Norway maple. Results are for upper soil section only (0-0.2 m), n=5.

	Italian Alder				Norway maple			
Chemical parameter	Control	Earthworm only	Compost only	Earthworm and compost	Control	Earthworm only	Compost only	Earthworm and compost
pH	-2.59	-0.46	-0.95	+0.93	-3.07	-1.17	-7.21	-27.86
Cond. (µs/cm)	+2.96	-2.34	-51.99	+1.52	+9.94	-68.00	-140.94	-53.20
Total N (%)	+33.33	+20.00	+16.67	+16.67	+33.33	+27.27	+33.33	+10.00
Total C (%)	+20.33	+6.21	+10.57	+9.33	+36.87	+26.41	+36.90	+2.30
C (Org) (%)	+34.19	+13.78	+16.12	+19.77	+49.55	+42.05	+50.47	+17.87
O.M. (%)	+34.19	+13.88	+15.87	+20.18	+49.57	+42.03	+50.48	+17.78
C:N ratio	+0.52	-3.33	-3.83	+2.78	+19.79	+15.96	+25.69	-16.14
Moisture content (%)	+26.49	+8.63	+13.10	+13.71	+38.18	+33.11	+34.55	+22.54
K (mg/kg)	-10.64	-4.57	-18.90	-12.25	-12.87	+8.30	-14.62	-26.23
Ca (mg/kg)	+11.77	+4.05	+1.04	+14.71	+19.84	+18.69	+18.73	-4.37
Mg (mg/kg)	+39.60	+32.84	+23.66	+28.58	+39.67	+48.52	+42.37	+22.74
Na (mg/kg)	+33.32	+23.34	+23.22	+28.93	+26.97	+33.51	+26.69	+12.93
[N(NH_4^+)] (mg/kg)	+43.40	+37.50	+31.05	+18.03	+43.04	+43.29	+50.50	+40.51
[N(NO_2^-)] (mg/kg)	-262.50	-11.11	-20.00	-12.50	-75.00	-112.50	-33.33	-300.00
[N(NO_3^-)] (mg/kg)	-26.09	-50.00	-39.13	-22.73	-2.63	+42.62	-34.88	-19.15
S(SO_4^{2-}) (mg/kg)	-59.55	-85.67	-325.12	-69.76	-3.67	-45.33	-119.65	-195.85
P(PO_4^{3-}) (mg/kg)	+4.06	+0.00	+4.28	-2.31	+4.21	+19.10	-6.93	-11.03
Soil density (g/ml)	-5.69	-4.84	-4.88	-4.84	-6.56	-5.74	-5.69	-32.65

APPENDIX IV. Conferences attended, presentations delivered (awards received) and examples of academic posters produced.

Date	Institution	Details
February 2013	British Society for Soil Science, University of Reading	Attending the one-day SEESOIL conference enabled me to expand my knowledge regarding current research in my field, and to undertake valuable networking.
8th April 2013	Forestry Commission England	A 5-minute oral presentation was given about this research in the field to the Director of the Forestry Commission England.
12th June 2013	**UCLan Grenfell-Baines School of Architecture, Construction and Environment research poster event (Winner)**	A research poster was prepared and presented, disseminating the Ingrebourne Hill field experiment to an audience of researchers within my school of the University. The poster was entitled "Investigating the effects of compost and earthworm addition on soil quality and tree growth on regenerated brownfield land".
4th July 2013	**UCLan Graduate Research School Office Research Conference (Winner)**	This required presentation of both a poster and oral presentation to a large audience at the conference. The presentation was entitled "Soil quality under brownfield land regeneration to woodland – provision of wider ecosystem services".
28th May 2014	Earthworm Research Conference, Isle of Rum, Scotland	This oral presentation (entitled "The influence of earthworm activity on reclaimed soil and woodland ecosystem service delivery") was delivered to an external audience of Scottish Natural Heritage (SNH) workers, members of UCLan's Earthworm Research Group (ERG) and researchers from an earthworm research group based in the University of Rzeszow, Poland.
22nd – 29th June 2014	10th International Symposium on Earthworm Ecology, Georgia, USA	This poster presentation was delivered to an international audience of professional researchers. The poster is entitled "Provision of soil and woodland ecosystem services on reclaimed land through the earthworm activity".
4th-7th November 2014	EU COST action BioLINK conference – University of Reading, UK	This poster presentation was delivered to an international audience of professional soil researchers. The poster is entitled "Provision of soil and woodland ecosystem services on reclaimed land through the earthworm activity".
17th-29th	EU COST action	This poster presentation was delivered to an international audience

