# SHORT ROTATION FORESTRY AND EARTHWORM DIVERSITY: IMPACTS AND RESPONSES

**Nalika Swarnamali Senevirathna Rajapaksha Mudiyanselage**

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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.

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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.

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# ABSTRACT

Short Rotation Forestry (SRF) has been introduced to the UK as a method to increase biomass production. However, some SRF species have raised concerns about potential impacts on the environment. A largely unknown aspect of SRF is the quality and quantity of leaf litter, and its impact on soil fauna, of which the earthworm community is a major component. Earthworms have direct impacts on the soil biogeochemistry of SRF systems, and the tree species can impact on the associated earthworm community. The aim of this study was to investigate the effects of SRF species and litter quality on earthworm communities, their diversity and activity. In addition, the effects of earthworms on SRF litter decomposition, carbon-nutrient cycling and tree growth were assessed. Field surveys, laboratory experiments and field experiments were utilised. Survey results suggested that SRF species, tree age, land-used history and soil type exhibited an interactive effect on overall earthworm community development. Further, growth of eucalyptus, as SRF on marginal-arable or reclaimed sites, led to relatively rapid earthworm colonisation and community development. SRF litter quality showed a direct effect on earthworm food selection, growth and reproduction. The native *Alnus glutinosa, Betula pendula* and *Fraxinus excelsior* litter supported earthworms and their activities over non-native *Acer pseudoplatanus, Castaneas sativa* and *Eucalyptus nitens*. Native British earthworms indicated a significant preference ($p < 0.05$) for *E. nitens* litter over *A. pseudoplatanus* and *C. sativa.* Earthworms showed a significant contribution ($p < 0.05$) to SRF litter decomposition, carbon and nutrient release within SRF systems and the degree of contribution varied with SRF species, earthworm density and diversity. Field studies demonstrated that a mixed earthworm community utilised non-native species but favoured particular native trees. Earthworm influence on nutrient uptake, tree growth and biomass production varied with SRF species. A one year field experiment showed that rapidly growing *E. nitens* benefited more from earthworm activity than relatively slow growing *B. pendula.* Overall, the current work supports the production of SRF, as with only one exception (*C. sativa*), results tended to show that SRF-earthworm interactions were positive. It is perhaps most interesting that non-native *E. nitens* showed a positive interaction with native British earthworms.

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You are generous beyond the belief and
made enormous sacrifices to give me the comfort during past 3 years

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# CHAPTER 1: INTRODUCTION

## 1.1 Background

Short Rotation Forestry (SRF) was recently introduced to the UK as a possible and efficient method to increase biomass production in the country (FC, 2010). In this silvicultural system, these trees are grown for 8 to 20 years, generally, much shorter than traditional forestry practices, but longer than alternative Short Rotation Coppice (SRC) (LTS International, 2006). Potential SRF practice includes fast growing native and non-native tree species. Selected species offer high yields in a relatively short period of time and potentially produce higher quality material for both electricity and heat generation than alternative biomass crops (Forestry Commision, 2010). The scope and overall objectives of this research originated from the interest of exploring and adapting appropriate SRF species for maximum productivity and their benefits/disbenefits to wider ecosystem services including soil sustainability, greenhouse gas benefits and biodiversity.

In the interest of combatting climate change, the UK government has committed to reduce greenhouse gas emissions by 80% of 1990 levels by 2050 (DECC, 2009). This has led to a rapidly developing market for alternative renewable energy sources. The UK has signed up to European targets to produce 15% of all energy from renewable sources by 2020 and the recent Renewable Energy Strategy proposed that 30% of renewable energy would come from bioenergy (DECC, 2013). Wood fuel is a sustainable and low carbon source of bioenergy that can make a substantial contribution to achieving these targets (TRADA, 2011). Fast growing SRF species have a great potential to contribute towards these targets as these trees can deliver greater volumes of

biomass from the same land area than alternative biomass crops such as SRC (McKay, 2011).

However, pressure is rising to expand planting of these trees, since the experience of SRF in the UK is limited. The impacts of these fast growing trees on the environment, especially on soils, hydrology, and biodiversity are unidentified. Environmental benefits/disbenefits of SRF largely depend on previous land use. Lands mainly available for SRF planting in the UK were formerly used for agriculture, either arable farming or pasture management. Growing of SRF species and harvesting for biomass over time may cause soil nutrient depletion and acidification in these lands (Hagen-Thorn *et al.*, 2004; Vanguelova and Pitman, 2011). Further, SRF may pose a number of potential threats and benefits to water quality and quantity. The water quality impact of SRF is expected to be beneficial compared to arable cropping due to less frequent land preparation and lower chemical usage. A potential risk for water quantity is expected from some deep-rooted water demanding SRF species such as eucalyptus, while the use of other native broad-leaved species could possibly benefit water resources (Nisbet *et al*., 2011). The land use transformation from agriculture to SRF has potential to improve below and above ground biodiversity by providing improved quality habitat. In addition, planting SRF on ex-agricultural lands increases soil carbon accumulation (Vanguelova and Pitman, 2011). However, in addition to previous land use, the net environmental impact of SRF may depend on many factors, mainly tree species, soil type, local hydrology and climate.

Forestry Commission Scotland (FCS) and England (FCE) have active sites on which SRF is trialled (Harrison, 2009). These SRF trials, across Great Britain involve planting a range of different tree species under different silvicultural practices and assessing

wider environmental issues along with growth/yield parameters. Forest Research (FR) is broadly investigating the environmental impact of these trees on biodiversity, hydrology and soils. One of the important but hitherto neglected aspects in current investigations is the impact of SRF on the soil faunal community, diversity and activity. Understanding of this interaction is vital as soil fauna may govern the long-term soil sustainability of these systems mainly through litter decomposition and nutrient cycling (Edwards and Heath, 1963). An important part of this soil fauna is the earthworm community.

Earthworms, which are considered as one of the best indicators of soil quality (Lavelle *et al*., 2006), account for the majority of soil faunal biomass in a wide range of temperate ecosystems (Mori *et al*., 2010). Their role within the soil system, including organic matter decomposition, nutrient cycling, and structural development, has been widely acknowledged (e.g. Lee, 1985; Edwards and Bohlen, 1996; Lavelle *et al*., 2006). Earthworms have a direct interaction with the above-ground plant community (Lee, 1985; Bardgett *et al*., 2005; Eisenhauer *et al*., 2009a). Different tree species may differently affect the distribution and diversity of the earthworm community, since trees are different in quality and quantity of litter produced (Muys *et al.*, 1992; Zou, 1993; Sarlo, 2006). Alternatively, presence of different earthworm species may have various impacts on soil structural development, litter decomposition, carbon and nutrient cycling and subsequently on plant growth and production (Marshall, 1971; Haimi *et al*., 1992; Welke and Parkinson, 2003). Further, earthworms are an important component in the diet of many terrestrial vertebrates (e.g. badgers). Because of the direct link with above-ground biodiversity and ecosystem processes, earthworms can be used as an important bio-indicator in assessing overall ecosystem sustainability (Paoletti, 1999). Moreover, because of their direct and relatively rapid response to physical and chemical

changes in soil, earthworms can be used as indicators in assessing the effects of land use change on below-ground biodiversity, hence soil sustainability (Jouquet *et al*., 2006).

However, the direct and indirect impacts of SRF species on the earthworm community and the feedback processes in these intensive forest systems are largely unidentified. The existing Forestry Commission SRF trials provided a platform for this investigation. Broad field surveys, well-designed laboratory experiments, and advanced field-based studies were carried out for this investigation to assess the effects of SRF species and their litter quality on population, diversity and activity of earthworms. In addition, the effects of earthworms on SRF litter decomposition, soil carbon and nutrient release and tree nutrient uptakes were assessed**.** The SRF-earthworm interaction-related information gained through the research for this thesis will be a valuable source for practitioners to assess overall SRF system sustainability in the process of its expansion within the UK.

## 1.2 Research questions

During the preliminary stage of this research, the following specific research questions were raised.

1) What are the effects of different SRF species, litter quality and quantity on earthworm population and diversity? Do these effects interact with soil types and physical and chemical properties?
2) Are there any changes in below-ground earthworm community due to land use conversion (e.g. from agriculture to SRF)?
3) What is the contribution of earthworms to leaf litter decomposition, plus carbon nutrient cycling within SRF systems?

4) What is the direct influence of earthworms on SRF nutrient uptake and plant growth?
5) Overall, how do earthworms integrate with other factors when modelling SRF ecosystems?

These research questions formulated a rather large and comprehensive set of second level research questions and issues which were explored during the research project.

### 1.3 Project aims

The research started with five major aims as follows:

- To explore the effects of SRF on different land-use systems on earthworm community development.
- To investigate the influence of SRF litter quality on earthworm food selection, growth and reproduction.
- To assess the contribution of earthworms to SRF litter decomposition, carbon and nutrient cycling within these forest systems.
- To study the direct effects of SRF trees (e.g. root chemistry, litter quality) on earthworm population establishment and the reciprocal effect of earthworms on SRF nutrient uptake and tree growth.
- To provide information to develop a model to predict impacts of SRF on the wider ecosystem, including soils and biodiversity.

The detailed scientific objectives related to these aims are presented in appropriate sections of the thesis in association with relevant experiments.

## 1.4 Thesis structure

This thesis is structured in the following way. The introduction presented in Chapter 1 provides an overview of the contextual background and the research issues that are dealt with in the research. Chapter 2 reviews literature that in essence, is selected pre-knowledge in different areas that are related to this research work. The major sections in the literature review include SRF practice, earthworm ecology, plant-earthworm interactions and associated techniques/methodologies. Chapter 3 describes the methodological approach and a summary of experimental design, highlighting the specific features and parameters of the laboratory and field-based experiments.

Chapters 4, 5, 6, and 7 present detailed experimental work undertaken with respect to the major aims stated in Chapter 1. These Chapters present specific objectives, methodologies and the important results of the each experiment. Experimental results, possible reasons and relevant literature are initially discussed under each experiment. Finally, Chapter 8 links and discusses all laboratory and field research results together and addresses the initial aims and specific objectives in experimental chapters.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Introduction

Foremost sections in the literature review include SRF practice in the UK, general earthworm ecology; influence of environmental factors on earthworm diversity/activity and their roles in the overall ecosystem. A major section of the review then discusses the techniques associated with earthworm research in the field and laboratory. Afterwards, this chapter discusses existing knowledge on interactions between plants and earthworms. The final section explains the potential impacts of SRF on soils and earthworms.

## 2.2 Short Rotation Forestry

Short Rotation Forestry (SRF) practice can be described as the cultivation of fast-growing tree species to reach an economically optimum size between 8 and 20 years (McKay, 2011). The trees are planted at optimum spacing that allows for rapid growth and easy harvesting. The resulting single stems are harvested for biomass at around 0.15 m diameter. After the first harvest, SRF trees are usually replaced by new planting.

### 2.2.1 Short Rotation Forestry and Short Rotation Coppice

SRF practice is different from the more widely occurring Short Rotation Coppice (SRC) also used for biomass production, where tree species are grown on a repeated coppice cycle of 3 – 4 years. SRC species generally require higher quality arable land for optimum growth and high yield (Harrison, 2009). However, SRF species do not

compete directly with food crops for the most productive agricultural lands, as these trees tend to be grown on lower-grade agricultural land, previously forested land or reclaimed land (McKay, 2011). SRC species of willow (*Salix* spp.) and poplar (*Populus* spp.) produce relatively poor quality biomass with a high moisture content, low wood density and high bark content (Kerr, 2011). Conversely, the wood-chips produced by SRF are high in quality and more homogeneous than wood-chips provided by SRC. Biomass from SRF can be used in power stations, alone or in combination with other fuels such as coal. Due to these reasons, SRF has become more attractive to both biomass producers and energy suppliers (Kerr, 2011).

SRF has the potential to produce a higher biomass yield, using marginal agricultural land, while benefiting the productive ecosystem (e.g. soil system, below and above-ground biodiversity). It has been suggested that SRF, particularly using eucalyptus, has the potential to deliver greater volumes of biomass from the same land area than alternative biomass crops (McKay, 2011). As such, SRF can be a more appropriate woody biomass source for growing in many parts of the UK. However, the field information available for SRF species, their growth, biomass production and environmental impacts are still insufficient in the UK.

### 2.2.2 SRF species

LTS International (2006) suggested a potential SRF species list (Table 2.2.1) to grow in the UK for biomass production. This SRF list includes native and non-native tree species. Trees that have developed over thousands of years in a particular region or ecosystem are considered as native species, while trees introduced with human help (intentionally or accidentally) to a new place or new type of habitat where it was not

previously found are considered as non-native species (NRCS, 2011). Non-native trees that do not need human help to reproduce and maintain themselves over time in an area where they are not native are called naturalised trees (NRCS, 2011). LTS International (2006) has further described yield data and rotation lengths (see Table 2.2.1) for selected SRF species. However, the authors have mentioned that this information was gathered through discussion with present practitioners and is based on very limited field data.

**Table 2.2.1 SRF species information (adapted from LTS International, 2006)**

| **SRF species** | **Origin** | **Biomass Productivity (dt $ha^{-1}$ $yr^{-1}$)** | **Rotation (years)** |
|---|---|---|---|
| Alder (*Alnus* spp.) | Native | 5 | 20 |
| Ash *(Fraxinus excelsior)* | Native | 7.4 | 20 |
| Birch *(Betula pendula)* | Native | 5 | 20 |
| Poplar (*Populus* spp.*)* | Native | 5.6 | 14 |
| Sycamore *(Acer pseudoplatanus)* | Naturalised | 7 | 20 |
| Cider gum *(Eucalyptus gunnii)* | Non-native | 9 | 12 |
| Shining gum *(Eucalyptus nitens)* | Non-native | 15 | 8 |
| Rauli *(Nothofagus* spp.*)* | Non-native | 11.8 | 12 |

dt – dry tonne

In a recent SRF review by Forest Research, Kerr (2011) discussed further potential SRF species (e.g. naturalised sweet chestnut and hybrid aspen) that were not included by LTS International (2006). Kerr has estimated the range of possible biomass productivity for considered SRF species (Table 2.2.2) using a combination of published information and new data. Kerr (2011) emphasised that often the quoted productivity for SRF

species in the UK, especially for introduced non-native species (e.g. eucalyptus) has not adequately accounted for the risks of frost and winter cold.

**Table 2.2.2 SRF species and biomass productivity (adapted from Kerr, 2011)**

| **SRF species** | **Biomass (dt $ha^{-1}$ $yr^{-1}$)** |
|---|---|
| Red alder *(Alnus rubra)* | 0.9 - 4.8 |
| Italian alder (*Alnus cordata)* | * |
| Hybrid aspen (*Populus tremula x tremuloides)* | * |
| Ash *(Fraxinus excelsior)* | 0.5 – 4.7 |
| Birch *(Betula pendula)* | 0.5 – 5.7 |
| Sycamore *(Acer pseudoplatanus)* | 0.5 - 5.7 |
| Sweet Chestnut (*Castaneas sativa)* | 1.2- 6 |
| Cider gum *(Eucalyptus gunnii)* | 1.5 - 8.2 |
| Shining gum *(E. nitens)* | * |
| Tingiringi gum (*E. glaucescens)* | 2.5 - 7.6 |
| Rauli *(Nothofagus alpina syn. N. procera)* | 3 - 10.5 |

* Insufficient information

### 2.2.3 SRF trials in the UK

Forestry Commission Scotland (FCS) and England (FCE) have established active sites (Table 2.2.3) across the UK on which SRF is trialled. This involves planting a range of potential tree species to assess tree performance, productivity and the wider environmental impacts on soil, biodiversity, water quality and quantity. Further, these trials aim to provide growth and yield information for potential SRF species and overall carbon and green-house gas (GHG) balance. The sites, cover a range of soil types, geographic areas and climatic zones, and consist of extensive (> 5.0 ha) and intensive (<

5.0 ha) areas. The intensive areas are used for research purposes, but some assessments and treatments are also conducted in the extensive areas, which are operational SRF sites. Unplanted representative areas are available at most sites as controls, to draw comparisons of factors such as hydrology, biodiversity, soil nutrition and soil carbon with the SRF planted area.

**Table 2.2.3 Newly established SRF trial sites and some older forest sites, consisting of potential SRF species (based on unpublished information provided by Forest Research)**

| **Site name** | **Site location** | **Established year** | **Species** |
|---|---|---|---|
| Mill Farm | Lincolnshire | 2009 | Various |
| Roan Farm | Cumbria | 2009 | Various |
| Carlshead farm | Yorkshire | 2009 | Various |
| Daneshill | Nottinghamshire | 2005 | *E. gunnii*, *E. nitens*, Other *Eucalyptus* spp., *Nothfagus*, *B. pendula* |
| Rogate Wood | W. Sussex | 2005 | Various *Eucalyptus* spp., *C. sativa* |
| Alcan | Lynmouth, Northumberland | 2004 | *E. gunnii*, *E. nitens* |
| Cannock Chase 2 | Staffordshire | ~2004 | *B. pendula* |
| Champion Court | Newham, Kent | 2001 | *E. nitens, E. gunnii.* |
| Usk College | Monmouth, Wales | ~2000 | *F. excelsior* and *A. pseudoplatanus* |
| Benton Wood | Pembroke, Wales | 1998 | *Nothofagus* |
| Glen Orchy | Argyll | 1998 | *Nothofagus* |
| Redmarley | Gloucester | 1984 | Various *Eucalyptus* spp. |
| Great Haldon | Exeter, Devon | 1983 | Various *Eucalyptus* spp. |

~ = approximately

## 2.3 Earthworms

Soil faunal communities as a functional group play a vital role in sustaining many terrestrial ecosystems. An important part of this soil fauna is the earthworm community which has a pronounced effect on physical, chemical and microbiological soil properties (Lee, 1985; Bartlett *et al*., 2010). Earthworms are the most widespread soil invertebrates which account for the majority of soil faunal biomass in a wide range of productive ecosystems (Shakir and Dindal, 1997; Mori *et al*., 2010), and are considered one of the best indicators of soil quality (Lavelle *et al.,* 2006). Earthworms play a significant role in decomposition of organic matter and nutrient cycling (Curry, 1987). Moreover, they assist soil structural development as a result of their feeding and burrowing activities which improve soil aeration, drainage and water holding capacity. Earthworms are commonly described as the most important ecosystem engineers; organisms that may modify their habitat/soil properties and thus influence the availability of resources to other species (Jones *et al*., 1994) in the soil matrix, due to their long-term effects on soil physical and bio-chemical properties (Emmerling *et al*., 2002). Positive effects of earthworms within the soil system are directly and indirectly interrelated with plant growth and yield (Lee, 1985; Haimi *et al*., 1992; Bardgett *et al*., 2005; Eisenhauer *et al*., 2009a).

### 2.3.1 Earthworm communities

#### 2.3.1.1 Ecological groups

Earthworm species show evidence of morphological and physiologically adaptations for existence within the soil system, effectively using available resources. According to their feeding behaviour, morphological characteristics and habitat use (vertical stratification in the soil system), earthworms have been grouped into three major categories; epigeic, endogeic, and anecic (Bouché, 1977; Lee, 1985; Curry, 1994; Paoletti, 1999). Epigeic earthworms live near the soil surface within/underneath the litter layer, are heavily pigmented, and generally feed on plant litter. Endogeic earthworms are active within the upper layer of the mineral soil where they feed on soil organic matter obtained by the ingestion of large amounts of soil (geophagy). Anecic earthworms tend to live in permanent vertical burrows, are dorsally pigmented, and feed on surface plant litter. The habitat preferences and feeding behaviour of different earthworms may result in a diverse but essential effect on ecosystem processes. Table 2.3.1 summarises the major characteristics of the above earthworm groupings.

**Table 2.3.1 Characteristics of the three major earthworm grouping (adapted from Bouché, 1977)**

| **Diagnostic Character** | **Earthworm grouping** | | |
|---|---|---|---|
| | **Litter dwelling (epigeic)** | **Shallow working (endogeic)** | **Deep burrowing (anecic)** |
| **Food eaten** | Decomposing surface litter | Mineral soil | Surface litter pulled into burrow |
| **Adult size** | Small to medium | Medium | Large |
| **Burrow** | None | Extensive horizontal (to 15 cm) | Large permanent vertical (to 2 m) |
| **Reproduction** | Rapid | Intermediate | Slow |
| **Longevity** | Short-lived | Intermediate | Long-lived |
| **British examples** | *Lumbricus rubellus, Dendrodrilus rubidus* | *Allolobophora chlorotica Aporrectodea caliginosa* | *Aporrectodea longa Lumbricus terrestris* |

### 2.3.1.2 Natural associations

Earthworm species have different effects on soil ecological functions. Although individual earthworm species contribute greatly to ecosystem functions, this contribution is often a combined effort of species association. Species association in a temperate ecosystem may include up to 15 species, but more commonly 2 - 6 species are found (Lee, 1985). Natural earthworm associations vary with soil type, vegetation, food supply, and climate (Curry, 1998). At a smaller scale, earthworm association is influenced by season, litter quality, humus, soil structure (Coderre *et al*., 1995) and even by land use history. Generally, earthworm species that represent different ecological groupings have a positive relationship (Uvarov, 2009) and are often found together. As an example, *L. rubellus* and *A. caliginosa* with different feeding habits show a positive

association (Raty and Huhta, 2003). Further, epigeic earthworm species can be found associated within *L. terrestris* middens (collection of organic and inorganic materials at the burrow entrance) as they generally provide food and shelter for the surface dwelling, small earthworm species (Butt and Lowe, 2007). The endogeic species *A. chlorotica* and *A. caliginosa* often show a negative association with each other, but also a positive relationship with anecic species (Lowe and Butt, 2003; Uvarov, 2009). *L. terrestris* frequently shows a negative association with *A. longa* and *L. rubellus* due to possible competition for food/litter at the soil surface (Edward and Lofty, 1972; Lowe and Butt, 1999; Uvarov, 2009).

### 2.3.2 The roles of earthworms in ecosystems

#### 2.3.2.1 Organic matter decomposition and nutrient cycling

Earthworms play a significant role in decomposition of organic matter and nutrient cycling within many terrestrial ecosystems (Edwards and Heath, 1963; Curry, 1987; Curry and Byrne, 1992; Eisenhauer *et al.*, 2009a). They consume various types of organic matter such as plant litter, crop residues and animal droppings and incorporate these into the soil. The resulting casts, which contain plant-available nutrients, are deposited on the soil surface and even within various layers of the soil profile, depending on their ecological grouping. Anecic species such as *L. terrestris* largely contribute to breakdown of, and incorporation of surface litter into the soil in many productive ecosystems. Scheu and Wolters (1991) suggested that *L. terrestris* has the greatest contribution for overall breakdown and incorporation of plant litter within mineral soils in many temperate woodlands, and is largely responsible for the formation of mull humus. Satchell (1967) estimated that incorporation of leaf litter into the soil by

*L. terrestris* may amount to 3,000 kg $ha^{-1}$ over three months in deciduous forests. Large presence of *L. terrestris* in lowland Oak forest on mineral gley soils in the South of England helps some of the 5,000 kg $ha^{-1}$ annual litter incorporation into the soils and the formation of a mull humus layer (Benham *et al*., 2012). Epigeic earthworms also consume considerable amounts of litter but do not incorporate it into the mineral layer. Endogeic earthworms feed mainly on fine organic matter and incorporate it with mineral soil. Anecic and endogeic earthworms have a synergistic effect on the distribution of organic matter throughout the soil profile (Lowe and Butt, 2003). Different earthworm species with various feeding behaviours (see Table 2.3.1) may lead to different but equally important effects on organic matter mineralisation and nutrient release (Laossi *et al*., 2009). The breakdown and mixing of organic matter by earthworms is vital for nutrient recycling within any productive ecosystem (Bernier and Ponge, 1994). Their contributions to nutrient cycling also includes enhancement of microbial activities (Postma-Blaauw *et al*., 2006).