March 2015	BioLINK conference – Krakow, Poland	of professional soil researchers. The poster is entitled "Earthworm colonisation of a restored landfill site in London".
28th April 2015	British Land Reclamation Society (BLRS) day event – Ecology in Land Restoration	I helped organise plan and arrange a day-long event for the BLRS, for members to visit a number of restored landfill sites and learn about the ecology of the sites. This involved me pre-event planning, conference calls, preparing materials and then, on the day, delivering a number of oral presentations over the course of the day, describing my research project and field experiments to around 20 land restoration professionals.
1st May 2015	British Society for Soil Science 'International Year of Soils', South Farnham Junior School	I visited the School with Dr Elena Vanguelova (a member of my supervisory team) and we delivered a 30-minute oral presentation to the assembled school (500 children + staff), to talk about the importance of soil and to raise the children's interest in science.
24th – 27th August 2015	Society for Ecological Restoration conference ('SER 2015'), Manchester, UK	I delivered an oral presentation at this conference to an international audience of land restoration professionals and academics. The presentation was entitled "Earthworm colonisation of a restored landfill site in London". I was successful in applying for funding to attend this conference, with the funding from the RIO 2015 grant fund at the University of Central Lancashire.
20th November 2015	Forest Research Seminar Series – Farnham, UK	I delivered an oral presentation at this seminar to an audience of academics across a range of scientific disciplines. The presentation was entitled "Earthworm colonisation of a restored landfill site in London".
12th April 2016	EU COST action BioLINK conference – Sofia, Bulgaria	This oral presentation was delivered to an international audience of professional soil researchers. The presentation is entitled "Woodland restoration on landfill sites: Earthworm activity and ecosystem service provision".

Winning poster presented at the UCLan Graduate Research School Office Research Conference, July 2013

Soil quality under brownfield land regeneration to woodland – provision of wider ecosystem services

uclan University of Central Lancashire

Forest Research The Research agency of the Forestry Commission

Ashwood, F. E.[1], Butt, K. R.[1], Doick, K. J.[2], and Vanguelova, E.[2]

[1]Earthworm Research Group, University of Central Lancashire, Preston, PR1 2HE, UK
[2]Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK

Background to the Project

Regenerating brownfield land into greenspaces such as woodlands may contribute a number of ecosystem services, such as improved biodiversity and climate change mitigation and adaptation. Creation of a suitable soil resource is essential for sustainable greenspace establishment, to provide necessary soil chemical and physical conditions and restore normal soil biological functions (Scullion, 1992). There is increasing industrial and scientific interest adding organic matter from waste streams to soil for regeneration projects. Earthworms play a crucial role in soil development and cycling essential plant nutrients and organic carbon within soils, yet there has been relatively little research into their potential for enhancing land regeneration to woodland.

Aim

This poster introduces a PhD project and presents the set-up and preliminary results of a field experiment. The aim of this PhD research project is to investigate the relationship between soil physical, chemical and biological status and the provision of key forest ecosystem services provided by woodland on regenerated brownfield land. Specific aims include…

Material and Methods

A combination of methods will be used throughout this research project. The opposite side of this poster presents a field experiment that has been set up at a regenerated brownfield site in the first year of the project., Other experiments will include nursery trials to explore the influence of worms and organic wastes on tree growth under controlled conditions, and microcosm experiments in a laboratory setting.

Application of the Research

This research is expected to contribute knowledge regarding the effects on tree growth, soil biodiversity, soil nutrient cycling and carbon sequestration potential on regenerated land through the action of earthworms and organic waste addition; and improve our understanding of the dynamics of naturally and artificially colonised earthworm populations in brownfield soils regenerated to woodland.

Field Experiment

A field experiment was set up, which aims to determine:

- The effects of organic matter addition and earthworm inoculation on tree growth and health, and soil physical, chemical and biological status on regenerated brownfield land.
- The effects of organic matter application and woodland establishment on earthworm population and community dynamics on regenerated brownfield land.

This experiment comprises four treatment types:

1) Control plots without treatment
2) Addition of earthworms
3) Addition of composted green waste
4) Addition of composted green waste and earthworms

Fig. 1. Experiment location within Ingrebourne Hill Community Woodland, a regenerated landfill site in Rainham, London

Composted green waste was added to the treated plots. Treated and untreated plots were cultivated, to relieve soil compaction and incorporate compost into the soil.

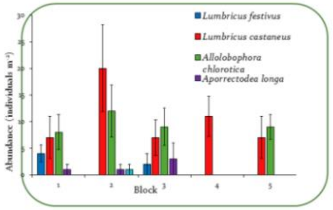
Earthworm barriers (polythene sheets) were dug into the perimeter of each experimental plot to prevent movement of worms between the experiment and surrounding woodland.

Tree planting of Norway Maple (Acer platanoides) and Italian Alder (Alnus cordata) took place in each experimental plot.

An earthworm survey was conducted to assess the existing background earthworm community composition within the experimental plots.