#### 2.3.2.2 Soil structural development

Earthworms, as key regulators of soil structure (Fonte *et al*., 2009) and as ecosystem engineers (Jones *et al*., 1994, see section 2.3), directly and indirectly affect soil physical properties. Darwin (1881) described earthworms as nature's plough, following his early observations of their habits. The effects of earthworms on soil structure result from their feeding, burrowing and casting activity. Earthworms directly improve soil aeration/porosity through their burrowing activity (Edwards *et al*., 1988; Knight *et al*., 1992). The extent to which burrowing influences pore space is dependent on soil and environmental conditions. Kretszchmar (1998) estimated that burrows can represent approximately 20% of air-filled space in soils even when air-filled porosity is at its

lowest with high moisture. Edwards *et al*. (1988) estimated $1.6 \times 10^6$ *L. terrestris* burrows $ha^{-1}$ in long-term, no till watershed at Coshocton, U.S.A. As a result of burrowing, earthworms increase water infiltration of the soil (Joschko *et al.*, 1989; Knight *et al*., 1992) and hence they reduce surface runoff and soil erosion (Pitkänen and Nuutinen, 1998; Jouquet *et al*., 2006). Hoogerkamp *et al*. (1983) monitored a significant increase in water infiltration in Dutch Polders reclaimed from the sea, 8 - 10 years after earthworm introduction. The study recorded that water infiltration rates over a 24 hour period were up to 136 times greater in earthworm inoculated pots than control plots with no earthworms. Earthworms contribute to soil profile development by incorporating organic matter into the mineral soil through surface removal of litter and deposition of casts on the surface and sub-surface of the soil profile. Through casting activity, earthworms directly promote soil aggregate formation and stabilise soil structure (Shipitalo and Protz, 1989; Tomlin *et al*., 1995). Changes in porosity, drainage and soil aggregates due to earthworm activity can increase soil water holding capacity (Van Rhee, 1969). However, quality and palatability of plant litter can influence soil aggregation by earthworms (Flegel *et al*., 1998) and they tend to produce a higher number of aggregates when they are fed with less palatable litter (Merciris *et al*., 2008). A decrease in food quality induces higher soil ingestion, followed by higher cast production and more construction of burrows (Marhan and Scheu, 2005).

#### 2.3.2.3 Soil food web relationship

Earthworms are important primary contributors to the existence and balanced function of soil food webs. They have many complex relationships with other soil organisms including microorganisms. Earthworms promote microbial activity in the soil system through initial fragmentation and surface mixing of organic resources (Mulder, 2006).

They disperse microorganisms and accelerate microbial activity within the soil system through burrowing and casting activity (e.g. Lavelle and Spain, 2001). Compared with surrounding soil, earthworm casts usually support greater populations of bacteria, fungi and actinomycetes (Tiwari and Mishra, 1993) as casts are generally rich in available nutrients and partially digested organic matter which provides an ideal substrate for growth of microorganisms. Further, some of the mucus secreted with casts stimulates microbial activity and growth (Scheu, 1991). Some researchers have shown that selected groups of actinomycetes and bacteria are stimulated during passage through the earthworm gut (Szabo *et al*., 1990). By contrast, microorganisms are an important source of food and provide nutrients for certain earthworms (Edwards and Fletcher, 1988). Curry and Schmidt (2007) in their review of the feeding ecology of earthworms emphasised that one of the main factors likely to influence earthworm food digestibility is the degree of microbial involvement in the process. They suggest that epigeic species which consume great amounts of raw organic matter have a broad range of enzymatic capacities, possibly originating from ingested microflora and also *L. terrestris* middens can stimulate microbial colonisation and degradation (the 'external rumen'), with mutually beneficial consequences for earthworms and microflora.

#### 2.3.2.4 Link to above-ground biodiversity

Earthworm communities have a close link with above-ground biodiversity and ecosystem processes. Earthworms can directly influence plant diversity by dispersal of plant seeds. Earthworms generally promote plant growth and yield mainly through enhancement of soil organic matter mineralisation, the modification of soil structure, the production of plant growth promoting substances, the stimulation and dispersal of beneficial microorganisms and control of pests and parasites (Scheu, 2003; Laossi *et al*.,

2010; Jana *et al*., 2010). Moreover, earthworms are an important component in the diet of many terrestrial vertebrates. They are a major source of food for many species of birds such as blackbird (*Turdus merula*), starling (*Sturnus vulgaris*), thrushes (*Turdus* spp.), crow (*Corvus corone*), robin (*Erithacus rubecula*), wood-cook (*Philohela minor*) and owl (*Strix aluco*) (MacDonald, 1983). Amongst the mammals, hedgehog (*Erinaceus europeus*), badger (*Meles meles*), mole (*Talpa europea*) and red fox (*Vulpes vulpes*) eat large number of earthworms (MacDonald, 1983). Hofer (1988) showed that earthworm contributed the bulk of the diet of the badgers in Wytham Woods, Oxfordshire and earthworm introduction into restored sites has on occasion been specifically designed for sustainable food provision for protected vertebrate species (Butt *et al.*, 2003).

### 2.3.3 The effect of environmental factors on earthworms

#### 2.3.3.1 Soil type (structure/texture)

Soil texture has shown a strong correlation with earthworm populations; earthworm populations are usually positively correlated with soil clay content (Hendrix *et al*., 1992; Baker *et al*., 1998). Guild (1951) suggested that medium loam soil supported a greater earthworm population than heavier clay, sandy or alluvial soils. Soil structure also has a large influence on earthworm populations and activity. Soils with increased water holding capacity, aeration and drainage provide better habitat for earthworms. Further, the soil bulk density can affect burrowing ability and cast production (Joschko *et al*., 1989; Kretzschmar, 1991). Soils with organic matter of the mull and moder types generally contain higher earthworm diversity and biomass compared with acidic soils and mor humus types (Paoletti, 1999). However, different species of earthworm prefer different type of soils. As an example, nutrient-rich soils are dominated by geophagous

earthworm species, while litter-feeding epigeic species are normally present in nutrient-poor soils (Fragoso and Lavelle, 1992). Deep burrowing anecic species such as *L. terrestris* need considerable soil depth to construct their burrows and do not commonly appear in shallow soils (Muys *et al*., 1992). The influence of soil type on earthworms may be due to its direct and indirect impacts on soil physical and chemical properties.

#### 2.3.3.2 Soil moisture

Soil moisture is a major factor which effects earthworm populations, but they have a considerable ability to survive under adverse conditions using different techniques. Nevertheless, prolonged drought can dramatically decrease earthworm population numbers. Adequate availability of moisture determines earthworm activity within the soil system and it can even affect earthworm reproductive success. The moisture requirement for general activities of earthworms varies with different species, but even within the same species it can be different according to their origin. Buckerfield (1992) indicated that certain species of earthworms are adapted to wide range of soil moisture contents and *A. rosea* can be active in soils with very low moisture levels (10%), in a semi-arid cereal field in Southern Australia. Different earthworm species use different strategies to survive under drought conditions. Edwards *et al.* (1995) reported that cocoons may act as the main survival stage during drought for some earthworm species such as *L. rubellus. L. terrestris* and *A. longa.* These species generally migrate to deeper soil when the surface soil is dry. Lack of moisture can cause some earthworm (e.g. *A. longa*) to enter a resting phase (diapause). Earthworms in diapause are tied up in a knot in a soil void space that is lined with mucus to avoid moisture loss. Other species (e.g. *A. chlorotica*, *A. caliginosa* and *A. rosea*) may enter into a less permanent quiescent state (Evans and Guild, 1947). Satchell (1967) reported that *L. terrestris* decrease

surface activity and cocoon production during drought periods. As reviewed by Curry (1998), most earthworms are active at a moisture tension approaching field capacity (~10 kPa), and activity declines rapidly as moisture tension exceeds 100 kPa and ceases for most species at a moisture tension below the permanent wilting point (1500 kPa).

Laboratory studies suggest that most temperate earthworm species are active and reproduce well under a moisture content of 25 - 30% (Butt *et al*., 1994; Berry and Jordan, 2001). Daugbjerg (1988) used a soil column with a continuous moisture gradient from 6% at the top to 30% at its base and studied the soil moisture preference of *A. caliginosa*, *A. longa* and *L. terrestris*. Adult *A. caliginosa* preferred a narrow range of soil moisture from 18 – 20% while *L. terrestris* spread out across the whole range, showing preference for a soil moisture content of 20%. The author suggests that *L. terrestris* is normally subject to dryer soil conditions as it feeds on the soil surface and therefore these are able to tolerate lower soil moisture conditions than endogeic *A. caliginosa*. Curry (1998) suggests that juveniles are less tolerant to drought, as they are unable to burrow deep down within the soil and enter into a dormant state.

#### 2.3.3.3 Soil temperature

The growth, maturity, reproduction and other activities of earthworms are influenced by soil temperature. Temperature and moisture are inversely related and high temperature and dry soil are much more limiting to earthworm populations than low temperature and water logged conditions (Nordstrom and Rundgren, 1974). Temperature can affect the number of cocoons produced and percentage viability (Butt, 1991) as well as embryonic development of some earthworms (Holmstrup *et al.*, 1991). Lee (1985) suggested that the optimum temperature for growth of natural populations of Lumbricidae in Europe

ranges from 10 °C - 15 °C. Butt (1991) cultured *L. terrestris* at various temperatures and showed that cocoon production was greatest at 15 °C and the length of cocoon incubation was shortest at 20 °C, with an ideal temperature for maximum production falling within 15 - 20 °C. Temperature also affects the food consumption of some species. As an example, Daniel (1991) reported that food consumption of *L. terrestris* increased linearly with temperature up to 20 °C, but declined above 22 °C. Earthworms have certain temperature limits for survival. The upper and lower limit of temperatures for earthworms depends on the species and the region. The upper lethal temperature was recorded as 28 °C for *L. terrestris* (Wolf, 1938; Grant, 1955), 26 °C for *A. caliginosa* (Grant, 1955) and 29.7 °C for *A. rosea* (Reinecke, 1975). The lower lethal temperature for earthworms in temperate regions is almost below freezing point (Holmstrup 1994; Nuutinen and Butt, 2009). Holmstrup (1994) investigated the cold tolerance of earthworms and recorded that *D. octaedra* was the most cold tolerant and it can survive at - 8 °C for three months and at - 13.5 °C for two weeks. He suggests that cocoons of earthworms that inhabit extremely cold environments have been shown to survive under freezing temperatures. However, extreme temperature conditions outside their survival limits greatly influence earthworm populations. Hopp (1947) suggested that earthworm populations in arable soil in the United States can be destroyed by frost in the absence of ground cover, as soil would freeze deeply enough to affect most of the species, but this is unlikely in pasture or woodlands.

#### 2.3.3.4 Soil pH

It has been shown that pH is a determining factor for earthworm diversity and population distribution (Karmegam and Daniel, 2007; Li *et al.*, 2010). Most species of earthworms prefer soils with neutral pH (Lowe and Butt, 2005; Karmegam and Daniel,

2007). Satchell (1955a) suggested that some species e.g. *A. longa, A. caliginosa* and *A. rosea* are intolerant to acidic condition (pH < 4.5), while others e.g. *D. octaedra, D. rubida* survive under acidic conditions. He suggests that *L. terrestris* is not very sensitive to soil pH. However, Satchell (1967) recorded that *L. terrestris* rarely occurs in the field at pH less than 4.3. Edwards and Lofty (1975) reported that not all species of earthworm responded to changes in soil pH in the same way and most could not tolerate a pH below 4. These authors further reported that the optimum range of pH for *A. caliginosa, A. rosea,* and *O. cyaneum* was 5 - 6. Most species of European earthworm have a good tolerance for a wide range of pH, but this has a great influence on earthworm distribution and abundance (Staaf, 1987).

#### 2.3.3.5 Seasonality

In the field, earthworm activity is greatly influenced by seasonality mainly due to variation in soil moisture and temperature. In temperate regions, earthworm activity reaches a maximum in spring and autumn and may become inactive in hot summers and cold winters (Satchell, 1967; Postma-Blaauw, 2006; Cesarz *et al*., 2007). In the Mediterranean region, soil fauna show a seasonal vertical migration; during the wet season they move up to the litter layer and move down again in the dry season (Sharon *et al*., 2001). In adverse conditions, earthworms have developed a range of survival strategies (see section 2.3.3.2).

#### 2.3.3.6 Soil organic matter/vegetation

Soil organic matter quality, quantity and distribution are important factors which determine earthworm abundance and diversity in any ecosystem (Muys *et al.*, 1992;

Tian *et al*., 1993). Past researchers have shown strong positive relationships between earthworm population size, biomass and organic matter content of the soil (Ghabbour and Shakir, 1982; Hendrix *et al*., 1992). However, this may not hold for organic matter rich peat soils with low pH where very low or no earthworms were recorded. Earthworms can feed on various types of organic matter such as animal dung, litter, and dead plant material. However, in less managed terrestrial ecosystems, leaf litter from plants is the main source of organic matter available to earthworms. Further, dead roots and rhizodeposition are also important (Curry, 1998). Since plants are the main source of organic matter to earthworms, vegetation can have a large influence on abundance and diversity. Plant species shows a diverse influence on development of earthworm communities as they differ in the quality and quantity of litter produced (Zou, 1993; Neirynck *et al*., 2000; Sarlo, 2006).

#### 2.3.3.7 Soil management practices

Undisturbed habitats such as permanent grasslands and many natural forests usually show an abundance and diversity of earthworms compared with cultivated lands (Paoletti, 1999; Curry *et al.*, 2002). Soil management practices associated with cultivation can have a significant impact on earthworms as these practices primarily change soil properties. Agricultural practices such as ploughing, application of pesticides, soil fertilisers and residue-burning directly or indirectly pose a threat to some species of earthworm (Edwards, 1983; Paoletti, 1999). In contrast, some agricultural practices, such as no-tillage management, crop rotation, mulching, liming and organic matter amendments may positively affect earthworm abundance and diversity (Paoletti, 1999; Ivask *et al.*, 2007). Lapied *et al.* (2009) have shown that agricultural fertilisation based on organic residue addition is highly beneficial for earthworms and soil quality.

Further, Simonsen *et al.* (2010) found that manure use was the most important management factor affecting endogeic earthworm numbers, while no-tillage was most important for juvenile and adult anecic groups and also had a significantly positive influence on endogeic earthworm number in a Midwestern cropping system.

#### 2.3.3.8 Land use history

Land use history may markedly affect the composition of earthworm communities in some ecosystems. Raty and Huhta (2004) compared the earthworm communities in birch (*B. pendula*) stands of different origins in Finland. They concluded that earthworm communities in a birch stand established on spruce forest soil and on arable soil differ clearly from each other even 30 years after reforestation. The study recorded significantly increased earthworm density and diversity under birch established on former arable soils compared with former spruce soils. The literature suggests that tree establishment on former arable soils has a great potential to increase earthworm density and diversity (Makeschin, 1994). In contrast, tree establishment on grassland soils generally decreases earthworm populations in longer-term (Yates, 1988; Muys *et al*., 1992).

## 2.4 Techniques associated with earthworm research in the field and laboratory

### 2.4.1 Field Earthworm sampling

It is vital to identify and quantify relative abundance and biomass of earthworms in a particular habitat for most ecosystem studies. An appropriate technique or combination of techniques is required to bring earthworms to the soil surface, as it is impossible to have direct counts of these soil-inhabiting creatures in situ. Currently, a number of techniques are available for earthworm sampling, some of which are used more often than others, but no standard has been established for all situations.

#### 2.4.1.1 Digging and hand sorting of soil

Digging and hand-sorting of soil is the simplest and perhaps most widely used method for earthworm sampling. Using a spade and quadrat, an area of 0.1 – 1.0 $m^2$ of soil (to a depth of 0.15 - 0.25 m) is removed to a plastic sheet in the field and then hand-sorted for earthworms (Butt, 2000). However, this is a very labour intensive, time consuming procedure and may recover only epigeic and endogeic earthworm species. Deep burrowing (anecic) species may fail to be noticed as they may retreat deep into burrows during digging (Butt, 2000; Butt and Grigoropoulou, 2010). In some situations, removal of a large soil monolith can overcome this problem (e. g. Lavelle, 1988). However, these methods may not be appropriate for every habitat as they may severely damage the upper soil layer of the sampling area.

#### 2.4.1.2 Use of a vermifuge

Application of a vermifuge to the soil surface is a popular method to extract earthworms as it is simple and effective. Once poured into soil, vermifuge chemicals bring earthworms to the soil surface as they act as a skin irritant when in contact with the animal (Butt and Grigoropoulou, 2010). Various chemicals have been used as a vermifuge. A dilute solution (0.04%) of formaldehyde (formalin) has been a widely used vermifuge for long period (Raw, 1959) and has been a standard for earthworm extraction (ISO, 2006). However, formaldehyde has been reported as carcinogenic. Also, when applied to the soil, it has a potential to cause negative effects to other soil fauna, soil respiration, and vegetation cover (Eichinger *et al*., 2007). Because of the negative effects of formaldehyde, attention has been given to other safer alternatives. Gunn, (1992) suggested that a suspension of table mustard in water can be used as a vermifuge. Butt (2000) has shown that a suspension of mustard powder (e.g. 50 g in 10 litres of water) is relatively inexpensive and more effective for earthworm sampling. Further, a chemical extract derived from mustard seed called Allyl isothiocyanate (AITC) has also been used effectively for earthworm collection (Zaborski, 2003).

However, from experience, many earthworm researchers have now concluded that the most effective earthworm sampling technique is a combination of digging and hand-sorting of soil followed by application of a vermifuge to the created pit (Butt, 2000; Pelosi *et al*., 2009). This combined technique allows extraction of most earthworms, including anecic species, from a representative area.

#### 2.4.1.3 Electrical extraction

This is not a new technique and has been used for over 50 years for earthworm collection (Satchell, 1955b). Later, it was modified and developed as the Octet apparatus, as a standard electrical extraction method by Thielemann (1986). Eight steel electrodes in this instrument are pushed into the soil in a regular circular pattern. It stimulates and brings earthworms to the surface. This method is attractive as it is non-destructive to the sampling area and only fallen leaves and overgrown vegetation need to be removed before sampling for easy detection of earthworms (Butt, 2000). However, this method has not been widely used mostly due to the equipment cost.

### 2.4.2 Field behavioural studies

#### 2.4.2.1 Soil surface activities: Casts deposition and Middens

Some species of earthworms deposit their casts (faeces) on the soil surface. In temperate soils, the earthworm *A. longa* produces clear visible casts. The presence of this species at high densities can cover almost all grass surfaces with their casts (Butt and Grigoropoulou, 2010). The amount of casting could give an indication of the species present in addition to their relative abundance in an area (Evans and Guild, 1947). After introduction of *A. longa* to an unpopulated site, casting activity can be used to follow dispersal through the soil over many years as shown by Butt *et al.* (1997) on a landfill site in Buckinghamshire. Further, deep burrowing *L. terrestris* provides another unique engineered sign at the soil surface called middens. These structures are normally constructed above the opening of their vertical burrow by the resident earthworm, gathering organic (mostly leaf litter/debris) and inorganic materials together and

cemented with casts (Butt and Grigoropoulou, 2010). Regulation of burrow temperature/ moisture content, protection from predators and provision of a food store may be some positive reasons for such a construction (Butt and Nuutinen, 2005), but the absolute function is still uncertain. Butt and Lowe (2007) have revealed that many other earthworm species are associated with *L. terrestris* middens compared with adjacent soil (see section 2.3.1.2).

#### 2.4.2.2 Below ground burrowing activities: Polyurethane resin and Dye

Earthworm investigations are sometimes necessary within the soil profile as most of the larger species are active below the soil surface. Researchers have used numerous techniques to explore below ground earthworm burrowing systems which are very useful for assessing water holding capacity and soil aeration. A polyurethane resin has been effectively used to study burrow morphology and volume. Liquid resin is poured down the burrow and allowed to set hard (Shipitalo and Butt, 1999), the solid representation of the burrow is dug out by excavation of a pit alongside the burrow location. Alternatively, to study the extent of burrow systems, dyes such as methylene blue in water can be poured into burrows or cracks in the soil and then exposed by digging (Shipitalo *et al*., 2004).

#### 2.4.2.3 Leaf litter decomposition: Litterbag technique

The litterbag approach has been commonly used to study leaf litter decomposition in various habitats. A known amount of freshly fallen, air dried, leaf litter is enclosed in nylon mesh bags and secured at the soil surface or buried at chosen soil depths. These permit access to certain groups of detritivorous soil organisms, and the bags are

collected at periodic intervals for measurement of the leaf mass remaining (Bocock and Gilbert, 1957; Edwards and Heath, 1963). The mesh size of the litterbags is generally selected to optimize the access of all organisms to the litter while minimising excessive particle loss (Karberg *et al*., 2008). However, mesh size can be manipulated to exclude certain groups of soil decomposer fauna depending on study objectives (Table 2.4.1).

**Table 2.4.1 The appropriate mesh sizes for invertebrate exclusion using litterbags (adapted from Edwards and Heath, 1963)**

| **Size of mesh openings** | **Soil organisms which can enter the litterbag** |
|---|---|
| 7 mm | All microorganisms and invertebrates |
| 1 mm | All microorganisms and invertebrates, except earthworms |
| 0.5 mm | Only microorganisms, mites, springtails, enchytraeids and small invertebrates |
| 0.003 mm | Only microorganisms |

The size of the bag is also an important component of litterbag studies and overall bag size should be appropriate to the considered litter species and ecosystem. Generally 0.2 m x 0.2 m size bags have been widely used, but diverse plant communities or larger leaf sizes may require larger litterbags (Karberg *et al*., 2008).

Litterbags can be used to measure direct actions of earthworms on organic matter incorporation into soils within a particular ecosystem. This requires some knowledge of the ecological groups present and the species of earthworm at the study site (Butt and Grigoropoulou, 2010). Litterbag studies allow quantification of litter decomposition pattern with time. This technique may allow the comparison of decomposition of different species of litter across different habitats.

### 2.4.3 Laboratory techniques

#### 2.4.3.1 Maintaining a breeding population of earthworms

Rearing earthworms under laboratory conditions is essential for many earthworm-related studies. However, their activities such as feeding, growth, reproduction, and even survival rates under controlled condition are determined by a number of abiotic and biotic factors. Soil substrate, food, moisture, temperature, pH, earthworm density and species composition are some of the critical factors affecting earthworm culture (Lowe and Butt, 2005). Under laboratory conditions, manipulation of these factors to an optimum level is possible and important to achieve increased rates of earthworm production and survival. After a review of the literature, Lowe and Butt (2005) provided guidelines for sustainable culture of four temperate earthworm species (Table 2.4.2).

**Table 2.4.2 Culture guideline for four temperate earthworm species (adapted from Lowe and Butt, 2005)**

| **Culture Parameters** | **Anecic** | | **Endogeic** | |
|---|---|---|---|---|
| | ***A. longa*** | ***L. terrestris*** | ***A. chlorotica*** | ***A. caliginosa*** |
| Soil Type | Loam (pre-treated to remove macro and meso-invertebrate) | | | |
| Soil Depth (cm) | >10 | >10 | >3 | >3 |
| pH | 6-7 | 6-7 | 6-7 | 6-7 |
| Soil Moisture (%) | 25 | 25 | 25 | 25 |
| Food | Dried and re-wetted animal dung (cattle or horse) | | | |
| Food amount (adult$^{-1}$month$^{-1}$) | >20 g | >20g | >10 g | >10 g |
| Food location | Surface-applied | | Mixed into soil | |
| Food particle size (mm) | <10 | <10 | <1 | <1 |
| Temperature ( °C) | 15 | 15 | 15 | 15 |
| Light | 24 h dark | 24 h dark | 24 h dark | 24 h dark |
| Vessel type | Sealed, opaque, plastic with ventilation holes in lid | | | |
| Stocking density (adult L$^{-1}$) | 4 | 3 | 10 | 6 |

### 2.4.3.2 Cocoon collection, incubation and hatchling storage

Earthworm reproduction can be determined by the collection of cocoons from experimental soils in which adult earthworms are present. Cocoons can be separated from the soil by washing the substrate with a jet of water through a series of sieves (wet-sieving) to retain any cocoons (Butt, 2002). This procedure allows for separation and identification of cocoons. Sieves are stacked in descending mesh size and the soil placed into the uppermost. Cocoons of most species can be identified from their size,

colour and shape (Sims and Gerard, 1999). Collected cocoons are incubated on moist filter papers in Petri dishes and filter papers are re-hydrated as required (Lowe and Butt, 2005). Butt (1991) provided excess moisture to prevent chances of dehydration and suggested that submerged conditions did not negatively affect cocoon development, or survival of emerging hatchlings. For temperate species of earthworm, most researchers have used a sub-optimal temperature of 15 °C for incubating cocoons (Baker *et al*., 2002: Lowe and Butt, 2002). Emerging hatchlings can be stored at low temperature (5 °C) to inhibit growth until required (Butt, 1991).

#### 2.4.3.3 Earthworm experiments

Earthworm experiments under laboratory condition are primarily conducted to investigate or compare behaviours, responses, performances and other activities. This can be within a species, between species or in combination, and it may be with or without other environmental changes. However, in any kind of earthworm related-experiment, earthworms should be characterised with respect to the following parameters; taxonomic identity (species name), ecological group, development stage (cocoon, hatchling, juvenile, sub-adult and adult), biomass (initial and end), physiological status and origin (Fründ *et al.*, 2010). In general, experimental earthworms should be healthy and free from injuries. Any chemical expellants used for field collection should be washed off immediately with water. Earthworms should be acclimated to the experimental conditions for at least one week before the start of the experiment (Fründ *et al*., 2010).

#### 2.4.3.4 Burrowing behaviour: Evans' boxes and X-ray tomography

A re-created visible structure of a soil profile, constructed with two sheets of glass separated by a very small distance (5 - 8 mm) has been used for some earthworm studies (Butt and Grigoropoulou, 2010). Evans (1947) used such a structure to observe the burrow formation of earthworms. Grigoropoulou *et al*. (2008) successfully used Evans' boxes (also referred to as 2D microcosms) to study the effects of adult *L. terrestris* on cocoons and hatchlings. These simple structures allow observation of earthworm burrow-related behaviours. Further, three-dimensional demonstrations have been achieved with soil-filled PVC cylinders inoculated with earthworms and subsequently analysed with computerised X-ray tomography (Fründ *et al*., 2010; Capowiez *et al*., 2011**).**

#### 2.4.3.5 Feeding behaviour

For more than a century, scientists have used various methods to study earthworm feeding behaviour. Darwin (1881) and Satchell and Lowe (1967) determined earthworm food preference by offering leaf litter particles at the soil surface and made observations of which particles were disturbed/removed after a certain period of time. Further, Doube *et al*. (1997a) used simple choice chamber techniques to study earthworm food preference under laboratory conditions. This technique allowed different types of known food materials to be offered to earthworms and allowed quantification of the intake with time. Butt *et al.* (2005) used direct observation experiments to determine food choices of some earthworms, such as *L. terrestris* which feeds directly from the soil surface. These researchers offered food (paper pulp and manure) at the soil surface

(around the burrow as a circle) and observed which material had been disturbed after the event or more directly through video recording of the actual behaviours in progress.

Relatively inexpensive, sophisticated "webcam" techniques have been successfully used for various earthworm surface behavioural studies. Nuutinen and Butt (1997) and Butt *et al*. (2005) have used infrared video recording to observe mating and feeding behaviour of *L. terrestris* at the soil surface. Valckx *et al*. (2010) have also used infrared sensitive webcams to monitor the locomotive behaviour of *L. terrestris*.

### 2.4.4 Microcosm/Mesocosm techniques

Enclosed or partially enclosed model ecosystems, often called "microcosms", have been widely used for studying soil biota, their interaction with plants and their effects on various ecosystem processes (Teuben and Verhoef, 1992; Verhoef, 1996). This experimental approach has become a major research tool in soil ecology because of its practicality, simplicity and replication ability (Beyers and Odum, 1993: Kampichler *et al*., 2001). Laboratory-based, relatively small, enclosed "microcosms" with certain manipulative treatments have been widely used to develop hypotheses about behaviour and function of real ecosystems (Kampichler *et al*., 2001). However, mid-sized, partially enclosed outdoor experimental units often called "mesocosms" have been employed to bridge the gap between laboratory "microcosms" and the large, complex, real-world "macrocosm" (Odum 1984; Verhoef, 1996; Kampichler *et al*., 2001). In earthworm ecology, various sizes and types of laboratory-based "microcosms" as well as greenhouse/field-based "mesocosms" have been used as experimental units to study numerous activities and ecological processes including burrowing (Bastardie *et al*., 2003), feeding (Doube *et al.*, 1997a), mucus excretion and casting (Scheu, 1991)

mating (Butt and Nuutinen, 1998), cocoon deposition (Butt, 2002), earthworm interactions (Capowiez and Belzunces, 2001) and even nutrient cycling (Hale *et al*., 2008).

### 2.4.5 Field earthworm inoculation

Reviews of earthworm research have shown that the appropriate reintroduction of earthworms to sites where they were absent can bring obvious positive changes in soil properties (Curry, 1988; Baker *et al*., 2006: Nuutinen *et al*., 2006). Several methods are available to introduce earthworms to locations where they are absent and at low density. The simplest method can be described as "collection and broadcast" using an available collection technique. Turf transfer is another simple technique that involves digging and translocating soil with grass attached (Butt and Grigoropoulou, 2010). The Earthworm Inoculation Unit (EIU) (Butt *et al.*, 1997) known as "worms in bags" allows culturing of a starter culture of adults under optimal conditions over a period of a few months. After this time, a population develops within the plastic unit which includes all life stages of earthworms (adults, cocoons, and hatchlings). The EIUs are then ready for introduction. Inoculation requires the contents of the EIUs to be inserted into appropriately sized holes in the intended inoculation site. The plastic bags are split at the bottom and carefully removed. The contents provide a protective microenvironment for introduced earthworms to survive and recolonise the introduced habitat.

## 2.5 Plant-earthworm interactions

Earthworms, the dominant soil macrofauna group in many terrestrial ecosystems, have major interactions with plant communities (Lee, 1985; Bardgett *et al.*, 2005; Eisenhauer *et al*., 2009a). Different earthworm species have diverse impacts on soil dynamics and subsequently on potential plant growth (Haimi *et al*., 1992; Welke and Parkinson, 2003). Equally, different plant species have various impacts on below-ground earthworm communities (Muys *et al*., 1992; Zou, 1993; Sarlo, 2006). Although this earthworm-plant relationship has been widely investigated, the relative impacts and feedback processes are extremely complex due to the number of mechanisms involved and the number of other factors such as soil properties, plant species and earthworm species which likely influence these mechanisms (Haimi *et al*., 1992; Jana *et al*., 2010).

### 2.5.1 Impacts of earthworms on plant growth and yield

Earthworms are generally regarded as beneficial to plant growth (Lee, 1985; Bardgett *et al*., 2005; Eisenhauer *et al*., 2009a). Many researchers have illustrated a direct and positive relationship between earthworm activity and plant growth. Haimi *et al.* (1992) studied the effect of the earthworm *L. rubellus* on net production of birch (*Betula pendula*) seedlings in laboratory microcosms and revealed that they grew significantly faster in the presence of earthworms, and had longer stems, and larger, greener leaves. The mean height of the seedlings after 51 weeks was 0.36 m in the treatment with earthworms, and 0.21 m in the control without earthworms. After two growing periods (51 weeks), the combined production of leaf biomass was 33% greater in the earthworm treatment compared with the control. A similar trend was observed for stem biomass, although the root biomass showed the opposite trend. At week 51, total nitrogen in

seedlings grown with earthworms was 11.4 mg more than seedlings grown without them. They concluded that birch seedlings benefit from earthworm activity in raw humus forest soil. Further, Welke and Parkinson (2003) investigated the effect of the endogeic earthworm *Aporrectodea trapezoides* on growth of Douglas-fir (*Pseudotsuga menziesii*) seedlings grown in three different soils (soils from a pure Douglas-fir stand, pure birch stand and mixed stand). The study demonstrated that mean root biomass increased much more dramatically from 5 months to 10 months in worm-worked soils (197%) compared to control (72%). However, no significant difference was observed in the change in shoot biomass, although it slightly increased in worm-worked soil (123%) compared with control (97%). Needle calcium level was higher in an earthworm treatment compared with control. However, a clear trend was not observed in needle nitrogen content. Bisht *et al*. (2006) evaluated the effect of the earthworm *Octolasian tyrtaeum* casting on crop growth and showed that compared to control, total dry weight in earthworm treatments increased by 65% in maize, 58.1% in wheat and 61.7% in barley. Eisenhauer et al. (2009a) observed the effect of earthworms on regrowth of grassland plant communities and concluded that earthworms significantly enhanced each of the plant re-growth parameters such as plant coverage and height of vegetation. The effects of earthworms on plant growth and performance involve a number of direct and indirect mechanisms, so it is difficult to establish the direct link under field conditions, but this can be studied under controlled conditions.

#### 2.5.1.1 Organic matter mineralisation: plant nutrient supply

One of the predominant mechanisms of earthworms which increase plant growth is organic matter mineralisation. This process induces the spatio-temporal nutrient availability through plant litter fragmentation and incorporation into the soil (Barois *et*

*al*., 1999; Brown *et al*., 2000), in addition to the stimulation of microbial mineralisation of soil organic matter (Postma-Blaauw *et al*., 2006). Further experiments have shown that the release of mineral nitrogen is essentially a major mechanism of earthworms responsible for an increase in plant biomass production (Brown *et al*., 1999). Different ecological groups of earthworms may have different, but important, effects on organic matter mineralisation, nutrient release and plant growth (see 2.3.1.1). Further investigations have revealed significant interactions between earthworms and soil type (Jana *et al*., 2010) and have suggested that the increase of plant growth through earthworm-mediated mineralisation should be superior in nutrient-limited, poor quality soil. In nutrient-rich soil, plants are less limited by the availability of mineral nutrients and earthworm-induced mineralisation may have less or no influence on plant growth (Brown *et al*., 2004). Jana *et al*. (2010) conducted an experiment to investigate the complex mechanism of interactions of an earthworm species on plant growth using a model plant *Arabidopsis thaliana* and a common temperate earthworm *A. caliginosa*. They used two types of soils for this experimental system and concluded that in poor soil with a low content of mineral nutrients and organic matter, earthworms significantly increased soil nitrate content and enhanced plant above-ground biomass production. By contrast, in the richer soil, earthworms had no significant effect on production of above-ground biomass. These results indicate that earthworm-induced mineralisation is a determining factor for plant growth particularly in nutrient-limited poor soil.

#### 2.5.1.2 Soil structural modification: seed-bed, plant-bed provision

Earthworms indirectly affect plant growth through soil structural modifications. The activities of earthworm that have the greatest influence on soil structure are burrowing, feeding and casting. During these processes, earthworms thoroughly mix the soil, form organo-mineral aggregates, increase soil porosity, aerate the soil and improve its water holding capacity. Those activities which affect plant root growth and water balance tend to enhance plant growth in most situations (Brown *et al*., 2004). Springett, (1985) suggested that earthworm burrows facilitate plant root growth by providing channels in soil where roots are able to grow with minimal resistance. These channels often have a lining rich in organic matter and available nutrients which provide ideal conditions for root growth (Edwards and Shipitalo, 1998). Furthermore, Laossi *et al*. (2010) have suggested that soil texture can modulate earthworm effects on soil structural development. In clayey soils, earthworms might lead to very stable aggregates while in sandy soils structures created by earthworms may be more fragile. This could influence on soil nutrient status, hence plant growth.

#### 2.5.1.3 Production of plant growth promoting substances

Nelson (1965) proposed that plant growth-promoting substances are present in tissues of *A. caliginosa, L. rubellus and E. fetida*. Dell-Agnola and Nardi (1987) further investigated this and confirmed that earthworm activity produces hormonal effects on plant growth. Muscolo *et al*. (1999) identified an auxin-like compound in earthworm casts. Recent research has suggested that some earthworms produce plant growth regulators via the stimulation of microbial activity (Laossi *et al*., 2010). Jana *et al*. (2010) suggested that earthworms release phytohormone-like compounds through the

stimulation of bacteria. All of these findings suggest that earthworms can enhance plant growth possibly through direct production of plant growth promoting substances or via stimulation of microbial activity which produce such compounds or in combination of both. Further, Jana *et al*. (2010) confirmed that release of phytohormone-like compounds and organic matter mineralisation which influence plant growth are complementary mechanisms stimulated by earthworms.

#### 2.5.1.4 Plant pests and parasite control

Earthworms are known to affect plant growth through alleviation of the negative effect of pests and parasites (Laossi *et al*., 2010). Earthworms may greatly reduce plant parasite density by ingestion and death or producing unfavourable conditions in cast material or burrow linings (Stephens and Davoren, 1997). Jana *et al*. (2010) suggested that earthworms allow plants to resist parasitic nematode attack via several mechanisms; by decreasing nematode populations, by enhancing the strength of plants or by stimulating microbes that are antagonistic to root pathogens. Earthworms can disperse beneficial disease-biocontrol agents and positively affect plant growth, while they can negatively affect plant growth by transmitting many plant pathogens (Edwards and Bohlen, 1996). As an example, earthworms can spread soil fungi, including pathogens throughout the soil by dispersing spores and hyphal fragments.

#### 2.5.1.5 The effects on plant seeds

Anecic earthworms are increasingly recognised as important dispersers and predators of plant seeds (Eisenhauer *et al*., 2009b). Grant (1983) reported decreased and delayed germination of seeds of some grassland plant species after gut passage through *L.*

*terrestris* and *A. longa*. In contrast, Eisenhauer *et al*. (2009b) proposed that certain plant species might benefit from gut passage since slight damage of the seed coat may break seed dormancy. They experimented with endogeic earthworms and concluded that gut-passage and cast products modified plant seed germination due to mechanical force such as scratching the seed coat and chemical stimuli such as increased nutrient concentrations. Moreover, they suggested that phytohomone-like substances and enzymes produced by microorganisms associated with the earthworm gut and the earthworms themselves may contribute to break seed dormancy.

### 2.5.2 Effect of trees on earthworms

#### 2.5.2.1 Tree species, litter quantity, and quality

Tree species affect the earthworm community since trees differ in quality and quantity of litter produced (Muys *et al.*, 1992; Zou, 1993). The quantity of litter produced can vary with tree species, age and planting density. Vanguelova and Pitman (2011) recorded the total litterfall (leaves, branches, cones and frass) from the UK Intensive Level II sites; oak (2.7 and 7.1), beech (3.0 - 5.3), scot pine (2.9 - 6.3) and sitka spruce (2.7 - 5.8) t $ha^{-1}$ $y^{-1}$. They suggest that difference in climate and deposition could influence the amount of litterfall, e.g. total annual litterfall of pine (3.8) and of beech (2.9) in low N deposition areas compared to (8) and (3.9) t $ha^{-1}$ $y^{-1}$, respectively in a high nitrogen deposition area. Davis and Trettin (2006) in South Carolina investigated the litterfall of sycamore (*Platanus occidentalis*) and sweetgum (*Liquidambar styraciflua*) established on former agricultural land and recorded that a sycamore plantation starts with 0.4 in the first year and can reach 7.77 t $ha^{-1}$ by the fifth year while sweetgum litterfall can increase from 0.06 to 1.85 t $ha^{-1}$ over a period of five years.

Earthworm abundance, activity, litter-specific decomposition and nutrient release rates are largely influenced by the chemical composition of plant litter (see Table 2.5.1). especially, nitrogen concentration, carbon to nitrogen ratio, lignin content, phenolic compounds and calcium content. Hendriksen (1990) reported that carbon to nitrogen ratio and final polyphenol concentration of the litter are the most important factors which influence detritivore aggregation. He recorded detritivore number under *Fraxinus, Tilia, Alnus, Quercus* and *Fagus* litter as 102, 82. 63, 50 and 29 $m^{-2}$ respectively, where litter carbon to nitrogen ratios were 19.0, 23.7, 19.2, 32.9 and 47.0 correspondingly.

**Table 2.5.1 Estimated litter quality parameters of some common European tree species (NR – Not Recorded)**

| **Species** | **Litter N (%)** | **Litter C %** | **Litter Ca (%)** | **Lignin (%)** | **Polyphenol (%)** | **References** |
|---|---|---|---|---|---|---|
| *Fraxinus* spp | 1.24 – 2.42 | 31.1 – 46.1 | NR | 20.9 | 0.14 | Hendriksen, 1990; Cornelissen, 1996 |
| *Alnus* spp | 2.35 | 45.2 | | 21.1 | 2.15 | Hendriksen, 1990 |
| *Acer pseudoplatanus* | 0.94 | 46.2 | 1.9 – 2.2 | 16.4 -16.7 | NR | Cornelissen, 1996; Reich *et al*., 2005; Hobbie *et al.*, 2006 |
| *Tilia cordata* | 1.2 – 1.8 | 43.2 | 1.8 - 2.2 | 30.0 – 41.7 | 0.31 | Hendriksen, 1990; Reich *et al*., 2005; Hobbie *et al*., 2006 |
| *Carpinus betulus* | 1.10 – 1.52 | 42.3 - 46.9 | 0.9 | 14.4 | 2.62 | Hendriksen, 1990; Reich *et al*., 2005; Hobbie *et al*., 2006 |
| *Betula pendula* | 1.0 – 1.4 | 47.8 – 52.8 | 1.2 | 38.4 – 40.8 | NR | Cornelissen,1996; Reich *et al*. 2005; Hobbie *et al*., 2006 |
| *Corylus* | 1.34 - 1.52 | 42.3 | NR | 28.5 | 2.62 | Hendriksen, 1990; Vanguelova and Pitman, 2011 |
| *Quercus robur* | 1.0 – 1.44 | 36.6 – 51.1 | 1.1 | 19.8 – 23.0 | 1.15 | Hendriksen 1990; Hobbie *et al*., 2006; Reich *et al*., 2005; Vanguelova and Pitman, 2011 |

In Belgium, Muys *et al*. (1992) investigated the effects of grassland afforestation with different tree species and showed that after 20 years of forest development on a sandy loam soil, the earthworm communities and litter decomposition rates were significantly different under specific tree species. The study recorded that total earthworm biomasses under *Alnus*, *Fraxinus, Tilia* and *Prunus* varied between (673 – 1334 kg $ha^{-1}$) after 20 years. However, it was low under *Quercus* (342 kg $ha^{-1}$) compared with original meadow (1014 kg $ha^{-1}$). These effects were directly linked with quality and quantity of the litter fraction produced by the different trees. The study suggests that poor quality *Quercus* litter (C: N >32) start to accumulate and form moder humus. Pigott (1989) compared the soil development under lime (*Tilia cordata*) and beech (*Fagus sylvatica*) trees established in arable land in Hampshire, England and recorded that after a period of 56 years, soil under lime was full of large earthworms ( *L. terrestris* and *Allolobophora* spp.) while under beech there were only a few small pigmented earthworms. This suggested that palatability of lime litter encouraged the earthworm community development while dry, papery beech litter with high polyphenols did not. In Poland, Reich *et al*. (2005) examined variation in soils and earthworms under different tree species, 30 years after tree establishment and recorded rapid and widespread changes in soil beneath specific tree species. This suggested that tree species affect soil both directly through the litter chemistry and indirectly through the effect of litter on earthworms. Earthworm biomasses were recorded as under *Tilia* (4.81), *Acer* spp. (3.41 - 7.38), *Betula* (0.46), *Quercus* spp. (0.08 – 2.34) and *Pinus* spp. (0.05 – 0.12) g $m^{-2}$ correspondingly while the litter calcium was recorded; *Tilia* (22. 4), *Acer* spp. (19.0 - 20.5), *Betula* (12.6), *Quercus* spp. (10.8-11.5) and *Pinus* spp. (3.7 - 5.8) mg $g^{-1}$ respectively. This study concluded that tree species which produce calcium-rich litter were associated with increased native earthworm abundance and diversity, as well as increased soil pH, exchangeable calcium, base saturation and forest floor

turnover rate. Neirynck *et al*. (2000) examined the impact of hardwood tree species on earthworm biomass and physico-chemical properties of topsoil of a loamy acid brown forest 60 years after establishment in Brussels. They recorded earthworm biomasses under different tree species; *Tilia* (10.6), *Acer* (36.8), *Quercus* (0.4 - 4.2) and *Fagus* (0.2) g $m^{-2}$ respectively. The study concluded that the presence of mull-forming tree species (e.g. *Tilia* and *Acer)* generally led to a higher earthworm biomass and more favourable physico-chemical top soil properties compared to moder-forming hardwoods (e.g. *Quercus* and *Fagus)*. In addition to broad field studies, some laboratory experiments have suggested that litter quality is a determining factor for earthworm community development as it has strong influence on feeding activity (Satchell and Lowe, 1967) as well as growth and reproduction success of earthworms (Butt, 2011).

#### 2.5.2.2 Other forestry activities

In addition to tree species as food source for earthworms, management activities such as land preparation, planting, soil fertilisation, weeding, liming and harvesting can have positive or negative impacts on earthworms as these management practices primarily change soil properties. As an example, Robinson *et al*. (1992) studied the earthworm communities in limed coniferous soils in the UK, France, and Northern Ireland and showed that liming of coniferous forest soils had significantly increased total earthworm number and biomass that the soil can support. In France, under acid brown earth soil of *Picea abies* earthworm density increased to 5 – 11 $m^{-2}$ compared with 6 $m^{-2}$ in nearby deciduous woodland and 0 $m^{-2}$ in unlimed *P. abies* plots. Similarly in Northern Ireland, liming increased earthworm density in peat soil under *Picea sitchensis* (79 $m^{-2}$ compared with 5 $m^{-2}$ in unlimed soils). Nachtergale *et al*. (2002) reported that single tree uprooting in a forest (Belgium) can invoke small scale environmental changes and

strongly reduce earthworm abundance for period of six years especially at a micro-site level.