In total, 2,000 earthworms of the anecic species Aporrectodea longa (black-headed worm) were collected from the surrounding community woodland and translocated to the experimental plots

- Measurements of tree growth, soil physical and chemical parameters, earthworm and soil microbial populations will be taken at regular (ca. 6 monthly) intervals throughout the 3-year duration of the experiment
- Baseline surveying revealed five earthworm species within the experimental site, at the following mean levels: *Lumbricus festivus* (1 m^{-2}), *L. castaneus* (10 m^{-2}), *L. terrestris* (0.2 m^{-2}), *Aporrectodea longa* (1 m^{-2}) and *Allolobophora chlorotica* (8 m^{-2}) (Fig.4).
- Differences in the population density of each worm species per block was analysed statistically. The abundance of *L. festivus* was shown to be significantly different between the plots (p = 0.014). All other species displayed no significant differences in abundance between blocks (p > 0.05)..



Winning poster presented at the UCLan Grenfell-Baines School of Architecture, Construction and Environment research poster event

uclan University of Central Lancashire | School of Built and Natural Environment

Forest Research — The Research agency of the Forestry Commission

Investigating the effects of compost and earthworm addition on soil quality and tree growth on regenerated brownfield land

Ashwood, F. E.[1*], Butt, K. R.[1], Doick, K. J.[2], and Vanguelova, E.[2]

[1]Earthworm Research Group, University of Central Lancashire, Preston, PR1 2HE, UK
[2]Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK
[1*]E-mail contact: feashwood@uclan.ac.uk

Introduction and Objectives

Creation of a suitable soil resource is essential for sustainable greenspace establishment, in order to provide necessary soil chemical and physical conditions and restore normal soil biological functions (Scullion, 1992). There is increasing industrial and scientific interest in improving the soil materials for regeneration projects, particularly through the addition of organic matter from waste streams. Earthworms play a crucial role in soil development and the cycling of essential plant nutrients and organic carbon within soils, yet there has been relatively little research into their potential for enhancing land regeneration to woodland. This poster presents the set-up and preliminary results of a PhD field experiment, which aims to determine:

- The effects of organic matter addition and earthworm inoculation on tree growth and health, and soil physical, chemical and biological status on regenerated brownfield land.
- The effects of organic matter application and woodland establishment on earthworm population and community dynamics on regenerated brownfield land.

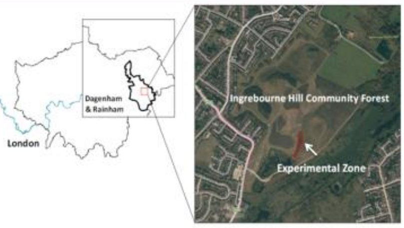


Fig. 1. Experiment location within Ingrebourne Hill Community Woodland, a regenerated landfill site in Rainham, London

Materials and Methods

Fig. 2. (a) Application of composted green waste; (b) Digging earthworm barriers into perimeter of experimental plots.

This experiment comprises 5 blocks (replicates), each containing four treatment plots:

1) Control plot without treatment
2) Addition of earthworms
3) Addition of composted green waste
4) Addition of composted green waste and earthworms

- **Composted green waste** was added to the treated plots at a rate of 500 kg Total N ha^{-1}, the maximum permissible rate for a Nitrate Vulnerable Zone (figure 2a). Treated and untreated plots were cultivated, to relieve soil compaction and incorporate compost into the soil.
- **Earthworm barriers** (polythene sheets) were dug into the perimeter of each experimental plot to prevent movement of worms between the experiment and surrounding woodland (figure 2b), a technique demonstrated by Butt & Grigoropoulou (2010).
- **Tree planting** of Norway Maple (*Acer platanoides*, figure 3a) and Italian Alder (*Alnus cordata*) took place in each experimental plot.
- An **earthworm survey** was conducted to assess the existing background earthworm community composition within the experimental plots.
- **In total, 2,000 earthworms** of the anecic species *Aporrectodea longa* (black-headed worm) were collected from the surrounding community woodland and **translocated to the experimental plots** (figure 3b).

Fig. 3. (a) Norway Maple (*Acer platanoides*) in an experimental plot; (b) Translocated *Aporrectodea longa* in an experimental plot.

Preliminary Results

- Baseline surveying revealed five earthworm species within the experimental site, at the following mean levels: *Lumbricus festivus* (1 m^{-2}), *L. castaneus* (10 m^{-2}), *L. terrestris* (0.2 m^{-2}), *Aporrectodea longa* (1 m^{-2}) and *Allolobophora chlorotica* (8 m^{-2}) (Fig.4).
- Differences in the population density of each worm species per block was analysed statistically using ANOVA. Where the assumptions of ANOVA were not met, non-parametric Kruskal-Wallis was applied. The abundance of *L. festivus* was shown to be significantly different between the plots ($p = 0.014$). All other species displayed no significant differences in abundance between blocks ($p > 0.05$).
- Measurements of tree growth, soil physical and chemical parameters, earthworm and soil microbial populations will be taken at regular (ca. 6 monthly) intervals throughout the 3-year duration of the experiment.