#### 2.5.2.3 Forest tree species diversity: pure vs. mixed

Diverse food quality positively affects decomposer fauna and therefore, it is generally accepted that mixed forests ought to support a more diverse decomposer community compared with pure stands. Cesarz *et al*. (2007) studied the influence of tree species diversity on the earthworm community in a deciduous mixed forest in Germany and suggested that increasing proportions of high quality litter were positively correlated with high densities of both epigeic and endogeic earthworm species. Aubert *et al*. (2002) in France investigated the effect of canopy composition on earthworms and other macro-invertebrates in two deciduous temperate forests; a pure beech (*Fagus sylvatica*) and mixed beech-hornbeam (*Carpinus betulus*). The results revealed that spatial variability of soil macrofauna communities was greater for the pure system than the mixed. These results do not support the general agreement that resource diversity and patchiness increases soil fauna biodiversity and heterogeneity. However, here the investigators had only 30% of hornbeam in the beech-hornbeam mixed forest which may not have been enough as a key determinant for soil macrofauna community. Further, Laossi *et al*. (2008) examined the effects of plant diversity on plant production and soil macrofauna density and diversity in Brazil. They used four plant species; an herbaceous legume, a perennial grass, a legume shrub and a non-legume shrub in a field as individuals and mixed assemblages and reported that plant diversity did not significantly affect density and diversity of soil macrofauna. Raty and Huhta (2004) showed that quality of litter as food played an important role for litter-feeding earthworm species such as *L. terrestris* and *L. rubellus*. They further suggested that

earthworm populations are often food-limited, but litter quality rather than its quantity most often limit earthworm populations. Laganiere *et al*. (2009) linked the abundance of aspen (*Populus tremuloides*) with soil faunal communities along black spruce (*Picea mariana*) - aspen gradients in three forests in eastern Canada. Their results suggest that aspen favours the expansion of a macrofaunal community, because of leaf quality. Tree diversity may not always effect earthworm communities positively, but the quality of the litter of individual tree species either as a pure or mixed stand is the key determinant.

### 2.5.3 Potential impacts of SRF on soils and earthworms

Woody biomass crops could affect the soil system below them directly through leaf litter inputs, tree rooting, plant nutrient uptake and even through water uptake. Further, those crops which are grown for biomass harvest can indirectly affect the soil system through associated silvicultural practices. These direct and indirect effects can cause changes in soil organic matter, nutrients fluxes, soil pH, soil moisture, soil biodiversity and even in soil erosion and compaction (Vanguelova and Pitman, 2011). However, SRF impacts and relative changes in soil systems largely depend on previous land use. The agricultural lands which are mainly used for SRF planting in the UK are generally rich in base cations, nitrogen and phosphorus. Planting of fast-growing tree species and harvesting for biomass may lead to significant soil nutrient depletion and soil acidification over time (Mitchell *et al*., 1999; Hagen-Thorn *et al.*, 2004). Conversely, compared with arable farming, SRF with relatively lengthy rotation has a greater potential to improve soil physical, chemical and biological properties due to less frequent soil disturbance, leaf litter accumulation and less application of chemicals (Makeschin, 1994; Perttu, 1998). The network of fine roots in the upper soil layers

along with surface litter cover may reduce the soil erosion in SRF systems compared with arable farming. However, the net impacts of SRF on the soil are dependent not only on previous land-use, but also on many other factors, mainly SRF species, soil type/texture, silvicultural practices and local climate (LTS International, 2006).

The above mentioned SRF impacts on soils and relative changes may largely effect earthworm populations and the diversity of these systems. The leaf litter inputs, soil organic matter, soil pH and soil moisture changes associated with tree species can inevitably effect soil faunal community development under these plantations.

#### 2.5.3.1 Leaf litter inputs and soil organic matter changes

Conversion from arable to SRF has significant potential to increase soil organic matter through leaf litter inputs, woody debris inputs and tree rooting (Vanguelova and Pitman, 2011). The increased level of organic matter can provide more abundant resources to soil organisms in SRF systems than agricultural crops, and this could improve the abundance and diversity of soil faunal communities (Makeschin, 1994; Bardgett, 2002). Soil systems with high organic matter content and increased levels of soil faunal activities generally have positive impacts on soil structure, water-holding capacity, and the storage and availability of nutrients (Bernier and Ponge, 1994; Mann and Tolbert, 2000). However, the effects of SRF on soil organic matter, faunal activity and diversity, nutrient dynamics may vary with tree species, since trees are different in quantity and quality of litter produced (see section 2.5.2.1 and Table 2.5.1).

Broad-leaved tree species generally provide rapidly decomposable litter which can be incorporated into the upper soil horizon, relatively quickly through soil faunal activity

(Drift, 1961). Compared with native broadleaves, deciduous non-native broadleaves, such as *Nothofagus* spp., show a similar effect on soils (Peterken, 2001). The leaf litter from non-deciduous broadleaves such as eucalyptus takes relatively longer to decompose in the soil system compared with native broadleaves (Cornelissen, 1996; Louzada *et al*., 1997). However, eucalyptus litter decomposes relatively quickly compared with native conifers (Wedderburn and Carter, 1999). The canopy density and leaf litter decomposition rates for selected SRF species are summarised in Table 2.5.2. The canopy density determines the quantity of annual litter fall and water interception rate and may indirectly affect the soil moisture content.

**Table 2.5.2 Canopy density and leaf litter decomposition rates for SRF**

| **Species** | **Canopy density** | **Rate of litter decomposition** | **References** |
|---|---|---|---|
| *F. excelsior* | Light | Rapid | Cornelissen, 1996 |
| *Alnus* spp. | Moderately light | Rapid | Cornelissen, 1996 |
| *A. pseudoplatanus* | Dense | Rapid | Cornelissen, 1996; Hobbie *et al*., 2006 |
| *B. pendula* | Light | Intermediate | Cornelissen, 1996; Hobbie *et al*., 2006 |
| *Populus* spp. | Moderately light | Intermediate | Cornelissen, 1996 |
| *Castaneas* spp. | | Intermediate | Vanguelova and Pitman, 2011 |
| *E. nitens* | Dense | Slow | Wedderburn and Carter, 1999; Lopez *et al.*, 2001 |
| *E. gunnii* | Light | Slow | LTS International, 2006; Vanguelova and Pitman, 2011 |

#### 2.5.3.2 Soil pH changes

Conversion from arable to woodland is likely to reduce the soil pH with time (Hagen-Thorn *et al*., 2004). Poplar and willow establishment on former arable soils in Germany recorded a decrease in top soil pH up to 0.5 units after 10 years (Jug *et al.*, 1999). Soil pH reduction with time under SRF plantations established on previously arable land was also noted in Sweden (Alriksson and Olson, 1995). Pigott (1989) compared the soil development under lime (*Tilia cordata*) and beech (*Fagus sylvatica*) trees established in arable land in Hampshire, England and recorded that after a period of 56 years, soil pH under tree species were significantly different; lime (5.1) and beech (3.8). Vanguelova and Pitman (2011) also suggest that the effects of tree species on soil pH are highly species-specific, and as an example they emphasised that under *Salix* and *Populus*, significant acidification was noticed, while under *Fraxinus, Tilia, Alnus, Betula* and *Nothofagus* soils became less acidic with time. These pH changes differ when SRF trees are established on ex-grassland sites, depending on the tree species. In Belgium, Muys *et al.* (1992) recorded a decrease (0.13 units) in soil pH under oak (*Q. palustris*) but an increase (0.14 – 0.90 units) under *Alnus, Fraxinus* and *Tilia* after 20 years of their establishment in old pasture land. Moffat and Boswell (1990) recorded decreases in soil pH under *Alnus* planted in grassland compared with a grassland control. These tree-associated soil pH changes can effect long-term soil faunal community development. However, Vanguelova and Pitman, (2011) suggest that most of the SRF species used in the UK have a large tolerance to soil pH as long as sufficient nitrate is available.

#### 2.5.3.3 Soil moisture changes

In terms of water usage, trees and forests are likely to use more water than that of shorter types of vegetation, mostly due to interception of rain water by their rough canopies and also high transpiration rates by deeper rooting (Nisbet *et al*., 2011). Interception of a high proportion of rainfall water by tree canopies would also reduce water reaching the soils under trees compared to arable or grassland use. The fast growing and most productive non-native SRF species, such as *Eucalyptus nitens,* are identified as high potential water users, partly due to high transpiration rates associated with deep rooting (Lima, 1984: Calder *et al*., 1997). Almeida *et al.* (2007) in Brazil studied *Eucalyptus grandis* hybrid and recorded average annual crop water use of 1092 mm compared to 1147 mm precipitation, leaving only 3% as runoff. Calder (1992) report that roots of young eucalyptus established on deep soils penetrated to a greater depth, so that annual evapotranspiration exceeded rainfall by a significant margin. However, the water use of many SRF broadleaved crops in UK is unlikely to differ greatly from conventional broadleaved woodland (Nisbet *et al*., 2011). Literature shows that water use of broadleaves in the UK is greatly influenced by tree species, soils and geology (Wullschleger, 1998; Roberts and Rosier 2005). The impact of tree species on soil moisture can greatly influence soil faunal diversity, activity and other soil processes.

#### 2.5.3.4 Soil temperature changes

Soil temperature is one of the principle factors which determine the activity of soil biota and the rate of decomposition (Swift *et al*., 1979). It is well known that the shading and insulation afforded by the forest canopy buffer temperature extremes at the ground

surface (Prescott, 2002b). Therefore, the soil temperate under forest is less responsive to air temperature changes compared to the soil temperature in open habitat. Unlike woodlands, soils in cultivated lands (in the absence of ground cover), could freeze at deapth during the winter and may greatly influence soil faunal population (Hopp, 1947). Vesterdal *et al*. (2012) studied the soil respiration and carbon turnover among six common European tree species; *Fagus, Quercus, Tilia, Acer, Fraxinus* and *Picea* and found no significant differences in soil temperature between tree species. However, this study recorded a little higher temperature under *Fraxinus* (10.3 °C ) compared with *Picea* (9.3 °C) due to differences in light transmittance associate with leaf area index.

#### 2.5.3.5 Silvicultural practices

In addition to the above mentioned tree impacts, SRF-associated activities such as site preparation, planting, soil fertilisation, weeding, harvesting and rootstock removal can effect soil faunal community development (Makeschin, 1994; Perttu, 1998) (see section 2.5.2.2). However, compared to arable farming, the frequency of soil disturbance is very low for SRF (see section 2.3.3.7).

Although the potential effect of biomass crops on soils has been recently reviewed by several authors (LTS International, 2006; Vanguelova and Pitman, 2011), data concerning the potential effects of SRF species on soil fauna and the reciprocal effects are very limited.

# CHAPTER 3: RESEARCH METHODOLOGY

## 3.1 Introduction

This chapter initially describes the methodological approach used for the duration of this research. Thereafter, it details the practical aspects considered in development of advanced experimental plans. Finally, it summarises the overall experimental design which is used in subsequent experimental chapters.

## 3.2 Methodological approach

This research approach was considerably broad at the outset, since it intended to explore the interactions between a wide range of SRF species, soils and earthworm communities. Therefore, a systematic approach was followed to obtain achievable outcomes within a given period of time.

- During the initial stage of the research, a comprehensive literature survey was conducted on earthworms, trees, soil processes and their interactions as this was vital before planning any experimental aspects of the research.
- Standard field earthworm sampling, preservation, and identification were reviewed. In addition, live earthworm collection techniques, laboratory culturing as well as inoculation techniques were practiced.
- Earthworm-related laboratory microcosm and field mesocosm experiments were intensely studied.

- Forestry science methodologies/techniques such as tree identification, soil/ plant/ leaf litter sample collection, preparation and chemical analysis were practiced. Further, litter decomposition and nutrient cycling investigations such as litterbag experiments on forest floor were studied.
- Baseline earthworm/soil surveys were conducted at selected SRF trial sites across England.
- Breeding populations of experimental earthworm species were cultured under laboratory conditions, initially from field-collected animals.
- Adequate amounts of required species of SRF leaf litter were collected in autumn (2009), air-dried and stored for future experiments.
- Preliminary laboratory experiments were conducted to inform further advanced studies.
- Based on the initial earthworm/soil surveys and laboratory trials, an advanced experimental design was developed to investigate the possible interactions between SRF species and earthworms.

### 3.3 Development of an experimental design: Practical considerations

Although this research project began with a broad view on all possible SRF-earthworm interactions, it was essential to narrow down the experimental species and locations in order to balance the time and resource availability, without affecting the quality of the final results. The experimental SRF species, locations, earthworm species and soil types were carefully selected to be representative of existing communities.

### 3.3.1 SRF species selection

An extensive list of potential SRF species (see Table 2.2.1 and 2.2.2) was initially proposed by Forest Research. After investigating the literature and visiting a number of available sites, seven SRF species which have been trialled extensively in England were selected for the baseline earthworm/soil surveys (Table 3.3.1).

**Table 3.3.1 Experimental SRF species and their origin**

| SRF species | Abbreviation used | Origin |
|---|---|---|
| Birch *(Betula pendula)* | Br | Native |
| Ash *(Fraxinus excelsior)* | Ah | Native |
| Common alder (*Alnus glutinosa*) | Ad | Native |
| Shining gum *(Eucalyptus nitens)* | En | Non-native |
| Cider gum *(Eucalyptus gunnii)* | Eg | Non-native |
| Sycamore *(Acer pseudoplatanus)* | Sy | Naturalised |
| Sweet chestnut (*Castaneas sativa)* | Sw | Naturalised |

Six SRF species, all except *E. gunnii* (from Table 3.3.1) were used in most of the laboratory experiments. Although, several potential eucalyptus species were suggested, highly attractive (produce high biomass yield within short period of time) *Eucalyptus nitens* was chosen for comparative laboratory studies. However, in the latter stage of this research, which involved the commissioning of more advanced and resource-demanding experiments, two or three major SRF species were chosen. Appropriate combinations of SRF species for each experiment were selected to achieve specific objectives. Tree origin (native, non-native and naturalised see section 2.2.2 for details) was considered as a major criterion when selecting experimental tree species.

### 3.3.2 SRF site selection

As SRF is a relatively new concept to the UK, at the beginning of the study, the newly established network of SRF sites was very young (2 - 6 years old) and the availability of appropriate sites to conduct a complete comparative survey between selected species was limited. The comparative trial sites which were at an appropriate age, consisted mostly of two or three of the SRF species listed in Table 3.3.1. However, using existing sites and resources, the field studies were conducted to achieve maximum potential outcomes. Five Forestry Commission sites (see Table 3.3.2) were selected for field surveys/experiments based primarily on the SRF species present, land-use history and soil type. When selecting sites for more intensive field experiments, practical considerations (e.g. frequent accessibility and potential disturbance) were also taken into account.

**Table 3.3.2 Experimental sites, locations and SRF species present**

| **Site name** | **Location (English County)** | **National Grid Reference** | **SRF species present** |
|---|---|---|---|
| Alcan | Northumberland | NZ 291890 | *E. gunnii, E. nitens* |
| Daneshill | Nottinghamshire | SK 680856 | *E. gunnii, E. nitens* |
| Rogate | West Sussex | SU791257 | *C. sativa, B. pendula* |
| Gisburn | Lancashire | SD 731566 | *B. pendula, A. pseudoplatanus, F. excelsior* |
| Carlshed | Yorkshire | SE 380488 | SRF potential; ex-agriculture (no SRF species present) |

### 3.3.3 SRF species litter collection and preparation

Collection and preparation of tree litter was a major aspect of this project as most of the laboratory and field experiments were designed around SRF litter. Litter quality had to be homogeneous in order to make comparisons across experiments. Therefore, ample amounts of SRF species litter had to be collected at an initial stage, so that these would be sufficient for the entire research project.

Freshly fallen SRF species litter was collected from known forest sites in autumn 2009. Collected litter was taken to the laboratory and air-dried. Then, twigs, soil particles, grasses, leaves of other tree species and other extraneous materials were removed. Clean, air-dried SRF species litter was stored in separate plastic bags at room temperature and clearly labelled for future use. Sub-samples from each species were ground (after drying at 70 °C) and analysed for litter chemistry following the standard procedure used by Forest Research laboratory. Total C and N were determined using a CN Elemental Analyser. Major cations (e.g. P, K, Ca, Mg, Al, Fe, Mn, B, Na, Cd, Cr, Cu, Ni, and Zn) were measured through acid digestion and followed by Inductively Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) analyses. Mean litter chemistry results are presented in Table 3.3.3.

**Table 3.3.3 Mean litter chemistry results for experimental SRF species (all units are mg $kg^{-1}$, unless otherwise stated; n = 3)**

| Elements | SRF species | | | | | |
|---|---|---|---|---|---|---|
| | **Ad** | **Ah** | **Br** | **En** | **Sw** | **Sy** |
| **N (%)** | 2.76 | 1.51 | 1.59 | 1.33 | 0.94 | 1.45 |
| **C (%)** | 50.5 | 47.3 | 51.5 | 52.5 | 48.7 | 47.9 |
| **K (%)** | 0.41 | 0.24 | 0.22 | 0.42 | 0.42 | 0.22 |
| **Ca (%)** | 1.91 | 2.66 | 1.17 | 1.36 | 0.93 | 2.55 |
| **Mg (%)** | 0.17 | 0.24 | 0.21 | 0.20 | 0.27 | 0.20 |
| **P (%)** | 0.12 | 0.15 | 0.10 | 0.09 | 0.09 | 0.08 |
| **Al** | 226 | 276 | 378 | 1302 | 928 | 776 |
| **Fe** | 261 | 250 | 318 | 357 | 388 | 561 |
| **Mn** | 202 | 125 | 1575 | 386 | 2504 | 89 |
| **B** | 21.7 | 11.9 | 12.5 | 20.8 | 23.0 | 13.4 |
| **Na** | 487 | 162 | 218 | 548 | 698 | 255 |
| **Cd** | 0.07 | 0.13 | 1.08 | 0.09 | 0.11 | 0.15 |
| **Cr** | 2.26 | 1.85 | 1.90 | 2.84 | 5.04 | 4.74 |
| **Cu** | 13.2 | 10.6 | 7.53 | 3.54 | 6.05 | 9.63 |
| **Ni** | 1.63 | 1.04 | 2.35 | 6.51 | 3.70 | 0.96 |
| **Zn** | 73.5 | 36.3 | 236 | 17.6 | 47.4 | 31.9 |
| **C/N (no units)** | 18.3 | 31.3 | 32.5 | 39.5 | 52.0 | 33.0 |

*A glutinosa* (Ad), *F. excelsior* (Ah), *B. pendula* (Br), *E. nitens* (En), *C. sativa* (Sw) and *A. pseudoplatanus* (Sy).

### 3.3.4 Earthworm species selection

Based on results of baseline earthworm surveys at selected forest sites, the four most abundant earthworm species (see Table 3.3.4) were selected for laboratory and field experiments. The selected earthworm species are widespread in Britain and represent different ecological groupings (section 2.3.1.1). These earthworm species were used for

laboratory and field experiments in monoculture or in combinations, depending on the objectives of given experiments.

**Table 3.3.4 Details relating to selected earthworm species (adapted from Sims and Gerard, 1999)**

| Earthworm species | Abbreviation used | Common name | Ecological group | General description |
|---|---|---|---|---|
| *Lumbricus terrestris* | Lt | Lob worm/ Dew worm | Anecic | Deep burrowing species, feeds on surface plant litter, common in undisturbed terrestrial habitats such as grasslands and orchards, forms surface middens. (Preferred pH range 6.2 - 10). |
| *Aporrectodea longa* | Al | Black-headed worm /Long worm | Anecic | Deep burrowing species, feeds on surface plant litter, common in garden, cultivated soil, pasture and woodlands, produces surface casts. (Preferred pH range 6.7- 9.4). |
| *Aporrectodea caliginosa* | Acal | Grey worm/ Turgid worm | Endogeic | Shallow working species (common in top 70 mm of soil), feeds on soil organic matter obtained by the ingestion of large amount of mineral soil, common in gardens and cultivated lands. (Preferred pH range 5.9 - 11.1). This has several different physiological types. |
| *Allolobophora chlorotica* (green morph) | Ach | Green worm/ Stubby worm | Endogeic | Often found in the rhizosphere, co-dominant with *A. caliginosa* in gardens, grassland and woodland usually within 60 mm of the surface, feeds on mineral soil. (Preferred pH range 4.5 - 8.2) |

### 3.3.5. Experimental earthworm collection and culturing

The majority of adult species of earthworms were collected from mixed deciduous woodland located in Bretherton, West Lancashire (National Grid Ref. SD 462202, for site details see Grigoropoulou and Butt, 2010). One species, *A. chlorotica*, was collected from a grassland site located in Poulton-Le-Fylde, North Lancashire (National Grid Ref. SD 368416). The shallow-working species were collected by digging and hand sorting of soils while deep-burrowing species (e.g. *L. terrestris*) were collected using a vermifuge extraction technique (see section 2.4.1.2). Here, a suspension of mustard powder was directly applied into *L. terrestris* burrows using a 100 ml syringe. Emerging earthworms were immediately washed with clean cold water to remove any expellant and transported to laboratory. Healthy adults (fully developed clitellum) were selected and reared in 10 °C constant temperature incubators. A sterilised loamy soil (25% moisture) was used as a standard substrate (see section 3.3.6) and a mixture of SRF species litter was supplied as a food source for breeding earthworm populations. Field-collected adult earthworms were acclimated to laboratory conditions for at least four weeks before the start of any experiment, as suggested by Fründ *et al*. (2010). The experimental hatchlings were laboratory cultured as it is difficult to identify earthworms at this age to species level in the field. It also proved advantageous to have a cohort of animals, with similar biomass/growth stage and life history for comparative laboratory experiments.

### 3.3.6 Experimental soil type

Soil type and texture are known to be major factors, which influence earthworm abundance, in addition to activity (Hendrix *et al*., 1992; Baker *et al*., 1998). In general, medium loamy soils with neutral pH, support larger earthworm populations (Guild, 1951; Bouché, 1977). Pre-sterilised, Kettering loam was selected as an experimental medium for all of the laboratory experiments (Butt *et al*., 1994). Sub-samples from Kettering loam were analysed for initial soil chemistry data following the standard procedure used by Forest Research Laboratory Service. Air-dried soil samples were sieved through a 2 mm sieve and soil pH was measured in water solution. Organic matter was measured through loss on ignition. Total C and N were determined using a CN Elemental Analyser. Exchangeable cations were measured through $BaCl_2$ extraction. Cation Exchange Capacity (CEC) was determined as the sum of the extractable amounts of ($K^+$, $Ca^+$, $Mg^{2+}$, $Na^+$, $Al^{3+}$, $Fe^{3+}$, $Mn^{2+}$, $H^+$) from $BaCl_2$ extraction. Percentage Base Saturation (BS) was calculated using base cations of ($K^+$, $Ca^+$, $Mg^{2+}$, $Na^+$) (Hagen-Thorn, *et al*., 2004). Mean chemical nutrient analysis results for Kettering loam are presented in Table 3.3.5.

**Table 3.3.5 Mean Chemical nutrients analysis results for Kettering loam (n = 3)**

| **Soil parameters** | |
|---|---|
| Organic Matter - OM (%) | 5.0 |
| pH ($H_2O$) | 7.3 |
| Total N (%) | 0.2 |
| Total C (%) | 2.5 |
| Cation Exchange Capacity - CEC (cmol+ $kg^{-1}$) | 24.4 |
| Base Saturation - BS (%) | 99 |
| **Exchangeable cations (mg $kg^{-1}$)** | |
| K | 153 |
| Ca | 4465 |
| Mg | 155 |
| Na | 52 |
| Al | 0.0 |
| Fe | 0.0 |
| Mn | 0.5 |

A consistent soil source, free from soil macro and micro invertebrates, is very important for this kind of comparative study. Kettering loam has been used by earthworm researchers successfully for laboratory experiments for more than 15 years (Butt *et al*., 1994). Equally, a loamy soil was appropriate for this research, as SRF trees are planned for expansion primarily on nutrient-rich, ex-agricultural lands which consist of similar soil types.

## 3.4 Experimental design

The experimental design was developed to achieve comprehensive and valid results within a given period of time using available resources to address the initial research questions (see Chapter 1). The design included laboratory and field experiments in order

to provide information in a systematic manner by balancing scientific findings. Almost all of the laboratory experiments were conducted in the Soil Research Laboratory, School of Built and Natural Environment, University of Central Lancashire. The field experiments were conducted at selected Forestry Commission sites (Table 3.3.2). Most of the chemical analyses relating to laboratory and field experiments were conducted at the Forest Research laboratory, Alice Holt, Farnham. Table 3.4.1 summarises overall experimental design including SRF/earthworm species selected, location, timing and aims. Each individual experiment was planned with a broad understanding of relevant literature and with expert advice. When using novel/modified techniques, preliminary trials were used to assist development of the most appropriate methodology/technique.

**Table 3.4.1 Summary of the overall experimental design**

| Experiment | SRF species | Earthworm species | Type of experiment & location | Exp. duration & timing | Research Aims |
|---|---|---|---|---|---|
| 1. Earthworm Survey | Ad, Ah, Br, Eg, En, Sw, Sy + Control | All available earthworm species | Field<br>Alcan, Northumberland;<br>Daneshill, Nottinghamshire;<br>Gisburn, Lancashire;<br>Rogate, West Sussex. | 2 years<br>Spring 2010<br>Spring 2011 | • To investigate the effects of growing SRF in different land-use systems on earthworm community development. |
| 2. Earthworm growth and reproduction (I, II) | Ad, Ah, Br, En, Sw, Sy | *L. terrestris* hatchlings & adults | Laboratory<br>SBNE, UCLan | 7 months<br>Jan. 2011 to July 2011 | • To assess and compare the impact of SRF litter on growth/ reproduction of a major litter feeding earthworm. |
| 3. Earthworm food preference: Leaf litter choice chambers ( I, II, III) | Ad, Ah, Br, En, Sw, Sy, | *L. terrestris*, *A. longa* *A. caliginosa*, *A. chlorotica* | Laboratory<br>SBNE, UCLan | 6 months<br>Dec. 2010 to June 2011 | • To assess and compare the SRF litter preference by selected earthworm species. |

| | | | | | |
|---|---|---|---|---|---|
| 4. Earthworm food preference: Web cam recording (I, II, III) | Ad, Ah, Br, En, Sw, Sy, | *L. terrestris* | Laboratory<br>SBNE, UCLan | 3 months<br>Nov. 2011 to Feb. 2012 | • To record SRF litter selection behaviour by *L. terrestris.* |
| 5. Earthworm casting/nutrient cycling | Br, En, Sy | *L. terrestris* | Laboratory<br>SBNE, UCLan | 2 months<br>Sep. 2011 to Nov 2011 | • To measure and compare the influence of SRF litter on N cycling in earthworm cast. |
| 6. Field Litterbag studies (I, II) | Ah, Br, En, Sw, Sy, | All available earthworm species | Field<br>Alcan, Northumberland; Gisburn, Lancashire; Rogate, West Sussex; Carlshead, Yorkshire. | 1 year<br>Jan. 2011 to Jan. 2012 | • To investigate the effect of earthworms on SRF litter decomposition under field conditions. |
| 7. Field-based tree-earthworm experiment | Br, En | *L. terrestris*, *A. chlorotica* | Field<br>Headley nursery, Headley Down, Hampshire. | 1 year<br>May 2011 to May 2012 | • To record the direct interaction between SRF trees (En and Br) and earthworms under field condition. |

SRF species: *A glutinosa* (Ad), *F. excelsior* (Ah), *B. pendula* (Br), *E. gunnii* (Eg), *E. nitens* (En), *C. sativa* (Sw) and *A. pseudoplatanus* (Sy).

# CHAPTER 4: EFFECTS OF SRF SPECIES AND LITTER QUALITY ON EARTHWORM COMMUNITY DEVELOPMENT

## 4.1 Introduction

Tree species have a pronounced influence on soils and earthworm community development beneath, depending on the quality and quantity of litter produced (Muys *et al*., 1992; Zou, 1993). Studies (e.g. Pigott, 1989; Muys *et al*., 1992; Neirynck *et al*., 2000; Reich *et al*., 2005) suggested that growth of some forest tree species, such as *Alnus, Fraxinus* and *Tilia,* generally led to a higher earthworm density/diversity and more favourable physico-chemical top soil properties compared with *Quercus, Fagus* and *Pinus* (see section 2.5.2.1 for more details). These studies emphasised that this difference was mostly associated with tree litter quality rather than quantity.

Field surveys (soil plus earthworm sampling) have been widely used for temporal and spatial comparisons of influence of land-use change and tree development on soils and earthworms (e.g. Yates, 1988; Makeschin, 1994; Muys *et al*., 1992; Mboukou-Kimbatsa *et al*., 1998; Neirynck *et al*., 2000; Reich *et al*., 2005). In addition, some researchers have used different approaches, such as litterbags (Hendriksen, 1990) and laboratory microcosms (Butt, 2011), to investigate the influence of tree litter quality on earthworms and their activities.

A vital, but largely unidentified aspect of SRF species is the quality and quantity of litter they produce, and their impact on soils and soil fauna, of which the earthworm community is an important component. The aim of the present study was to investigate the direct and indirect influence of SRF species and litter quality on soil and earthworm

community development. Baseline field surveys at various Forestry Commission sites were used to investigate impacts of SRF growth on soil and earthworm development. It also allowed comparisons of various SRF plantations with appropriate control areas. In addition, two laboratory experiments were conducted to examine the direct influence of SRF litter quality on earthworm growth and reproduction.

This chapter initially presents the findings from soil and earthworm surveys undertaken at various Forestry Commission trial sites across England and then demonstrates the results of laboratory growth and reproduction experiments.

## 4.2 Impacts of SRF species on soil, earthworm density and diversity: A field survey

### 4.2.1 Introduction

SRF trees tend to be grown on lower-grade agricultural land, previously forested land or reclaimed land (McKay, 2011). Earthworms, which inhabit soils and organic matter layers in most ecosystems, are very sensitive to such land use transformations, since tree species and associated activities directly and indirectly change physical and chemical properties of the soil system. Because of their direct and relatively rapid response, earthworms are considered to be useful indicators in assessing the effects of land-use change on soil biodiversity and hence overall soil sustainability (Jouquet *et al*., 2006). Since they have a direct link with above-ground plants and animals, earthworms can be used as an important bio-indicator in assessing overall ecosystem sustainability (Paoletti, 1999) in forest ecosystems. Baseline soil and earthworm surveys at various SRF sites were conducted to achieve the following objectives:

a) To investigate the effects of growing SRF in different land-use systems on earthworm community development.
b) To assess and compare the impacts of selected SRF species on earthworm density and diversity.

### 4.2.2 Materials and Methods

#### 4.2.2.1 Survey sites

Earthworm surveys were conducted at four forestry sites across England. Selected sites consisted of monoculture plantations of two to three SRF species. Most of the selected tree species covered an area of 0.3 – 0.5 ha with an average planting density of 3,000 trees $ha^{-1}$. An appropriate adjacent control for each site was sampled for the purpose of direct comparison. Figures 4.2.1 – 4.2.4 show the original forest view at the sampling time. Table 4.2.1 summarises information relating to each of the sites.

**Table 4.2.1 Details of Forestry Commission SRF survey sites**

| Site | National Grid Ref. | SRF species | Mean annual ppt (mm) | Mean annual Temperature ( °C) | Min/Max Temperature ( °C) | Prior use | Year established |
|---|---|---|---|---|---|---|---|
| Alcan (Lynemouth, Northumberland) | NZ 291890 | En, Eg | 672 | 9.8 | 6.0/13.7 | Agriculture (arable) | 2004 |
| Daneshill (Retford, Nottinghamshire) | SK 680856 | En, Eg | 591 | 10.5 | 6.5/14.6 | Reclaimed (mining site) | 2005 |
| Rogate (West Sussex) | SU 791257 | Sw, Br | 639 | 11.2 | 7.1/15.3 | Pasture | Approx. 1990 |
| Gisburn (Lancashire) | SD 731566 | Br, Sy, Ah | 1321 | 9.7 | 6.2/13.2 | Mixed coniferous | Br, Sy (1995) Ah (approx.1980) |

SRF species: *A glutinosa* (Ad), *F. excelsior* (Ah), *B. pendula* (Br), *E. gunnii* (Eg) *E. nitens* (En), *C. sativa* (Sw) and *A. pseudoplatanus* (Sy).

(Details of rainfall and temperature adapted from Met Office, 2012).

**(a)** **(b)**

**Figure 4.2.1 Six years growth of *E. nitens*, Alcan, Northumberland (May 2010), (a) view from outside; (b) from within, showing a deep litter layer.**

**(a)** **(b)**

**Figure 4.2.2 Five years growth of *E. nitens*, Daneshill, Nottinghamshire (June 2010), (a) view from outside; (b) from within.**

**(a)** **(b)**

**Figure 4.2.3 Rogate site, West Sussex (April 2011), (a) *B. pendula*; (b) *C. sativa* (*C. sativa* had been coppiced once in 1995).**

**(a)** **(b)**

**Figure 4.2.4 *B. pendula*** at **Gisburn forest, Lancashire (April 2011), (a) tree stand; (b) forest floor view.**

#### 4.2.2.2 Earthworm sampling and identification

A combined method of collection that involved digging and hand-sorting of soil followed by application of a vermifuge was used throughout the survey (see Figure 4.2.5). Twenty, 0.1 $m^2$ quadrats were randomly located within 0.3 - 0.5 ha of each sampling site. The litter layer within the area, defined by the quadrat, was hand-sorted for litter-dwelling earthworms and then the soil below was dug to a depth of 0.2 m. The removed soil was hand-sorted for earthworms on a plastic sheet in the field. A mustard vermifuge (50 g mustard powder suspended in 10 L water) was applied to the soil pit created (Butt, 2000).


**(a)** **(b)** **(c)**

**Figure 4.2.5 Earthworm sampling procedure: (a) 0.1 $m^2$ quadrat placed on the ground, litter removed and sorted; (b) Soil dug to 0.2 m and hand-sorted, note large earthworm burrows (bw); (c) Mustard vermifuge (50 g in 10 L water) applied.**

Collected earthworms from each sampling point were preserved in 4% formaldehyde within 150 ml plastic bottles and taken to the laboratory. Specimens were indentified to species level, using the nomenclature of Sims and Gerard (1999). Individual earthworms were allocated as juvenile or adult (fully clitellate) and numbers/masses

were recorded. Earthworm density and biomass were calculated for each habitat sampled. All earthworm surveys were conducted in spring; Alcan (May 2010), Daneshill (June 2010), Rogate and Gisburn (April 2011).

#### 4.2.2.3 Soil sampling and analysis

Soil samples were taken from each site simultaneously with earthworm sampling. The surface organic matter layer was removed and soil samples were dug from a depth of 0 - 0.2 m using a trowel. Samples were placed in polythene bags, sealed and transported to the laboratory. Soil moisture was immediately measured from fresh soils by drying at 105 $^{o}$C. Further samples were air-dried to a constant weight and passed through a 2 mm sieve for chemical analysis. Standard procedures used in the Forest Research laboratory were followed for soil chemical analysis (see section 3.3.5 for details).

#### 4.2.2.4 Statistical analysis

Statistical analyses on experimental data were performed using the statistical software package Minitab 16. One way analysis of variance (ANOVA) was used to test the effect of tree species on soil properties, earthworm densities and biomass at the same site. If an assumption of ANOVA was violated with a valid reason, the regular analysis and statistical significances were confirmed with a Kruskal–Wallis test which was robust for the situation. Where appropriate, a Tukey-Kramer multiple comparison test was applied for all of the pair-wise comparisons.

### 4.2.3 Results

#### 4.2.3.1 Alcan

Table 4.2.2 shows the soil and earthworm survey results of three different habitats at the Alcan site. Soil moisture (%) was significantly higher ($p < 0.05$) under *E. nitens*. Soil organic matter, total C as well as CEC showed a slight increase under tree plantations compared with the arable control, although the difference was not significant ($p > 0.05$). Soil pH showed a slight decrease (0.4 units) under both tree plantations compared with control. Mean earthworm density was significantly higher ($p < 0.05$) under *E. nitens* (152 $m^{-2}$) compared with *E. gunnii* or control (47 and 51 $m^{-2}$ respectively). Mean earthworm biomass was significantly higher ($p < 0.05$) under *E. nitens* compared with control, but not when compared with *E. gunnii*.

**Table 4.2.2 Soil properties of selected habitats at the Alcan site (mean ± se, n = 4), plus earthworm community measurements (mean ± se, n = 20)**

| | *E. nitens* | *E. gunnii* | Arable Control |
|---|---|---|---|
| **Soil properties** | | | |
| Soil Moisture (%) | 18.1 ± 0.2[a] | 14.9 ± 0.1[b] | 14.4 ± 0.5[b] |
| Soil pH ($H_2O$) | 6.6 ± 0.1 [a] | 6.6 ± 0.3 [a] | 7.0 ± 0.1 [a] |
| Organic Matter (%) | 8.0 ± 0.6 [a] | 8.0 ± 0.2 [a] | 7.0 ± 0.2 [a] |
| Total N (%) | 0.2 ± 0.0 [a] | 0.2 ± 0.0 [a] | 0.2 ± 0.0 [a] |
| Total C (%) | 3.7 ± 0.2 [a] | 3.5 ± 0.1 [a] | 3.2 ± 0.1 [a] |
| CEC (cmol+/kg) | 15.1 ± 0.9 [a] | 13.8 ± 1.7 [a] | 12.3 ± 0.7 [a] |
| BS (%) | 98 ± 1.5 [a] | 98 ± 0.9 [a] | 99 ± 0.7 [a] |
| **Earthworm measurements** | | | |
| Density (No. $m^{-2}$) | 152 ± 17.1[a] | 47 ± 6.5[b] | 51 ± 12[b] |
| Biomass (g $m^{-2}$) | 89.7 ± 13.8 [a] | 65.7 ± 10.9 [ab] | 49.3 ± 9.4[b] |

Different letters in a row indicate significant difference between habitats (ANOVA, Tukey-Kramer test, $p < 0.05$).

In total, eight earthworm species were recorded at the Alcan site; two anecic, four endogeic, and two epigeic species. Each of the different plantations contained at least seven species of earthworms, although their compositions were slightly variable (Table 4.2.3). As shown in Figure 4.2.6, endogeic and epigeic earthworm numbers were significantly higher ($p < 0.05$) under *E. nitens* compared to both adjacent habitats. However, anecic earthworm numbers were not significantly different ($p > 0.05$) between habitats. *L. terrestris*, *A. rosea*, *A. caliginosa*, *A. chlorotica*, and *L. castaneus* were the dominant species present below *E. nitens*. *E gunnii* recorded a lower number of epigeic and endogeic earthworms (Figure 4.2.6). *L. terrestris* was the most dominant species found under *E. gunnii*. *A. chlorotica* and *L. terrestris* were the main species present in the arable control. However, the number of *L. terrestris* under both tree

plantations was twice that compared with the arable control plot (Table 4.2.3). The epigeic earthworm *L. festivus* was recorded only within the arable control area.

**Table 4.2.3 Details of Earthworm species within selected habitats at the Alcan site**

| **Earthworm species** | **Ecological grouping** | **Below *E. nitens*** | | **Below *E. gunnii*** | | **Arable Control** | |
|---|---|---|---|---|---|---|---|
| | | **No $m^{-2}$** | **g $m^{-2}$** | **No $m^{-2}$** | **g $m^{-2}$** | **No $m^{-2}$** | **g $m^{-2}$** |
| *Allolobophora chlorotica* | Endogeic | 36 | 4.2 | 1.5 | 0.2 | 23 | 4.4 |
| *Aporrectodea caliginosa* | Endogeic | 17.5 | 3.9 | 3.5 | 0.7 | 0.0 | 0.0 |
| *Aporrectodea longa* | Anecic | 1.0 | 2.0 | 1.0 | 0.1 | 3.0 | 5.3 |
| *Aporrectodea rosea* | Endogeic | 36 | 4.6 | 7.0 | 0.8 | 0.5 | 0.1 |
| *Lumbricus castaneus* | Epigeic | 8.5 | 1.3 | 2.0 | 0.3 | 1.0 | 0.1 |
| *Lumbricus festivus* | Epigeic | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 1.2 |
| *Lumbricus terrestris* | Anecic | 22 | 63.5 | 22.5 | 60.5 | 10 | 31.6 |
| *Octolasion cyaneum* | Endogeic | 4.0 | 4.4 | 1.5 | 1.5 | 2.5 | 4.0 |
| Juvenile *Lumbricus* species | N/A | 27.5 | 5.8 | 8 | 1.8 | 10 | 2.5 |
| **Total species count** | | **7** | | **7** | | **7** | |

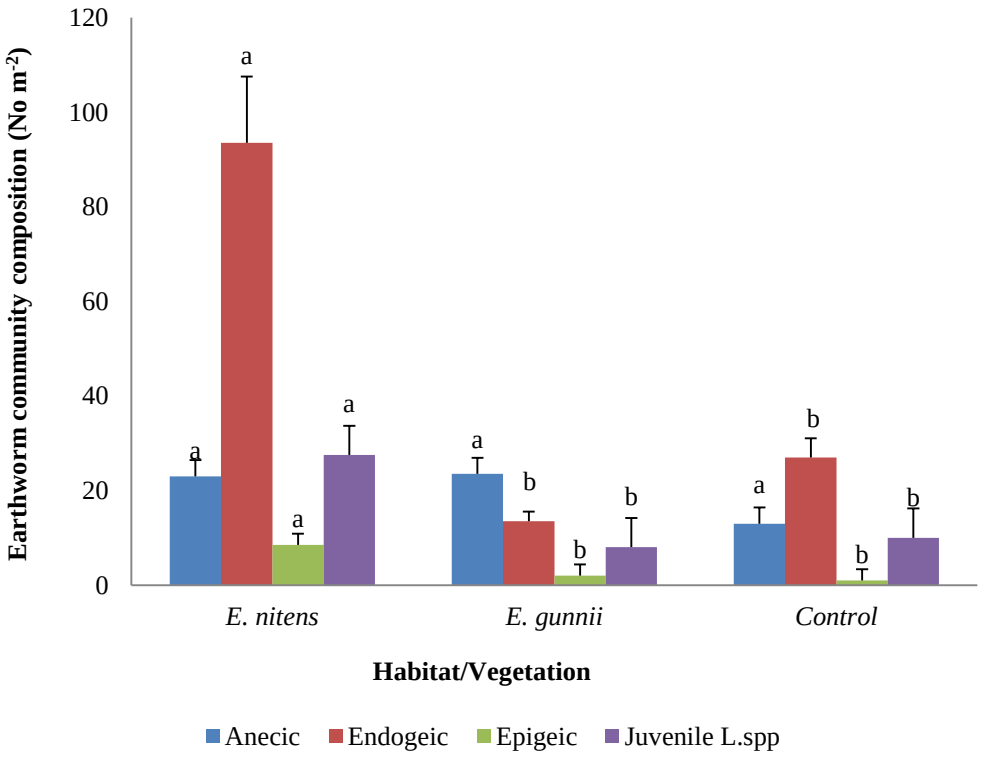



**Figure 4.2.6 Mean earthworm community composition within selected habitats at the Alcan site. Bars representing different ecological groups (same colour) that share the same letter are not significantly different (p > 0.05, Tukey-Kramer test). Error bars represent standard error of the mean.**

### 4.2.3.2 Daneshill

Table 4.2.4 shows the soil and earthworm survey results of selected habitats at the Daneshill site. Both tree plantations established on man-made sandy soil recorded significantly lower ($p < 0.05$) soil moisture (%), total N (%), total C (%) and CEC compared with adjacent pasture control. Soil pH was lowest under *E. nitens*. The total and earthworm densities and biomasses were not significantly different ($p > 0.05$) between habitat types in this study area.

**Table 4.2.4 Soil properties of selected habitats at Daneshill (mean ± se, n = 4), plus earthworm community measurements (mean ± se, n = 20)**

| | *E. nitens* | *E. gunnii* | Pasture Control |
|---|---|---|---|
| **Soil properties** | | | |
| Soil Moisture (%) | 12.1 ± 0.6 [a] | 12.0 ± 0.7 [a] | 17.9 ± 0.1 [b] |
| Soil pH ($H_2O$) | 5.4 ± 0.3 [b] | 6.2 ± 0.1 [ab] | 6.9 ± 0.2 [a] |
| Organic Matter (%) | 5.4 ± 0.7 [a] | 4.9 ± 0.2 [a] | 6.3 ± 0.3 [a] |
| Total N (%) | 0.16 ± 0.0 [b] | 0.17 ± 0.0 [b] | 0.21 ± 0.0 [a] |
| Total C (%) | 2.0 ± 0.2 [b] | 2.1 ± 0.1 [b] | 2.7 ± 0.1 [a] |
| CEC (cmol+/kg) | 5.9 ± 1.0 [b] | 8.2 ± 0.6 [b] | 11.8 ± 1.0 [a] |
| BS (%) | 88 ± 0.6 [a] | 98 ± 0.2 [a] | 98 ± 0.1 [a] |
| **Earthworm measurements** | | | |
| Density (No. $m^{-2}$) | 40 ± 7.5[a] | 47 ± 8.2 [a] | 55 ± 9.8 [a] |
| Biomass (g $m^{-2}$) | 25.1 ± 4.3 [a] | 22.2 ± 4.8 [a] | 24.7 ± 5.3 [a] |

Different letters in a row indicate significant difference between habitats (ANOVA, Tukey-Kramer test, $p < 0.05$).

In total, six earthworm species were recorded within the Daneshill site; two anecic, three endogeic and one epigeic species. Each of the different plantations contained at least five species of earthworm, although their composition differed (Table 4.2.4 and Figure 4.2.7). The dominant earthworm species under *E. nitens* was *A. caliginosa* (25.5 $m^{-2}$), whilst *A. rosea* was abundant (34.5 $m^{-2}$) in the control. Both of these endogeic earthworm species (*A. rosea* and *A. caliginosa*) were present in similar numbers under *E. gunnii* at densities of 19.0 and 15.5 $m^{-2}$ respectively.