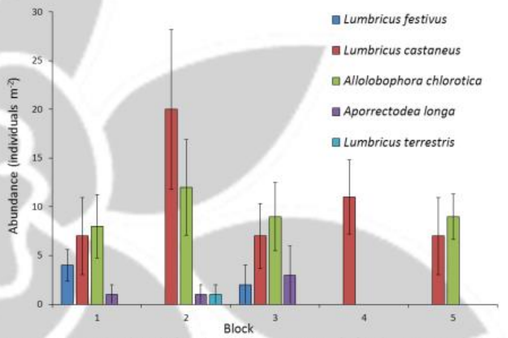


Fig. 4. Mean number (±S.E.) of each earthworm species m^{-2} of each experimental block

References

Butt, K.R. & Grigoropoulou, N., 2010. Basic Research Tools for Earthworm Ecology. *Applied and Environmental Soil Science*, pp.1–12.

Scullion, J., 1992. Re-establishing life in restored topsoils. *Land Degradation & Development*, 3, pp.161–168.

Research 2013

Poster presented at the EU COST BioLINK conference, March 2015

Earthworm colonisation of a landfill restored to woodland in East London

Ashwood, F. E. [1*], Butt, K. R. [1], Doick, K. J. [2], and Vanguelova, E. I. [2]

[1]Earthworm Research Group, University of Central Lancashire, Preston, PR1 2HE, UK
[2]Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK
[1*]E-mail contact: feashwood@uclan.ac.uk

uclan University of Central Lancashire

Forest Research

Introduction

Earthworms play a crucial role in soil development and the cycling of essential plant nutrients and organic carbon, however much is still unknown about their community dynamics and potential for improving the delivery of soil ecosystem services on restored land. To promote soil development on restored sites, it is beneficial that the soil material provided on these sites is suitable for earthworm colonisation. This work presents the set-up and preliminary results of an earthworm colonisation survey of Bonnet's Wood, a newly restored woodland on a former landfill site in East London (Fig. 1).

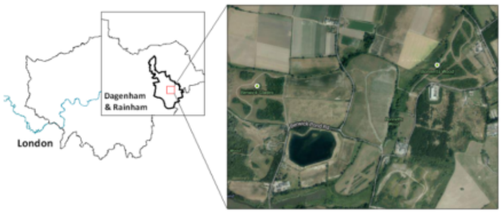


Fig. 1. Location of Bonnet's Wood, a restored landfill site in Rainham, London

Objectives and Methods

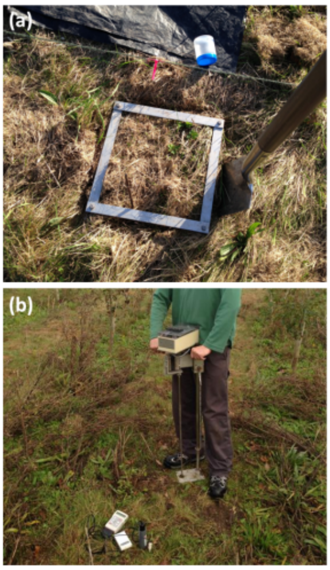


Fig. 2. (a) Digging for earthworms along a transect, (b) Measuring soil compaction using a manually-operated digital penetrometer

The objective of this survey was to investigate the effects of soil physical quality, vegetation cover, and distance from the site boundary on earthworm community composition and distribution.

- Transect lines of 20 m in length were placed at 50m intervals, radiating from the site boundary fence toward the centre of the site. Sampling was carried out at 5m intervals along each transect (fig. 3).
- At each sampling point, the earthworm community composition and population density, soil compaction (fig. 2b), soil moisture content, estimated cover of leaves, grass, and forbs for each quadrat, distance to nearest tree and species of the nearest tree was recorded.
- Earthworm sampling was a combination of digging and hand-sorting for earthworms, followed by mustard vermifuge application into soil pits dug below a 0.1 m^2 quadrat (fig. 2a).
- Earthworm species richness and abundance will be mapped across the site, using the ordinary kriging function in ArcGIS, which uses known data from sampling points to predict unknown values at other points within the sampling area.