**Table 4.2.5 Details of earthworm species within selected habitats at Daneshill**

| Earthworm species | *E. nitens* | | *E. gunnii* | | Pasture Control | |
|---|---|---|---|---|---|---|
| | No $m^{-2}$ | g $m^{-2}$ | No $m^{-2}$ | g $m^{-2}$ | No $m^{-2}$ | g $m^{-2}$ |
| *A. caliginosa* | 25.5 | 8.5 | 15.5 | 5.3 | 5.0 | 1.1 |
| *A. longa* | 2.5 | 2.8 | 4.5 | 2.1 | 6.0 | 4.2 |
| *A. rosea* | 2.5 | 0.3 | 19.0 | 3.5 | 34.5 | 5.1 |
| *L. castaneus* | 1.0 | 0.2 | 0.0 | 0.0 | 1.0 | 0.1 |
| *L. terrestris* | 4.5 | 12.2 | 4.5 | 10.1 | 7.0 | 13.9 |
| *O. cyaneum* | 0.5 | 0.7 | 1.5 | 0.7 | 0.0 | 0.0 |
| Juvenile *Lumbricus* spp | 3.5 | 0.4 | 3.0 | 0.5 | 1.5 | 0.2 |
| **Total species count** | **6** | | **5** | | **5** | |

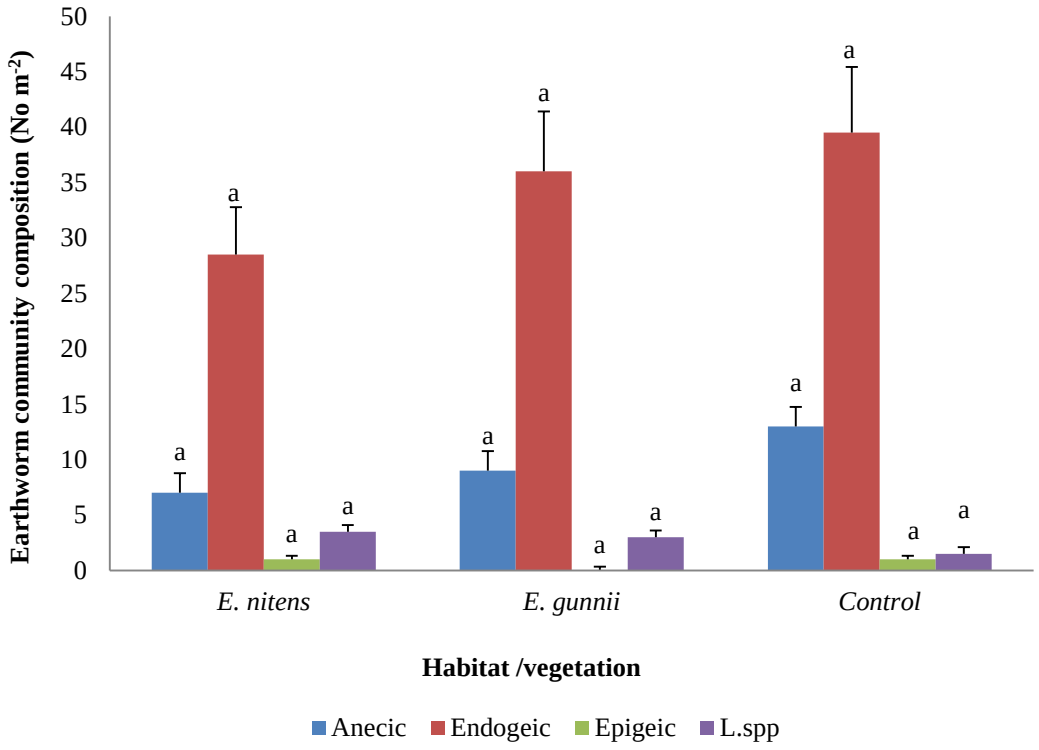



**Figure 4.2.7 Mean earthworm community composition within selected habitats at the Daneshill site. Bars representing different ecological groups (same colour) that share the same letter are not significantly different (p > 0.05, Tukey-Kramer test). Error bars represent standard error of the mean.**

#### 4.2.3.3 Rogate

Table 4.2.6 shows the soil and earthworm results of selected habitats at the Rogate site. Soil moisture and organic matter (%) were significantly higher ($p < 0.05$) under *C. sativa* compared with both *B. pendula* and pasture control. Total C (%) and CEC were slightly increased under *C. sativa*, although these were not statistically significant ($p > 0.05$). Soil pH was significantly lower ($p < 0.05$) under *C. sativa* and it was almost one unit lower than control and 0.9 units lower than under *B. pendula*. The highest earthworm density was recorded under *C. sativa* (23 $m^{-2}$) while highest earthworm biomass was recorded under *B. pendula* (6.0 g $m^{-2}$). Lowest density of earthworms was recorded in control soil which was an adjacent pasture plot. However, the mean earthworm densities and biomass were not significantly different ($p > 0.05$) between habitat types considered in the study area.

**Table 4.2.6 Soil properties of selected habitat at the Rogate site (mean ± se, n = 3), plus earthworm community measurements (mean ± se, n = 10)**

| | *C. sativa* | *B. pendula* | Pasture Control |
|---|---|---|---|
| **Soil properties** | | | |
| Soil Moisture (%) | 18.0 ± 2.2 [a] | 9.4 ± 1.3[ab] | 5.4 ± 0.7 [b] |
| Soil pH ($H_2O$) | 4.1 ± 0.1 [a] | 5.0 ± 0.1 [b] | 5.1 ± 0.1 [b] |
| Organic Matter (%) | 9.4 ± 4.0 [a] | 4.1 ± 0.8 [b] | 3.2 ± 0.1 [b] |
| Total N (%) | 0.1 ± 0.0 [a] | 0.1 ± 0.0 [a] | 0.1 ± 0.0 [a] |
| Total C (%) | 2.8 ± 1.1 [a] | 1.7 ± 0.3 [a] | 1.5 ± 0.1 [a] |
| CEC (cmol+/kg) | 3.1 ± 1.4 [a] | 1.9 ± 0.3 [a] | 1.8 ± 0.1 [a] |
| BS (%) | 47 ± 5.8 [a] | 56 ± 6.0 [a] | 68 ± 3.8 [a] |
| **Earthworm measurements** | | | |
| Density (No. $m^{-2}$) | 23 ± 3.6 **[a]** | 15 ± 5.0 **[a]** | 12 ± 6.6 **[a]** |
| Biomass (g $m^{-2}$) | 3.7 ± 1.4 **[a]** | 6.0 ± 2.4 **[a]** | 3.4 ± 1.8 **[a]** |

Different letters in a row indicate significant difference between habitats (ANOVA, Tukey-Kramer test, $p < 0.05$)

This sandy soil site recorded a relatively low density, in addition to a low diversity of earthworms. Only three epigeic earthworm species; *L. rubellus*, *L. castaneus* and *Dendrobaena octaedra* were recorded within the site. Each of the different plantation soils contained at least two species of earthworms (Table 4.2.7). Anecic or endogeic earthworms were not recorded in any habitat at Rogate. *D. octaedra* had a distribution restricted to *C. sativa*.

**Table.4.2.7 Details of earthworm species under different habitat at the Rogate site**

| **Earthworm species** | **Ecological grouping** | ***C. sativa*** | | ***B. pendula*** | | **Pasture Control** | |
|---|---|---|---|---|---|---|---|
| | | **No $m^{-2}$** | **g $m^{-2}$** | **No $m^{-2}$** | **g $m^{-2}$** | **No $m^{-2}$** | **g $m^{-2}$** |
| *D. octaedra* | Epigeic | 16 | 1.0 | 0.0 | 00. | 0.0 | 0.0 |
| *L. rubellus* | Epigeic | 1.0 | 1.0 | 2.0 | 1.7 | 1.0 | 0.9 |
| *L. castaneus* | Epigeic | 3.0 | 0.3 | 2.0 | 0.2 | 1.0 | 0.1 |
| Juvenile *L*. spp | N/A | 3.0 | 1.3 | 11.0 | 4.1 | 10.0 | 2.4 |
| **Total species count** | | **3** | | **2** | | **2** | |

### 4.2.3.4 Gisburn Forest

Table 4.2.8 shows the soil and earthworm results between different tree stands at Gisburn Forest. The lowest soil pH (3.9) and base saturation (16.4%) was recorded under *B. pendula.* Similarly derived *A. pseudoplatanus* recorded slightly increased soil pH (4.8) than *B. pendula*. Most of the soil properties, especially pH, CEC and BS were higher under *F. excelsior*. Earthworm density was significantly higher ($p < 0.05$) below *F. excelsior* compared with both *B. pendula* and *A. pseudoplatanus*. Both earthworm density and biomass were significantly lower ($p < 0.05$) under *B. pendula.*

**Table 4.2.8 Soil properties of selected habitats at the Gisburn Forest (mean ± se, n = 3), plus earthworm community measurements (mean ± se, n = 5)**

| | *F. excelsior* | *B. pendula* | *A. pseudoplatanus* |
|---|---|---|---|
| **Soil properties** | | | |
| Soil Moisture (%) | 32.6 ± 0.5 [a] | 34.9 ± 1.0 [a] | 35.2 ± 0.1 [a] |
| Soil pH ($H_2O$) | 5.8 ± 0.1 [a] | 3.9 ± 0.1 [c] | 4.8 ± 0.1 [b] |
| Organic Matter (%) | 10.0 ± 0.5 [b] | 10.2 ± 0.2 [b] | 12.0 ± 0.2 [a] |
| Total N (%) | 0.3 ± 0.0 [a] | 0.3 ± 0.0 [a] | 0.3 ± 0.0 [a] |
| Total C (%) | 4.0 ± 0.1 [a] | 4.2 ± 0.3 [a] | 4.5 ± 0.4 [a] |
| CEC (cmol+/kg) | 14.1 ± 1 [a] | 7.4 ± 0.5 [a] | 7.8 ± 0.2 [a] |
| BS (%) | 99 ± 2.1 [a] | 16 ± 2.2 [c] | 67 ± 3.8 [b] |
| **Earthworm measurements** | | | |
| Density (No. $m^{-2}$) | 66 ± 11.2 [a] | 6 ± 2.4 [b] | 26 ± 5.1 [b] |
| Biomass (g $m^{-2}$) | 13.9 ± 3.6 [a] | 0.9 ± 0.3 [b] | 8.7 ± 2.4 [ab] |

Different letters in a row indicate significant difference between habitats (ANOVA, Tukey-Kramer test, $p < 0.05$)

As shown in Table 4.2.9, a total of six earthworm species were found at the Gisburn Forest site and consisted of four endogeic and two epigeic species. Anecic earthworms were not recorded under any of the tree stands (Figure 4.2.8). A greater number of earthworm species (5) were found below *F. excelsior* while only (one) earthworm species (*L. castaneus*) was found below *B. pendula.* The major earthworm species recorded under *F. excelsior* were *A. rosea, A. caliginosa* and *L. castaneus* while *A. chlorotica* was recorded at high densities under *A. pseudoplatanus.*

**Table 4.2.9 Details of earthworm species in selected habitats at Gisburn forest**

| Earthworm sppecies | *F. excelsior* | | *B. pendula* | | *A. pseudoplatanus* | |
|---|---|---|---|---|---|---|
| | No $m^{-2}$ | g $m^{-2}$ | No $m^{-2}$ | g $m^{-2}$ | No $m^{-2}$ | g $m^{-2}$ |
| *A. caliginosa* | 8.0 | 2.7 | 0.0 | 0.0 | 0.0 | 0.0 |
| *A. chlorotica* | 2.0 | 0.3 | 0.0 | 0.0 | 14.0 | 3.1 |
| *A. rosea* | 38.0 | 6.3 | 0.0 | 0.0 | 6.0 | 1.0 |
| *L. castaneus* | 12.0 | 2.3 | 6.0 | 0.9 | 2.0 | 0.7 |
| *L. rubellus* | 0.0 | 0.0 | 0.0 | 0.0 | 2.0 | 2.5 |
| *O. cyaneum* | 2.0 | 1.9 | 0.0 | 0.0 | 0.0 | 0.0 |
| Juvenile L. spp | 4.0 | 0.4 | 0.0 | 0.0 | 2.0 | 1.5 |
| **Total species count** | **5** | | **1** | | **4** | |

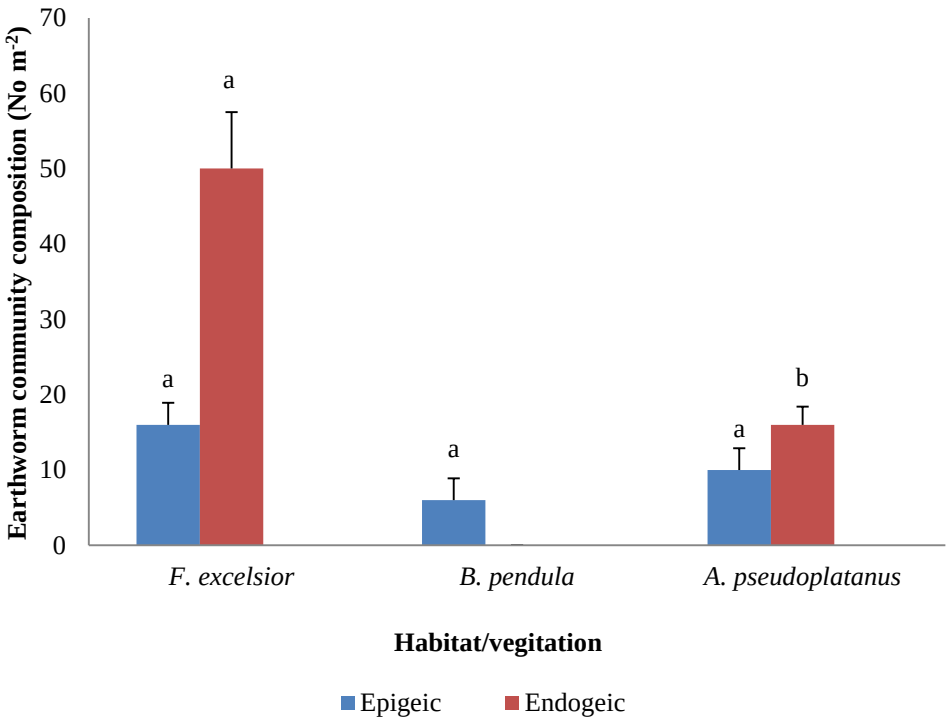



**Figure 4.2.8 Mean earthworm community composition within different habitats at the Gisburn Forest site. Bars representing different ecological groups (same colour) that share the same letter are not significantly different (p > 0.05, Tukey-Kramer test). Error bars represent standard error of the mean.**

### 4.2.4 Discussion

#### 4.2.4.1 Alcan

Earthworm survey results at the Alcan site suggest that conversion from arable to SRF can increase the earthworm density depending on tree species planted. At the study site, six years of *E. nitens* development on former arable loamy soil had increased earthworm density three fold compared to the unplanted control. However, parallel *E. gunnii* development had not significantly changed total earthworm density. In the same soil, under similar climatic condition, two eucalyptus species had different influences on earthworm community development. Soil survey results suggested that under *E. nitens*, soil moisture was enhanced, possibly due to a deeper litter layer accumulated below this species (Figure 4.2.9).

The differences in earthworm occurrence and soil analyses may be associated with the level of soil disturbance (Makeschin, 1994), quality and quantity of litter produced (Muys *et al*., 1992; Reich *et al*., 2005) and canopy density. The dense canopy and thick litter layer of *E. nitens* (Figure 4.2.9) provided a more suitable habitat for epigeic, endogeic and anecic earthworms (see Table 2.3.1), directly through food provision and indirectly through its positive effect on soil moisture. *E. gunnii* consisted of a relatively light canopy, which resulted in less amounts of litter deposited on the soil surface (Figure 4.2.9). This may be the reason for low soil moisture content recorded underneath this plantation. The inter-connected factors of light canopy density, less litter quantity, and low soil moisture below *E. gunnii* negatively affected litter-dwelling epigeic and shallow-working endogeic earthworm populations. However, results suggest that this impact was minimal for deep burrowing earthworms. Compared with

the arable control, fewer disturbances and increased food provision had positively affected the anecic *L. terrestris* population development under both tree species.

(a) (b) (c)

**Figure 4.2.9 Litter cover on the soil surface within selected habitat at Alcan (May 2010); Beloe (a) *E. nitens*, (b) *E. gunnii*, (c) Arable control.**

In Germany, Makeschin, (1994) studied the effects of energy forestry (SRC + SRF) on former arable soils and suggested that soil faunal density and diversity increased under tree plantations compared with arable land due to less frequent soil disturbances, leaf litter accumulation and reduced chemical application. Current work suggests that the effects of tree plantation on earthworm density and diversity can vary with tree species which concurs with Muys *et al*. (1992). Similar to the present soil survey findings, several studies have suggested an increase of soil organic matter and carbon content under tree plantations on former arable soil (e.g. Tolbert and Wright, 1998; Vanguelova and Pitman, 2011). Decreases in pH under SRF (planted on previously arable soils) have been recorded by Jug *et al.* (1999) in Germany and by Alriksson and Olsen (1995) in Sweden.

The effects of eucalyptus species on the soil have focused on allelopathy caused by its litter, which affects soil microorganisms and germination/growth of other plants (Cao *et al*., 2010; Zhang *et al*., 2010). Mboukou-Kimbatsa *et al*. (1998) studied the changes in

soil fauna when fast growing trees were planted on savanna soils in southern Congo and suggested that eucalyptus (a natural hybrid between *E. alba* and another undetermined parent) had no negative effects on earthworm development in a clay loam soil. The present work also suggests that *E. nitens* and *E. gunnii* had no negative effects on earthworm community development in a loamy soil (after 6 years).

#### 4.2.4.2 Daneshill

Earthworm survey results at the Daneshill site suggest that short-term *E. nitens* and *E. gunnii* growth on a poor, reclaimed (mineral extraction, backfilling and reforestation) site can support earthworm community development. After five years, earthworm densities and biomass under both tree plantations were not significantly different from adjacent pasture land. Increased provision of organic matter through growing trees (with litter fall and rooting) may be one of the factors to attract earthworms to this reclaimed site.

However, soil analyses suggest that most of the general soil properties such as soil moisture, soil pH, total C, total N, and CEC were slightly lower under tree plantations compared with pasture. In general, pasture lands are chemically and biologically rich systems and earthworms are a major component in these systems (Bouché, 1977). Butt (2000) recorded an earthworm density of (291 $m^{-2}$) and biomass of (86 g $m^{-2}$) in alkaline pasture in the Yorkshire Dales, and Butt *et al*. (2008) recorded an earthworm density of (310 $m^{-2}$) and biomass of (149.6 g $m^{-2}$) from equally alkaline loamy pasture in Kent. However, compared with these UK pasture sites, the Daneshill pasture site, which consisted of relatively poor sandy soil, supported a relatively low earthworm density

and biomass (55 $m^{-2}$ and 24. 7 g $m^{-2}$ respectively). This suggests that soil type can directly influence earthworm occurrence, in addition to the vegetation type.

Hendrychová, (2008) suggested that reforestation of reclamation sites may have a great potential to enable early colonisation of organisms from the surrounding landscapes and to support an increase in biodiversity. According to Hendrychová (2008), faster development of the tree layer and closure of the tree canopy is a basic aspect of forest reclamation and therefore, fast growing tree species have received much attention for use in land reclamation. Frouz (2006) compared development of humus and fermentation layers in plots reclaimed by planting alder (*Alnus glutinosa*), lime (*Tilia cordata*), oak (*Quercus robur*), larch (*Larix decidua*), pine (a mixture of *Pinus silvestris* and *P. nigra*) and spruce (a mixture of *Picea omorica* and *P. pungens*). The most rapid development of fermentation and humus layers of soil was found under alder and lime plantations. Larch also supported rapid soil development, whereas slower soil development was found under spruce and pine plantations. In alder plantations, macrofauna was more abundant, dominated by Diptera larvae, Diplopoda and earthworms. This study suggested that presence of earthworms resulted in more intensive soil mixing, which appears in rapid formation of a humus layer. It further emphasised that selection of appropriate forest species is important in land reclamation for rapid colonisation of soil fauna and hence overall soil development.

Mboukou-Kimbatsa *et al*. (1998) studied the change in soil macrofauna, including earthworms, when fast growing trees were planted in savanna soils in southern Congo and recorded that biomass of soil macrofauna was very low in both sandy and clay savanna soils, total biomass being 3.3 and 5.8 g $m^{-2}$ respectively. However, soil macrofauna biomass reached 29 g $m^{-2}$ in the 20 year Eucalyptus plots on sandy soils and

74 g $m^{-2}$ in 26 years old eucalyptus plantation on clay soils. The study suggested that there was a correlation between plant age and soil macrofauna biomass and that soil type had a clear influence on macrofauna development. The study recorded that under eucalyptus, changes in C content and the development of soil macrofauna occurred 7-10 years after planting. The current study suggests that earthworm community development under both *E. nitens* and *E. gunnii* and density/diversity was at the level of adjacent pasture land after 5 years. This shows that after this period of time a negative influence has not been recorded. However, future studies are required at this site to investigate the influence of plant ageing on earthworm populations, to see if an enhancement of macrofaunal community results.

#### 4.2.4.3 Rogate

The results suggest that over 20 years of *C. sativa* or *B. pendula* growth on less fertile sandy soil has not significantly changed total earthworm density or biomass, compared with an adjacent pasture control. However, earthworm species composition had slightly changed between habitats although it was totally restricted to the epigeic category. Earthworm density had slightly increased under *C. sativa,* mostly due to *D. octaedra* while earthworm biomass was slightly increased under *B. pendula,* mostly due to *L. rubellus.* The soil survey suggested that soil pH was considerably decreased under *C. sativa* (4.1) while soil moisture, organic matter, total C, total N and even CEC were slightly increased. Acid tolerant, *D. octaedra,* which was restricted to the *C. sativa* plantation, are generally associated with soils having high organic content such as peat, wet moorland, forested and acid hill pastures (Sims and Gerard, 1999). It is noteworthy that all the epigeic earthworms (*L. rubellus*, *L. castaneus* and *D. octaedra*) found at the Rogate site are acid-tolerant species (Sims and Gerard, 1999).

Yates (1988) reported a significant reduction in earthworm biomass and soil pH after 13 years under pine (*Pinus radiata*) plantations which were established on New Zealand grassland. Muys *et al.* (1992) recorded significant differences in earthworm biomass, community structure and even soil pH after 20 years of forest development (*Q. palustris, T. platyphyllos, P. avian, A. glutinosa, F. excelsior*) on a former grassland sandy soil in Belgium. These authors recorded a diminished earthworm biomass under oak (*Q. palustris*) (see section 2.5.2.1 for details). As previously mentioned, pasture lands are chemically and biologically rich systems (Bouché, 1977) and growing of trees generally leads to soil acidification and a reduction in earthworms. But similar to Muys *et al*. (1992), the current study at the Rogate site suggested that this could be controlled by selection of appropriate tree species.