Fig. 3. Transect layout and sampling design around site perimeter

Preliminary Results

- Within a year of restoration, there was an even distribution of earthworms from the site boundary to over 20 m into the site. Natural colonisation rates (typically 4-6 m^{-yr}) cannot account for this, and earthworms must have been introduced to the site within topsoil materials used during restoration.
- Earthworm abundance was reduced at the 5 m sampling point (149 m^2, compared with $\geq$206 m^2 at all other points), which was significantly ($p < 0.05$) more compacted to 30 cm depth than surrounding areas (Fig 4). The deep-burrowing, soil-developing earthworm species *Lumbricus terrestris* was found at all sampling points except at 5m, with this species' absence likely due to the higher levels of compaction.
- Earthworm species richness and abundance was higher at this site than at an adjoining 5 year old restored landfill site which had a lower standard of restoration (7 species compared to 5 species, and 207 earthworms m^2 compared with 48 m^2).
- This research shows that a higher quality of soil material used during the restoration process may facilitate earthworm colonisation, and thereby promote soil ecosystem service provision on restored land.

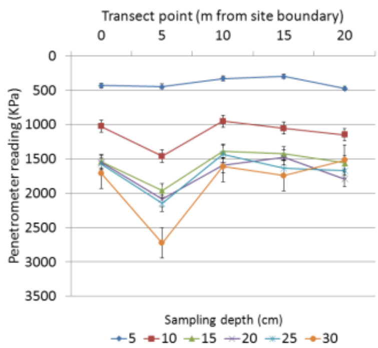


Fig. 4. Average soil compaction (± 1 S.E.) to 30cm depth across transect lines

Appendix V. Confirmation of ethical approval for this research project

uclan
University of Central Lancashire

7 June 2013

Kevin Butt / Frank Ashwood
School of Built and Natural Environment
University of Central Lancashire

Dear Kevin / Frank

Re: STEM Ethics Committee Application
Unique Reference Number: **STEM 132**

The STEM ethics committee has granted approval of your proposal application '**Soil quality under brownfield land regeneration to woodland – provision of wider ecosystem services**'.

Please note that approval is granted up to the end of project date or for 5 years, whichever is the longer. This is on the assumption that the project does not significantly change, in which case, you should check whether further ethical clearance is required.

We shall e-mail you a copy of the end-of-project report form to complete within a month of the anticipated date of project completion you specified on your application form. This should be completed, within 3 months, to complete the ethics governance procedures or, alternatively, an amended end-of-project date forwarded to roffice@uclan.ac.uk quoting your unique reference number.

Yours sincerely

Tal Simmons
Chair
STEM Ethics Committee

Appendix VI. Selected risk and COSHH assessments

Job: Visiting Brownfield Sites

Location: Various

Brief description of the job: Any visits to brownfield sites for project meetings or gathering information. Brownfield sites are derelict, underused, damaged, neglected and possible vacant environments. The sites can be ex-industrial, commercial or domestic environments.

HAZARD	Who could be harmed?	Level of Risk	CONTROLS	IMPLEMENTING / MONITORING
Terrain rough, boulders, tree stumps and logs – trip and slip hazards	All visitors to site	Medium	Keep to paths wherever possible, be watchful, walk with care and attention, wear appropriate footwear, ideally with ankle support.	Ensure all visitors are aware of the hazards and advised of the controls; Footwear maintained and in good order.
Glass, metal, flints, plastics in soil – laceration hazard	All visitors to site	Medium	Keep to paths wherever possible. Take precautions to prevent slips/trips (see above). First aid kit must be available.	Ensure all visitors are aware of the hazards and advised of the controls.
Undergrowth uncut trip and slip hazard, laceration hazard	All visitors to site	Medium	Where possible keep to paths, be watchful, walk with care and attention.	Ensure all visitors are aware of the hazards and advised of the controls.
Water filled ditches/drainage channels – slip hazard, drowning hazard	All visitors to site	Medium	Wear footwear with suitable tread. Do not try to leap over ditches. Do not get too close or enter ditch unnecessarily. Beware trip and slip hazards when approaching ditches/drainage channels	Visitors to be advised of hazard and advised of the controls and not to approach alone. Leader to be aware of the whereabouts of visitors.
Leachate in drainage channels	All visitors to site	Low	Avoid contact with skin and eyes. If this should occur, wash area with water, seek medical attention.	Visitors not to enter drainage channels and to minimise risk of accidentally entering channels. Visitors to be in pairs/groups at all times.
Weil's Disease	All visitors to site	Low – major disease unlikely	Risk minor. No formal risk assessment required. Controls in place through issue of HSM 28 and attached information note to all staff. Ensure provision of water-proof gloves and first aid kits to treat cuts and abrasions.	Ensure availability of HSM 28 (CFCC Office Manager) Regular and frequent checks of First Aid kits
Soil Contamination	All visitors to	Low	Previous operations may have resulted in some contamination of the site with heavy metals, organic	No eating, drinking or smoking to be allowed on site. Staff to wash hands

	site		contaminants or asbestos. Ensure all staff are aware of risks associated with heavy metals (e.g. lead, cadmium). Limit dermal-soil contact. Be aware of potential gaseous emissions (e.g. methane, hydrogen sulphide). Move away from affected areas. In dry conditions, where inhalation/ingestion of dust may be likely masks should be worn on site. No eating, drinking or smoking to be allowed on site.	before leaving the site and certainly before eating. Masks to be worn if soil is dry and it is windy. Staff to be aware of signs of landfill gas, such as patches of dead or no vegetation growth and the smell of rotten eggs.
Giant hogweed and ragwort	All visitors to site	Low	Avoid contact. Wear suitable protective clothing. Do not handle.	Ensure all visitors are aware of the presence and how to identify these species.
Heavy plant traffic	All visitors to site	Low	Activity should be confined to designated area(s) only, care to be taken not to enter active area of site. Take extreme care when using or crossing site access roads. Wear high visibility jacket.	Regularly monitor traffic movement about the site, keep in contact with group. Responsible person to keep informed about any changes in traffic access routes. Site manager must be aware of groups presence on site and course of travel
Illegal off-road use by motorbikes and other vehicles	All visitors to site	Medium	Be mindful of local activities. Try to ensure a safe distance at all times. Wear high visibility jacket.	Ensure all visitors are aware of the hazards and advised of the controls.
Contact with strangers	All visitors to site	Medium	Read and sign Risk Assessment No. 98 (have buddy system in operation).	
Ticks, insect bites and stings Tetanus Dehydration, sunburn Hypothermia			Read and sign Job Risk Assessment No 1 (Lone Working) which addresses these issues.	Responsible H&S person to check and ensure that all staff have signed JRA 1.

I have read the above-approved assessment.

I have been instructed and trained in the safe operation of the activity identified by this assessment, and agree to follow controls specified by the assessment. I will inform Kieron Doick of any hazards encountered which are not covered by this risk assessment.

Name	**Signed**	**Date**
Francis Ashwood		29/05/2013

Job: Lone fieldwork.	Location: Anywhere

HAZARD	Who could be harmed?	Level of Risk (assuming no control)	CONTROLS	IMPLEMENTING / MONITORING
When lone working there is a lack of personnel to provide first aid or assistance in the event of an emergency.	Lone worker	High	Where possible avoid lone-working but in cases where this is not possible make the following arrangements. 1. Carry emergency equipment appropriate to the location, season and activity **Emergency Equipment** • Survival blanket • Food/Water • First Aid Kit • Whistle • Compass 2. A mobile (as a minimum 112/999) must be available at all sites. Reception to mobile phone networks is sometimes not known. Staff must check with the local forest district in advance and may need to consider the use of a satellite telephone. 3. CFCC lone working system must be followed There are two types of Lone working covered by the system A. Spending a significant amount of time 'remote from help' **You must register and use the G24 system in advance.** Details and guidance on the use of this system can be found on http://alpacorn.forestry.gov.uk:7777/portal/page?_pageid=33,334673&_dad=portal&_schema=PORTAL **And on Appendix 1 attached, along with CFCC Lone Working Emergency Plan.** The CFCC G24 administrators are: Alice Holt: Sue Bellis NRS: Madge Holmes The Guardian 24 system can be operated using either (1) a	• Annual review of Divisional Safety HoC • PPE to be checked • Annual review first aid kits by designated person All above to be managed via resumption system. • Line manager to ensure lone working systems are set up and adhered to and this issue will be reviewed at each PMS review.

			landline, (2) a mobile phone or (3) a satellite phone. B. Undertaking low risk work and you are out for up to 1 day, 'near to help', i.e. visiting an experiment near Alice Holt, working at a location where there is frequent public access or an area where you are visible from a busy public road. This may enable the use of the **CFCC Buddy system** • A buddy card should be filled out providing agreed written contact details, time & location of visit (with Grid ref where possible) expected time of return, and your contact mobile phone number and handed to 3 responsible Buddies (these should be work colleagues and **NOT** family or friends) who will know your location and what action to take in the event of non-contact. • Red Buddy cards are available from the Centre Office at AH or the health and safety file in Room 8 at NRS. • It is the responsibility of the lone worker to ensure that their chosen buddies fully understand their role, location and agreed contact schedule • If you are moving around an area, then you must keep your buddies informed of your current location • If research involves meeting several people in the course of your work, make an itinerary and timetable of contact points. (If possible the itinerary should contain the contact details of the person being visited). • The card will identify the primary buddy who should act as the main contact and keep the other buddies informed. 4 All records must be kept and forwarded to the Safety Administrator Lynn Jordan.	
Dehydration, sunburn due to working out-of-doors	All	Minor	Ensure adequate fluids are taken. Wear Sun block and suitable clothing.	As above
Hypothermia	All	Major	Avoid working in severe weather conditions Wear suitable protective clothing and carry emergency equipment.	As above