#### 4.2.4.4 Gisburn Forest

The *B. pendula* plantation showed the lowest number of earthworms, although its litter is reported to generally support earthworm feeding (Satchell and Lowe, 1967), growth and reproduction (Butt, 2011). Considerably low soil pH (3.9) and low base saturation (16.4%) under *B. pendula* may have negatively affected earthworm development. *A. pseudoplatanus,* with relatively improved soil pH and base saturation, recorded moderate earthworm density and diversity. The highest earthworm density and diversity was recorded under *F. excelsior* where soil pH, CEC and base saturation were high. The soil status, especially low pH, which was likely to have been associated with previous conifer establishment, had greatly influenced earthworm community development under these plantations. Similar to the findings of this study, Muys *et al*. (1992) suggested that *F. excelsior* is one of the forest species which supports earthworm development. Satchell (1980) studied earthworm development under *B. pendula,* planted on the soil of

a podzolized heather moor in North Yorkshire and concluded that after 20 years, *B. pendula* had not materially improved the site as an earthworm habitat. Rati and Huhta (2004) studied the earthworm communities in 30 year old *B. pendula* stands in Finland, established on former coniferous plantation and on arable soil. They recorded that earthworm diversity and density were lower under *B. pendula* established on an ex-coniferous stand than on arable soil.

Major findings of the earthworm survey:

- The baseline survey at the Alcan site showed that conversion from arable to eucalyptus can increase earthworm density and diversity, but this may be a function of reduced tillage, in addition to tree planting.
- The survey at Daneshill demonstrated that short-term (five years) eucalyptus growth on a poor reclaimed site can support earthworm community development similar to adjacent pasture land.
- The survey at selected forest sites suggested that species of SRF trees exhibit species-specific effects on earthworm community development.
- Overall survey results suggested that the effect of SRF trees on earthworms is a complex process which depends not only on tree species, but also on tree age, soil type and land-use history.

Further studies are recommended to confirm interactive effects of SRF species, tree age, soil types, land-use history and local climate on earthworm community development.

## 4.3 Effects of SRF species litter on growth and reproduction of *L. terrestris* under controlled environmental condition

### 4.3.1 Introduction

Tree litter quality is one of the key components which influence earthworm community development in many terrestrial ecosystems, since it has a direct impact on feeding (Satchell and Lowe, 1967), growth and reproductive success of earthworms (Butt, 2011). To examine this with respect to SRF species, two experiments were conducted under controlled environmental conditions with the litter-feeding earthworm *Lumbricus terrestris*. For the current study, *L. terrestris* was selected as it was one of the most abundant earthworm species found within SRF sites (see earthworm survey results in Table 4.2.3). According to the literature, *L. terrestris* are widely distributed in UK forests and make a great contribution to tree litter decomposition (see section 2.3.2.1 for details). These set out:

a) To assess and compare the effect of SRF litter on earthworm growth.
b) To assess and compare the effect of SRF litter on earthworm reproduction.

### 4.3.2 Materials and Methods

Two separate experiments were conducted to examine (1) growth and (2) reproduction of *L. terrestris*. For each experiment, six SRF litter treatments; *A. glutinosa* (Ad), *F. excelsior* (Ah), *B. pendula* (Br), *E. nitens* (En) *C. sativa* (Sw) and *A. pseudoplatanus* (Sy) were used. Standard sterilised Kettering loam (see Table 3.3.5 for details) with a moisture content of 25% was utilised as a substrate for earthworms and sealed opaque

plastic containers (Lakeland Plastics Limited, UK) with ventilation holes in lids (made with a mounted needle) were used as experimental vessels. These vessels were maintained in darkness at 15 °C in temperature-controlled incubators (Lowe and Butt, 2005).

#### 4.3.2.1 Growth

Experimental *L. terrestris* hatchlings were collected from laboratory-produced cocoons which had been incubated at 15 °C. Emerging hatchlings were collected on a daily basis and kept in a sealed plastic vessel of water (with a few small ventilation holes in the lid) at 5 °C, until the required number was gathered. The experiment began in January 2011, with hatchlings of mean individual mass of 0.06 g. Plastic vessels of 0.4 L (depth 0.04 m) were filled with 0.3 L of moist soil and a single hatchling was introduced into each vessel. As hatchings are unable to consume whole plant leaves, they were fed with previously collected and air-dried SRF litter that was ground (MAGIMIX 4150W food processor) and passed through a 2 mm sieve before feeding to prevent any influence of particle size on earthworm growth. A known amount of sieved litter from each tree species was soaked in water for 10 minutes and surface applied to experimental vessels. Initially, the hatchlings were fed with 2 g (dry basis) of litter individual$^{-1}$ 4 weeks$^{-1}$, which from sixteen weeks onwards was increased to 4 g individual$^{-1}$ 4 weeks $^{-1}$.

Experimental vessels were examined every four weeks and earthworm survival, mass and their development stages were recorded before each one was returned to its vessel. After each four week interval, soils were replenished. Four replicates per treatment were maintained and the experiment was terminated after 28 weeks (when one treatment had recorded 100% clitellate animals). Earthworm masses after 28 weeks were used for

direct comparison of treatments using the Tukey-Kramer multi-comparison one way analysis of variance (ANOVA).

#### 4.3.2.2 Reproduction

Field-collected *L. terrestris* adults (n = 40) were acclimated to laboratory conditions for four weeks (4 adults in 2 L vessels). A mixture of experimental SRF leaves was supplied to ensure that no pre-conditioning occurred with respect to litter (Butt, 2011). Earthworms (mean individual mass 5.15 g) were randomly assigned as 12 pairs and each pair kept in a 0.75 L vessel (depth 0.1 m). Six litter species were used separately as feed treatments, so that two pairs were fed with each experimental litter species. A known amount of air-dried whole leaves (2 g adult$^{-1}$ 4 weeks$^{-1}$) was soaked with water for 20 minutes and surface applied to experimental vessels. This set up continued for four weeks to ensure adequate mating opportunity. After four weeks, each pair was separated and animals were introduced to individually coded 0.75 L vessels and fed, as before, with the same litter species (n = 4 per litter species). Experimental vessels were examined every four weeks. At sampling, any litter remaining at the soil surface was collected and air-dried for weighing, to allow earthworm litter removal calculations. Earthworm survival, and mass changes were recorded and they were re-housed in fresh soils and fed as before. Soil removed from vessels on a four-weekly basis was wet-sieved through a series of 6.7, 4.0 and 2.8 mm meshes for collection of cocoons (Butt *et al*., 1994). The experiment was started in December 2010 and completed after 16 weeks. Final earthworm masses, monthly cocoon production, and monthly litter removal were compared across treatments using multi-comparison one way ANOVA.

### 4.3.3 Results

#### 4.3.3.1 Growth

Figure 4.3.1 shows the growth of *L. terrestris* hatchlings fed with six different types of SRF litter. A difference in mass was first discernible graphically after 4 weeks, and became more obvious thereafter. After 28 weeks, individual mean mass was significantly greater ($p < 0.05$) in *A. glutinosa*-fed earthworms, while it was significantly lower ($p < 0.05$) in *C. sativa*-fed earthworms. *F. excelsior, B. pendula*, *A. pseudoplatanus* and *E. nitens*-fed earthworms showed a relatively similar mean growth rate throughout the experiment. Tubercula pubertatis (glandular swellings on the ventral surface, either on or near the clitellum and key feature to identify maturity of earthworms) were first recorded for *A. glutinosa*-fed earthworms after 20 weeks ($n = 1$) and after 28 weeks for *F. excelsior*-fed earthworms ($n = 2$). The first clitellate animal was observed after 24 weeks with *A. glutinosa* ($n = 1$). Even after 28 weeks, *B. pendula*, *A. pseudoplatanus*, *E. nitens* and *C. sativa*-fed animals showed no signs of maturity, while *A. glutinosa*-fed animals were all clitellate. After 28 weeks, *A. glutinosa*-fed earthworms recorded the highest mean mass (5.02 g), while *C. sativa*-fed earthworms recorded the lowest mean mass (1.55 g) of the SRF species examined. Table 4.3.1 summarises the production results and significant differences ($p < 0.05$) for the growth experiment after 28 weeks. At termination of the experiment, 100% earthworm survival was recorded for all treatments.

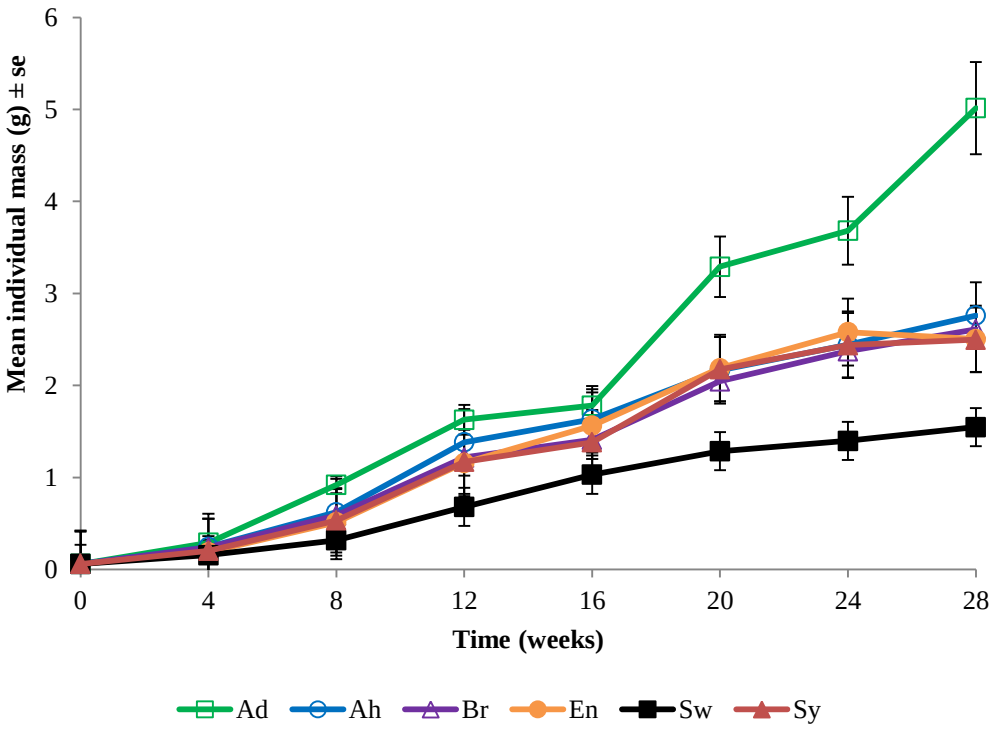



**Figure 4.3.1 Mean (± se) growth (g) of hatchling *L. terrestris* fed with *A. glutinosa* (Ad), *F. excelsior* (Ah), *B. pendula* (Br), *E. nitens* (En) *C. sativa* (Sw) and *A. pseudoplatanus* (Sy) over a period of 28 weeks.**

**Table 4.3.1 Summary production results for *L. terrestris* hatchlings supplied with six types of litter materials - after 28 weeks [Abbreviation of SRF species as for Figure 4.3.1]**

| Attribute | SRF species | | | | | |
|---|---|---|---|---|---|---|
| | **Ad** | **Ah** | **Br** | **En** | **Sw** | **Sy** |
| Mean growth rate (mg $g^{-1}$ $wk^{-1}$) | 2949 | 1607 | 1518 | 1455 | 885 | 1451 |
| Mean individual mass after 28 weeks | $5.02^{a}$ | $2.76^{b}$ | $2.61^{b}$ | $2.51^{b}$ | $1.55^{c}$ | $2.5^{b}$ |
| Maturation (fully clitellate) at 28 weeks (%) | 100 | 0 | 0 | 0 | 0 | 0 |

Different letters in a row indicate significant differences ($p < 0.05$, $n = 4$).

### 4.3.3.2 Reproduction

Figure 4.3.2 shows cocoon production for field-collected adult *L. terrestris* fed with 6 different litter types. Mean individual cocoon production, over the period of 16 weeks was recorded as *A. glutinosa* (11 $ind^{-1}$), *B. pendula* and *F. excelsior* (4 $ind^{-1}$) *E. nitens* and *A. pseudoplatanus* (2 $ind^{-1}$) and *C. sativa* (1 $ind^{-1}$) which equated to 2.69, 1.00, 0.44 and 0.31 $ind^{-1}$ 4 $weeks^{-1}$ respectively. Mean cocoon production was significantly higher ($p < 0.05$) for earthworms fed with *A. glutinosa* litter while *B. pendula*, *E. nitens*, *A. pseudoplatanus*, *C. sativa*, and *F. excelsior*-fed earthworms showed no significant difference ($p > 0.05$) in cocoon production.

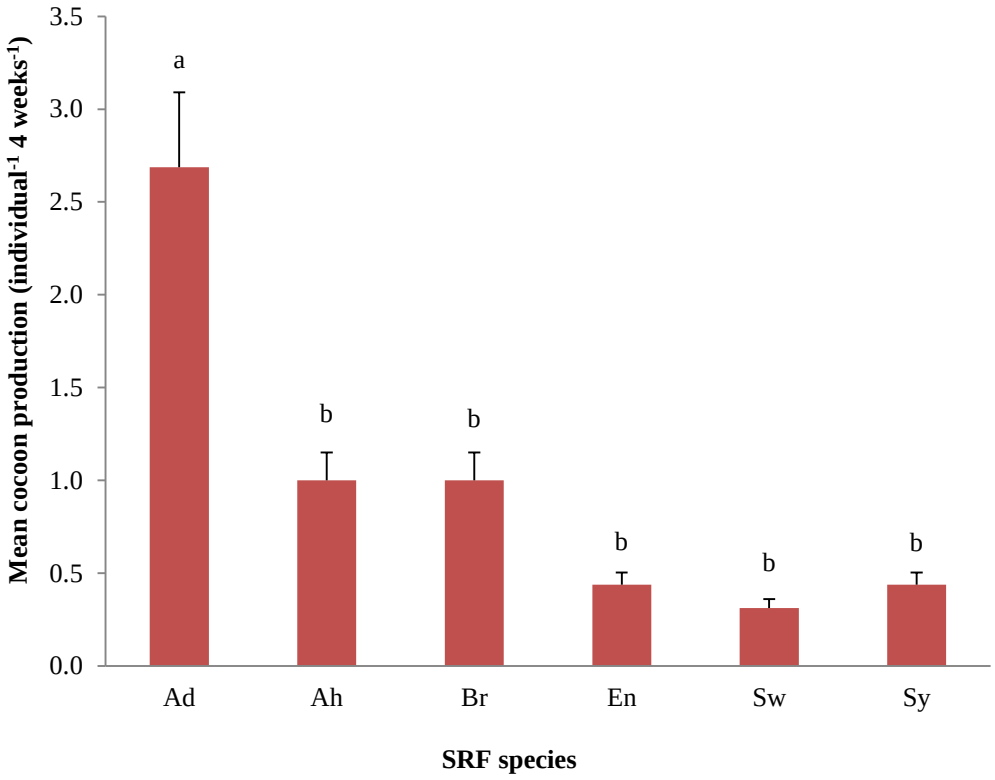



**Figure 4.3.2 Mean (± se) cocoon production by *L. terrestris* fed with six types of leaf litter after 16 weeks. Different letters denote significant differences between treatments ($p < 0.05$) [Abbreviation of SRF species as for Figure 4.3.1].**

Figure 4.3.3 shows mean mass change of earthworms over the experimental period. After 16 weeks, an 8% mass increment was recorded by *A. glutinosa*-fed earthworms, while all other treatments recorded a mass loss; *C. sativa* (44%), *A. pseudoplatanus* (37%), *B. pendula* (17%), *E. nitens* (11%.) and *F. excelsior* (12%.) At termination of the experiment, *C. sativa* recorded a 75% survival rate (1 animal died) while all other treatments recorded 100% survival.

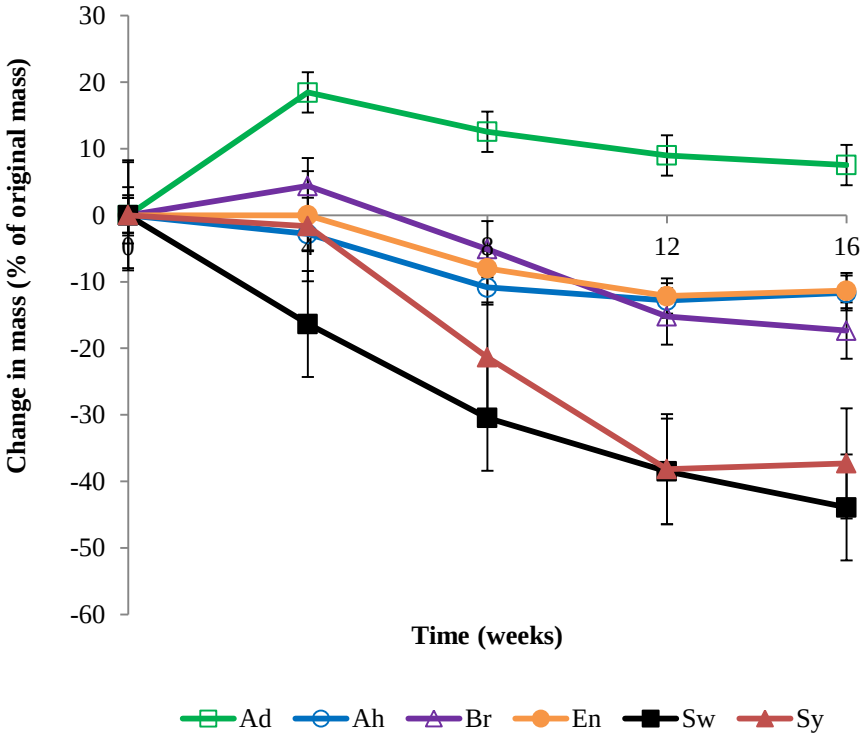



**Figure 4.3.3 Mean (± se) change of individual biomass (%) of adult *L. terrestris* supplied with six types of leaf litter over a period of 16 weeks [Abbreviations as for Figure 4.3.1].**

As illustrated in Figure 4.3.4, the mean monthly surface litter removal by individuals was recorded as 1.5 g for *A. glutinosa* (75%), 1.19 g for *B. pendula* (59%), 0.88 g for *F. excelsior* (44%), 0.69 g for *E. nitens* (35%), 0.44 g for *A. pseudoplatanus* (22%), and 0.19 g for *C. sativa* (10%). Table 4.3.2 summarises the results for reproduction experiment after 16 weeks.

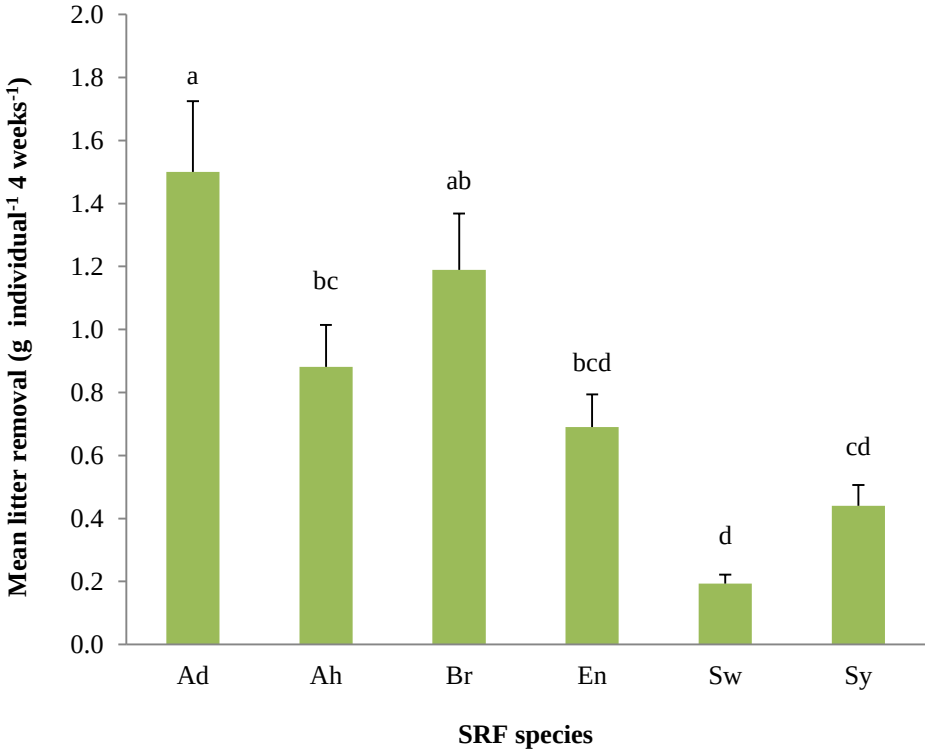



**Figure 4.3.4 Mean (± se) surface litter removal by *L. terrestris* supplied with six types of leaf litter after 16 weeks. Different letters denote significant differences between treatments ($p < 0.05$) [Abbreviation of SRF species as for Figure 4.3.1].**

**Table 4.3.2 Summary production results for *L. terrestris* adults supplied with six types of leaf litter - after 16 weeks [Abbreviation of SRF species as for Figure 4.3.1]**

| Attribute | SRF species | | | | | |
|---|---|---|---|---|---|---|
| | **Ad** | **Ah** | **Br** | **En** | **Sw** | **Sy** |
| Survivorship (%) | 100 | 100 | 100 | 100 | 75 | 100 |
| Initial mean mass (g ind. $^{-1}$) | 5.19 | 5.27 | 4.95 | 4.88 | 5.42 | 5.20 |
| Final mean mass (g ind. $^{-1}$) | 5.58 | 4.65 | 4.09 | 4.33 | 3.04 | 3.26 |
| Mass change of survivors (% of starting mass) | +8 | -12 | -17 | -11 | -44 | -37 |
| Mean litter removal (g ind$^{-1}$ 4 weeks$^{-1}$) | 1.50$^{a}$ | 0.88b$^{c}$ | 1.19$^{ab}$ | 0.69$^{bcd}$ | 0.19$^{d}$ | 0.44$^{cd}$ |
| Mean cocoon production (ind.$^{-1}$ 4 weeks$^{-1}$) | 2.69$^{a}$ | 1.00$^{b}$ | 1.00$^{b}$ | 0.44$^{b}$ | 0.31$^{b}$ | 0.44$^{b}$ |

Different letters in a row indicate significant differences ($p < 0.05$, $n = 4$)

As shown in Table 4.3.3, *A. glutinosa* litter had the highest total N (%) and lowest C:N while *C. sativa* recorded lowest total N (%) and highest C:N. The Ca (%) was high in *A. pseudoplatanus* and *F. excelsior* while it was lowest of the litter examined in *C. sativa*

**Table 4.3.3 Selected litter chemistry parameters for experimental SRF species (Refer to section 3.3.3 for details), [Abbreviation of SRF species as for Figure 4.3.1]**

| Litter parameter | SRF species | | | | | |
|---|---|---|---|---|---|---|
| | **Ad** | **Ah** | **Br** | **En** | **Sw** | **Sy** |
| Total N (%) | 2.76 | 1.51 | 1.59 | 1.33 | 0.94 | 1.45 |
| Total C (%) | 50.5 | 47.3 | 51.5 | 52.5 | 48.7 | 47.9 |
| C:N | 18.3 | 31.3 | 32.5 | 39.5 | 52.0 | 33.0 |
| P (%) | 0.12 | 0.15 | 0.10 | 0.09 | 0.09 | 0.08 |
| Ca (%) | 1.91 | 2.66 | 1.17 | 1.36 | 0.93 | 2.55 |

### 4.3.4 Discussion

#### 4.3.4.1 Growth

The results from this experiment suggest that SRF litter has an influence on *L. terrestris* growth. Litter chemistry results suggest that total N (%), C:N and Ca (%) in litter material are likely to be the reason for recorded differences in earthworm growth. *A. glutinosa* litter with the highest amount of N and lowest C:N led to a significantly greater final mass. *C. sativa* litter with highest C:N and lowest amount of N and Ca led to a significantly lower mass after 28 weeks. *B. pendula*, *F. excelsior*. *E. nitens* and *A. pseudoplatanus* which showed mid-range value for litter quality had an average effect on *L. terrestris* growth. Most of the previous field studies on earthworms and trees (e.g. Hendriksen, 1990; Tian *et al*., 1993; Zou, 1993; Reich *et al.*, 2005; Hobbie *et al.*, 2006; Sarlo, 2006) have suggested that leaf litter N content, C:N, and Ca content play a critical role in earthworm abundance and activity. These studies have further suggested that lignin content and phenolic compounds (tannins) have a potential influence on earthworms, which was not analysed in the current study. All of the aforementioned studies have focused on the influence of litter quality on earthworm density, diversity, total biomass and rate of litter decomposition, but not on individual earthworm growth or reproduction. However, Lakhani and Satchell (1970) conducted a long-term (three year) earthworm growth experiment and recorded mean adult mass of *L. terrestris* around 9.5 g from earthworms kept in mesh bags in the field, regularly fed with mixed deciduous tree leaves. These earthworms were on average 3 g heavier than animals collected from adjacent unmanaged field conditions.