Tick and other insect bites	All	Major	Check for ticks and remove on a daily basis; ensure all personnel are aware of the symptoms and dangers of Lyme's disease. Read Forest Research notes on Lyme's disease. Wear suitable clothing and insect repellent. *Report any cases of suspected Lyme's disease to H&S officer.*	Record issue of information notes. **RIDDOR reportable occurrence**
Tetanus	All	Major	**Ensure up to date immunisation**	Annual Reminders
Terrain- slipping and falling	All	Major	Wear protective footwear and carry emergency equipment.	• Annual review of Centre Safety HoC • PPE to be checked • Annual review first aid kits by designated person All above to be managed via resumption system. Line manager to ensure adherence to lone working procedure.
Verbal abuse	All	Minor	Avoid confrontation. If threatened leave the area and report incident.	Record issue of information notes.
Physical attack	All	Minor	Avoid confrontation. If threatened leave the area and report incident.	Record issue of information notes.

I have read the above approved assessment.

I have been instructed and trained in the safe operation of the activity identified by this assessment, and agree to follow controls specified by the assessment. I will inform [Sue Benham] of any hazards encountered which are not covered by this risk assessment.

Name	**Signed**	**Date**
Francis Ashwood		29/05/2013

Job: Driving at Work – Driving official, lease, hire or private vehicles on public and forestry class A1 roads				**Location: Variable**
The Hazard	**Who could be harmed?**	**Level of risk**	**Controls**	**Implementation/Monitoring as deemed appropriate by CFCC**
Vehicle Condition: Mechanical/ Safety defects Pre-journey checks	FC employee, passengers, Members of the Public (MoP)	Low	All vehicles to be serviced regularly according to Suppliers or MES instructions. Defects to be reported by drivers immediately and procedures in place to promptly rectify faults with safety implications. Before commencing a journey check 1) screen wash 2) oil 3) tyre condition and pressure 4) coolant level. 5) lights	Service record for CFCC Official vehicles to be maintained by Lynn Jordan. The defect to be reported to Lynn Jordan and noted in the vehicle log. Actioned by Lynn Jordan. Line manager to ensure that new drivers are familiar with how to maintain the vehicles they are asked to drive. Annual Issue of 'Information Note for Staff Driving at Work' by Lynn Jordan, signed for by Driver on Individual Authorisation to Drive Form and recorded by Lynn Jordan in the Record of Safety Training and Document Issue.
Driver Competence: Age Experience Health Conditions	FC employee, passengers, MoP	Med	Drivers must produce a valid driving licence and if driving a private vehicle on official business use must first produce a valid insurance certificate covering business use. Both must be re-produced annually. New or inexperienced drivers must not be expected to travel long distances, particularly on motorways, during severe weather or at night. Employee to inform supervisor of short term medical problems / drugs used that would affect driving abilities	Records of authorisations and categories maintained by Lynn Jordan and held in the Record of Safety Training and Document Issue and Official register of drivers. Copies of driving licences and insurance certificates will be taken and held centrally by Lynn Jordan. New or inexperienced drivers to be monitored by line manager. Health conditions or changes in medication, which may affect driving ability, must be notified to and discussed with Line Manager.
EHS Div driving policy in regard to: Tiredness and fatigue Mobile phones Inclement Weather	FC employee, passengers, MoP	Med	Employees advised on long journeys (>3hours) to take breaks of at least 15 minutes every 2 hours. Employee advised to avoid combined work/driving days > 11 hours excluding formal breaks > 30 minutes. Employees instructed to stop driving at first safe opportunity and take a short break after onset of drowsiness. Employee instructed never to use a hand held mobile phone while driving. Only pull over to answer it if it is safe and legal to do so.	Employees to discuss with Line manager if concerned that a work program endangers adherence to this control. Employee to enforce this protocol. Employee to source weather bulletin before travel and hence enforce this protocol. See CFCC Job Risk Assessment 1 Lone Working. Annual Issue of 'Information Note for Staff Driving at Work' by Lynn Jordan, signed for by Driver on Individual

Lone driving			Employees advised to drive appropriate to the conditions. Do not commence journeys in adverse weather conditions. Employees advised if weather is extreme to cancel meetings and return to the office or find shelter. Lone drivers should take appropriate action as guided by CFCC Job Risk Assessment 1 Lone Working.	Authorisation to Drive Form and recorded by Lynn Jordan in the Record of Safety Training and Document Issue.
Accident due to unsecured Cargo	FC employee, passengers, MoP	Med	All loads must be stowed safely and secured if necessary, to avoid movement whilst in transit.	Line Manager to check periodically
Accident in FC 4 Wheel Drive vehicle	FC employee, passengers, MoP	High	Employee must have the appropriate training to drive this category of vehicle showing competency on both public roads and off road situations.	Training record to be maintained by Lynn Jordan and held centrally.

I have read the above approved assessment.

I have been instructed and trained in the safe operation of the activity identified by this assessment and agree to follow the controls specified by the assessment. I will inform Lynn Jordan of any hazards encountered, which are not covered by this risk assessment.

Name	**Signed**	**Date**
Francis Ashwood		29/05/2013

A. PROCESS ASSESSMENT REFERENCE: WEBs/02/02

B. PROCESS: Preservation of animal tissue with 10% formal saline.

C. SUBSTANCES USED, PRODUCED OR DISPOSED OF BY THE ABOVE PROCESS:

SUBSTANCE	HAZ	MEL	OES	CARCINOGEN
Formaldehyde Solution 37-41% (contains 11-14% methanol)	TO, FL	2ppm		YES
Sodium Chloride	NH			

D. PROCESS DETAILS: 10% formal saline solution is prepared by diluting 10ml formalin (37 – 41% formaldehyde saturated solution) with 100ml distilled water containing 0.9g sodium chloride. Animal tissue is then preserved by immersion in the prepared solution in heavy-duty plastic bags, bottles or tubes at the rate of 3 parts by volume of solution to 1 part by volume of tissue.

E. RISK ASSESSMENT:

1. Formaldehyde solution 37-41% is Toxic by ingestion, inhalation and prolonged exposure to vapour. The diluted (mixed) solution (at least 5% but less than 30%) is not classed as Toxic but is Harmful if ingested or exposure to it is prolonged.
Both formaldehyde solution (37-41%) and formal saline solution liberate formaldehyde gas. Formaldehyde is irritating to eyes and respiratory systems and may cause sensitisation by skin contact.

2. Preparing the dilute formal saline solution is an extremely hazardous process due to the toxicity of the formaldehyde solution and to its possible carcinogenic effects (BDH US data).

F. CONTROLS REQUIRED TO MINIMISE RISKS: Respirator, disposable PVC gloves, apron and goggles or face shield will be worn when dispensing formaldehyde and mixing the preservative solution. Dispensing formaldehyde and mixing small quantities will only be done in a laboratory fume cupboard with the fan on.

Disposable PVC gloves, apron and goggles will be worn when preserving samples in formal saline.

When transporting large quantities by road, formaldehyde should be kept in sealed containers and transported by road trailer.

Specimens preserved in 10% formal saline will be washed with copious amounts of water before examination. Washing should be done in the fume cupboard whenever possible.

Spillages will be sluiced away with copious amounts of water and the area vacated until the fumes have dispersed.

Formaldehyde will be stored in air-tight containers in the chemical store; 10% formal saline will be stored in the laboratory chemical cupboard.

Ready-diluted formal saline solutions will be purchased as soon as existing stocks of formaldehyde are exhausted. Alternative, less toxic fixatives will be sought and used whenever practicable.

G. MAINTENANCE OF CONTROLS: M. Ferryman to ensure that protective clothing is available at all times. Also to liase with Central Services to ensure that the fume cupboard is tested at the appropriate intervals. M. Ferryman to ensure that formaldehyde and formal saline solutions and quantities are listed on the chemical inventories for the chemical store and the laboratory.

H. MONITORING OF EXPOSURE: A Drager gas pump will be used in conjunction with formaldehyde 0.5/a short-term tubes to ensure formaldehyde gas level is <2ppm in air.

An air sample will be taken at least once every 5 working days of use of formaldehyde or more frequently if conditions are considered to warrant the taking of a sample. A record of each air sample taken and the level of formaldehyde in ppm in air will be made on the relevant experiment record of operations forms.

I. HEALTH SURVEILLANCE: n/a

J. ASSESSMENT REVIEW DATE: February 2002

K. ASSESSOR: M. Ferryman

SIGNED: ____ DATE: ____________________

L. ASSESSMENT APPROVAL:

I am satisfied that the above assessment is suitable and sufficient under the terms of COSHH (1988) regulations.

SIGNED: ____ DATE: ____________________

NAME: GRADE:

M. INSTRUCTION AND TRAINING OF PROCESS OPERATORS:

[Signing here confirms that the operator has seen the assessment and has been suitably trained to operate the process under the terms of the assessment: it does NOT imply that the operator fully agrees with those terms]

I have read the above approved assessment (Ref.)

I have been instructed and trained in the safe operation of the process(es) identified by this assessment, and agree to follow the safety proposals set out by the assessment.

Name	**Signed**	**Date**
Francis Ashwood		29/05/2013