A number of laboratory experiments have been conducted to evaluate the influence of different organic sources, such as animal manure (Löfs-Holmin, 1983; Butt, 1998; Berry and Jordan, 2001), paper pulp (Butt, 1991) and paper pulp plus yeast extract, (Butt *et al*., 1992) on *L. terretris* growth. However, very few growth experiments have been recorded with tree litter (e.g. Curry and Bolger, 1984; Butt 2011). At 15 °C, Curry and Bolger (1984) fed *L. terrestris* (together with *A. caliginosa*) with excess *Salix* litter and recorded a mean increase in growth of 3 g (from 1.8 g to 4.8 g) over a period of 24 weeks. At 15 °C, Butt (2011) fed *L. terrestris* hatchings (initial mean mass 60 mg) with excess amounts of *B. pendula* litter or horse manure and recorded a mean mass of 4.19 g and 6.17 g respectively and 100% maturity after 28 weeks, showing that a diet of horse manure led to a significantly greater final adult mass, but that the rate of maturation was similar for both manure and *B. pendula* leaves as food. However, current experiments with the same species of earthworm with *B. pendula* litter recorded a comparatively lower mean mass (2.61 g) and no attainment of maturity at week 28. The quality and quantity of litter supplied could be the reason for this difference. The C:N of *B. pendula* litter used by Butt (2011) was low (21) compared with the same species of leaf litter (32.5) used in the current experiment. Further, Butt (2011) used excess amounts of litter, but in the present study, a limited amount of litter (2-4 g individual$^{-1}$ 4 weeks$^{-1}$) was supplied. However, it is noteworthy that a similar amount of *A. glutinosa* litter in the current experiment resulted in a mean mass of 5.02 g and 100% maturity of earthworms after 28 weeks while, *C. sativa* resulted in mean mass of 1.55 g and 0% maturity. Overall, the experiment suggests that SRF litter quality has a major influence on *L. terrestris* growth and maturation.

#### 4.3.4.2 Reproduction

This experiment demonstrated that SRF species litter has a major effect on reproduction of field-collected *L. terrestris*. *A. glutinosa* litter, with highest content of N, recorded significantly greater cocoon production, while *C. sativa* litter with lowest content of N, recorded the lowest cocoon production of the species examined. This study further suggests that SRF litter quality influences the amount of litter removal by *L. terrestris* and even body mass maintenance of mature animals. *A. glutinosa* litter recorded the highest value in terms of both of these aspects, while *C. sativa* recorded the lowest.

Butt (2011) suggested that food quality affects both somatic growth and reproductive potential of *L. terrestris*. In this study a significantly increased cocoon production and increased mean mass from *B. pendula* litter-fed earthworms, followed a switch to green leaves. An almost immediate reversal of this trend following a reversion to fallen litter was also noted by the author. This study suggested that the larger nitrate content in green leaves led to higher utilisation and rapid protein synthesis as required for growth and reproduction. This comparable study by Butt (2011) further recorded an average cocoon production of 5.15 $ind^{-1}$ $month^{-1}$ when laboratory cultured *L. terrestris* (mean mass 6.93 g) were fed with excess amounts of *B. pendula* litter. Generally at 15 °C, the average cocoon production of manure-fed *L. terrestris* (laboratory cultured) was recorded as more than 4 cocoons $ind^{-1}$ $month^{-1}$ (Butt *et al*, 1994, Butt, 2011). However, in the current study, except for *A. glutinosa*, all other litter species recorded a production of 1 or less cocoon $ind^{-1}$ 4 $weeks^{-1}$. It could be suggested that the origin of the earthworms (field-collected *L. terrestris* - mean mass 5.51 g), food quantity (2 g $ind^{-1}$ 4 $weeks^{-1}$) and quality had negatively influenced cocoon production in the current work.

Summary of major findings of two laboratory experiments:

- Native *A. glutinosa* litter had a very positive effect on *L. terretris* growth and reproduction and out-performed other selected SRF species.
- Naturalised *C. sativa* showed the most depressed effect on hatchling growth, adult mass maintenance and even cocoon production compared with other selected SRF species.
- Non-native *E. nitens* litter was as effective as native SRF species such as *B. pendula* and *F. excelsior* in terms of earthworm growth in addition to adult mass maintenance.
- Overall, this work showed that SRF species litter had a marked influence on *L. terresris* growth and reproduction depending on litter quality (e.g. N concentration C:N ratio and Ca concentration).

Further growth and reproduction studies are recommended with other earthworm species to confirm the influence of SRF litter quality on overall earthworm community development.

# CHAPTER 5: EARTHWORM PREFERENCE FOR SRF SPECIES LITTER

## 5.1 Introduction

Earthworms have been shown to demonstrate a preference for certain types of leaf litter over others (Darwin, 1881; Satchell and Lowe, 1967; Hendriksen, 1990). Selection depends on the palatability of the litter which is determined by some of its physical and chemical characteristics (Satchell and Lowe, 1967). Darwin (1881) observed that earthworms preferred some leaves more than others and suggested that these animals can distinguish between varieties. He further noticed that earthworms preferred plant leaves of a particular shape. A leaf litter selection experiment by Satchell and Lowe (1967) suggested that when provided with uniform disks of leaf material, *L. terrestris* preferred leaf litter from alder (*A. glutinosa*), ash (*F. excelsior*), elder (*Sambucus nigra*) and elm (*Ulmus glabra*) over larch (*Larix deciduas*), oak (*Quercus petraea*) or beech (*Fagus sylvatica*). In a litterbag experiment, Edwards and Heath (1963) found that *L. terrestris* preferred oak over beech. Hendriksen (1990) also used a litterbag technique to show that *Lumbricus* spp. prefered ash and lime over beech litter. These studies identified that the chemical composition of plant litter, especially nitrogen content, carbon to nitrogen ratio, lignin content, phenolic compounds and calcium content greatly influenced earthworm litter selection. Further, some investigators observed that earthworms preferred decomposed litter over fresh litter and concluded that bacterial and fungal activity on leaf litter enhances its palatability to earthworms (Satchell and Lowe, 1967; Wright, 1972; Hendriksen, 1990). Plant litter palatability has a strong influence on aggregation of decomposer communities and overall establishment of soil faunal population (Swift *et al*., 1979).

Previous researchers who investigated the preference of earthworms on various organic food sources such as manure, soil fungi and upland pasture plant species, used different species of earthworm for their studies (e.g. Doube *et al*., 1997a; Bonkowski *et al*., 2000; Neilson and Boag, 2003). However, most of the aforementioned tree litter preferential studies were limited to *L. terrestris* and barely investigated other earthworm species. Further, these studies have focused on common temperate forest tree species and the preference of European earthworms for non-native tree species such as *E. nitens* is almost unknown.

The aim of the present study was to investigate the preference of native British earthworms for SRF species litter. Based on a technique used by Doube *et al*. (1997a), choice chamber experiments were designed to explore SRF species litter preference by four species of earthworm; *Allolobophora chlorotica, Aporrectodea caliginosa, Aporrectodea longa* and *Lumbricus terrestris.* This system permitted quantification and comparison of litter removal by earthworms over time. In addition, a more sophisticated webcam recording technique was used to observe SRF litter selection of *L. terrestris* under cover of darkness. This recording approach allowed collection of direct evidence for litter selection behaviour.

This chapter therefore presents choice chamber experiments and then webcam recording experiments which observed *L. terrestris* feeding behaviour. All were conducted under controlled environmental conditions.

## 5.2 Choice chamber experiments

### 5.2.1 Introduction

The specifically designed leaf litter choice chamber used in this study was a modification of a simple choice chamber described by Doube *et al*. (1997a). The novel soil-mediated choice chamber system provided more natural and improved conditions for soil-dwelling earthworms and allowed quantification of the leaf litter selected as food over time, without disturbing the earthworms or the soil in which they were living. A series of experiments were conducted under controlled environmental conditions to assess and compare the SRF species litter preference by selected species of earthworms.

### 5.2.2 Materials and methods

Circular aluminium foil trays (diameter 0.16 m and depth 0.03 m) and Eppendorf tubes (diameter 0.01 m and depth 0.04 m) were used as the basis for the leaf litter choice chambers. Caps of Eppendorf tubes were separated and the centre was drilled-out, so that earthworms could move through. Holes were then made in the foil tray, so that the drilled caps could be fixed into equally spaced positions on the inner side of the tray wall, such that the Eppendorf tubes could be attached from the outside. This technique allowed removal of the Eppendorf tubes, weighing and re-fixing without disturbance to the experimental system (see Figure 5.2.1).

**Figure 5.2.1 Basic choice chamber consisting of an aluminium tray (diameter 0.16 m, depth 0.03 m) with six Eppendorf tubes (diameter 0.01 m and depth 0.04 m) attached; viewed from above.**

Empty Eppendorf tubes were fixed into each position to initially hold the cap in position. Trays were then filled with Kettering loam (25% moisture) as a substrate for earthworms (Butt *et al*., 1994). The experimental earthworms were introduced to each tray as per experiment (see Figure 5.2.2) and sprayed with water. Trays were covered with aluminium foil, which was held in place with a rubber band to prevent moisture loss and earthworm escape. Two holes were made with a mounted needle in the foil sheet to ensure air circulation. Trays were kept in darkness for 24 hours in 15 °C temperature-controlled incubators for earthworms to equilibrate to the system (Figure 5.2.5). Field-collected adult earthworms were used in the experiments and species used differed for each experiment (see specific details in sections 5.2.2.1 - 3). Animals had been acclimated to laboratory conditions for 8 weeks prior to experimentation (Fründ *et*

*al*., 2010). Leaves from the range of tree species to be tested were mixed and supplied as food during this period to ensure that no pre-conditioning occurred with respect to litter (Butt, 2011).

(a) (b)

**Figure 5.2.2 Earthworm species introduction to separate soil trays; (a) *A. chlorotica* (n = 30), (b) *L. terrestris* (n = 2).**

After 24 hours, previously collected, air-dried SRF litter was ground separately (using a MAGIMIX 4150W food processor) and passed through a series of sieves (2.8, 2.0 and 1.0 mm). Leaf particles (1 - 2 mm) were used for this experiment to prevent undue influence of particle size on earthworm food selection (Lowe and Butt, 2003). Chemical analyses were performed on sub-samples of this fraction using the method described in section 3.3.3.

A new set of Eppendorf tubes were weighed separately and filled (0.20 - 0.25 g) with dry leaf litter particles (1 - 2 mm) and reweighed. Leaf litter-filled tubes were then soaked with water for two hours (see Figure 5.2.3). After two hours, excess water was drained by invertion (5 minutes) on absorbent tissue paper. Tubes with moistened litter

were then reweighed. The empty Eppendorf tubes from previously prepared earthworm-containing choice chambers were replaced with experimental moist litter-filled Eppendorf tubes (food tubes) and maintained in incubators as before.

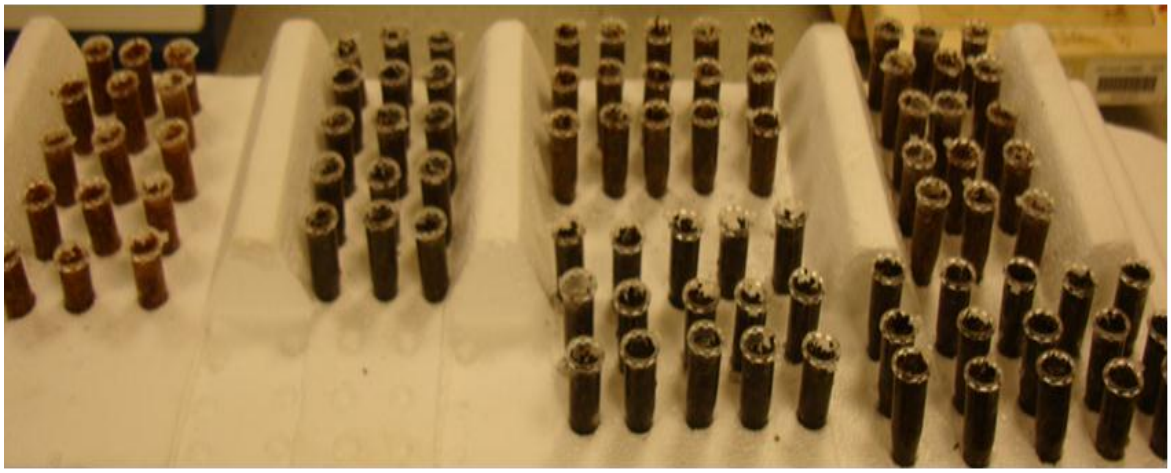


**Figure 5.2.3 Eppendorf tubes filled with air-dried leaf litter (0.20 – 0.25 g) and water-soaked for 2 hours before attaching into previously prepared choice chambers.**

SRF litter removal by earthworms was assessed by recording the weight loss of individually labelled food tubes over time. Choice chambers were examined two/three times per week; food tubes were removed, weighed and re-fixed in the same position. At this time, each tray was sprayed with an equal amount of water to maintain the soil moisture level throughout the study. A control was prepared without earthworms and treated similarly to measure the moisture variation throughout the experiment. Three sets of separate choice chamber experiments were conducted to investigate and compare the SRF litter preference by selected species of earthworms. At the end of each experiment, the number of surviving earthworms and their masses were also recorded.

#### 5.2.2.1 Choice Chamber Experiment 1

This initial experiment was conducted to investigate SRF litter preference by four species of earthworm from two ecological categories (see section 2.3.1.1). Those selected were; *A. chlorotica* and *A. caliginosa* (endogeic), *A. longa* and *L. terrestris* (anecic) with initial individual mean masses of 0.19, 0.43, 1.56 and 4.27 g respectively. The six SRF species selected were; alder (*A. glutinosa*), ash (*F. excelsior*), birch (*B. pendula*), eucalyptus (*E. nitens*), sweet chestnut (*C. sativa*), and sycamore (*A. pseudoplatanus*). Each earthworm species was introduced to separate choice chambers in the following numbers; *A. chlorotica* (30), *A. caliginosa* (15), *A. longa* (4) and *L. terrestris* (2). The earthworm numbers were selected so that each treatment received an approximately similar earthworm biomass and that an effect might be observed in a reasonable time frame. The food tubes containing different SRF species litter were randomly arranged around the tray, (n = 6; one from each SRF species, see Figure 5.2.4). Three replicate trays were prepared for each earthworm species. SRF litter preference was assessed by mean weight loss of individual food tubes, measured three times per week over four weeks.

(a) (b)

**Figure 5.2.4 Leaf litter choice chambers: (a) Details of 6 SRF species: alder (*A. glutinosa*), ash (*F. excelsior*), birch (*B. pendula*), eucalyptus (*E. nitens*), sweet chestnut (*C. sativa*), and sycamore (*A. pseudoplatanus*), arranged randomly around the tray; (b) Complete set of choice chambers for Experiment 1 (Four earthworm species; *A. chlorotica, A. caliginosa, A. longa,* and *L. terrestris* plus control).**

**Figure 5.2.5 Choice chambers covered with aluminium foil and maintained at 15 °C; 24 hour darkness in incubators.**

### 5.2.2.2 Choice Chamber Experiment 2

Based on results from experiment 1, this experiment was conducted to provide more evidence on earthworm SRF litter choices. The experiment used three selected SRF species; eucalyptus (*E. nitens*), sweet chestnut (*C. sativa*), and sycamore (*A. pseudoplatanus*). Two major litter feeding earthworms used for the experiment were *A. longa* and *L. terrestris* with initial mean masses of 1.63 g and 3.78 g respectively. Each earthworm species was introduced to separate choice chambers in the following number; *A. longa* (4) and *L. terrestris* (2). Similar to Experiment 1, the earthworms were introduced to individual trays so that each replicate for the same earthworm species

received an approximately similar biomass. The food tubes with different SRF species litter were arranged sequentially around each tray as each tray had a total of nine food tubes (three for each SRF species, see Figure 5.2.6). The new food tube arrangement in this experiment was used to test if earthworm SRF choice is random. Four replicate trays were prepared for each earthworm species. SRF litter selection was assessed by recording weight loss of individual food tubes twice per week for five weeks.

(a) (b)

**Figure 5.2.6 Leaf litter choice chambers with three SRF species: eucalyptus (*E. nitens*), sweet chestnut (*C. sativa*), and sycamore (*A. pseudoplatanus*) (a) Sequential food tube arrangement around the tray (three from each SRF species); (b) Complete set of choice chambers for Experiment 2 (two earthworm species; *A. longa* and *L. terrestris*).**

#### 5.2.2.3 Choice Chamber Experiment 3

This experiment was also based on results from the first choice chamber experiment and to provide further evidence of SRF litter choice by earthworms. The experiment was conducted using three selected SRF species; alder (*A. glutinosa*), ash (*F. excelsior*), and birch (*B. pendula*). The earthworm species were *A. longa* and *L. terrestris* with initial mean masses of 1.52 g and 4.11 g respectively. Each earthworm species was introduced to separate choice chambers in the following number: *L. terrestris* (1) and *A. longa* (1). The food tubes with different SRF species litter were arranged sequentially around each tray as one tray received a total of nine food tubes (three for each SRF species) which was similar to Experiment 2. Four replicate trays were prepared for each earthworm species. SRF litter selection was once again assessed by recording weight loss of individual food tubes twice per week for four weeks.

#### 5.2.2.4 Statistical analysis

Leaf litter selection behaviour was assessed by calculating the weight of litter remaining in individual food tubes over time. The remaining litter amount was considered to be associated with earthworm preferences, as highest remaining (%) for non-preferred and lowest remaining (%) for preferred. The point of 50 % total litter removal was taken as the criterion for statistical analysis. This was similar to the point used by Doube *et al*., (1997a). One way analysis of variance (ANOVA) was used to test the SRF litter preference by each species of earthworm separately. A Tukey-Kramer multiple comparison test was applied for all of the pair-wise comparisons. The amount of litter removed by each earthworm species was calculated by subtracting the remaining weight from the original weight.

### 5.2.3 Results

Table 5.2.1 shows selected litter chemistry parameters for the 1 - 2 mm fraction of experimental SRF species. The results were similar to total litter analysis results (see Table 3.3.1). *A. glutinosa* (Ad) litter had the highest N (%) and lowest C:N while *C. sativa* (Sw) had lowest N (%) and highest C:N. The Ca (%) was highest in *A. pseudoplatanus* (Sy) and *F. excelsior* (Ah), while it was lowest in *C. sativa* (Sw).

**Table 5.2.1 Selected litter (1-2 mm fraction) chemistry parameters of experimental SRF species; *A glutinosa* (Ad), *F. excelsior* (Ah), *B. pendula* (Br), *E. nitens* (En), *C. sativa* (Sw), and *A. pseudoplatanus* (Sy)**

| **Litter parameter** | **SRF species** | | | | | |
|---|---|---|---|---|---|---|
| | Ad | Ah | Br | En | Sw | Sy |
| N (%) | 2.55 | 1.47 | 1.46 | 1.36 | 0.89 | 1.43 |
| C (%) | 50.3 | 47.4 | 51.5 | 52.7 | 49.0 | 48.4 |
| K (%) | 0.41 | 0.22 | 0.20 | 0.45 | 0.40 | 0.19 |
| Ca (%) | 1.99 | 2.65 | 1.18 | 1.35 | 0.93 | 2.32 |
| Mg (%) | 0.16 | 0.24 | 0.20 | 0.21 | 0.26 | 0.17 |
| P (%) | 0.12 | 0.16 | 0.10 | 0.10 | 0.09 | 0.08 |
| C: N | 19.7 | 32.2 | 35.3 | 38.9 | 54.8 | 33.9 |

#### 5.2.3.1 Choice Chamber Experiment 1

Figures 5.2.7 to 5.2.10 illustrate the temporal pattern of leaf litter removal by four species of earthworm supplied with six types of SRF litter. The results show that the novel soil-mediated choice chamber approach was successful in helping to quantify earthworm litter selection behaviour. At the termination of the experiment (after 28 days), 100% survival was recorded for all four species of earthworms. The earthworm

mass changes were recorded as 1 – 7% loss across all species with respect to the original masses (see Table 5.2.2). Endogeic *A. chlorotica* had the highest mass loss, at 6.5%.

**Table 5.2.2 Summary of earthworm parameters for Choice Chamber Experiment 1**

| Earthworm attribute | Earthworm species | | | |
|---|---|---|---|---|
| | **Ach** | **Acal** | **Al** | **Lt** |
| Number (ind tray$^{-1}$) | 30 | 15 | 4 | 2 |
| Initial mean mass (g tray$^{-1}$) | 5.83 | 6.40 | 6.25 | 8.54 |
| Final mean mass (g tray$^{-1}$) | 5.45 | 6.36 | 6.08 | 8.42 |
| Mean mass change (% of original mass) | -6.5 | -0.6 | -2.7 | -1.5 |
| Survival (%) | 100 | 100 | 100 | 100 |

[*A. chlorotica* (Ach), *A. caliginosa* (Acal), *A. longa* (Al) and *L. terrestris* (Lt)]

Figure 5.2.7 shows the pattern of leaf litter removal from choice chambers by *A. chlorotica* over the experimental period. The experiment started with 1.37 - 1.43 g of litter (wet basis) in individual food tubes. After 28 days, the remaining litter masses (wet basis) were recorded as *A. glutinosa* (48%), *F. excelsior* (27%), *B. pendula* (28%), *E. nitens* (64%), *C. sativa* (99%) and *A. pseudoplatanus* (63%) which equated to mean litter removal of 52%, 73%, 72%, 36%, 1% and 37% respectively (see Table 5.2.3 for statistical analysis).

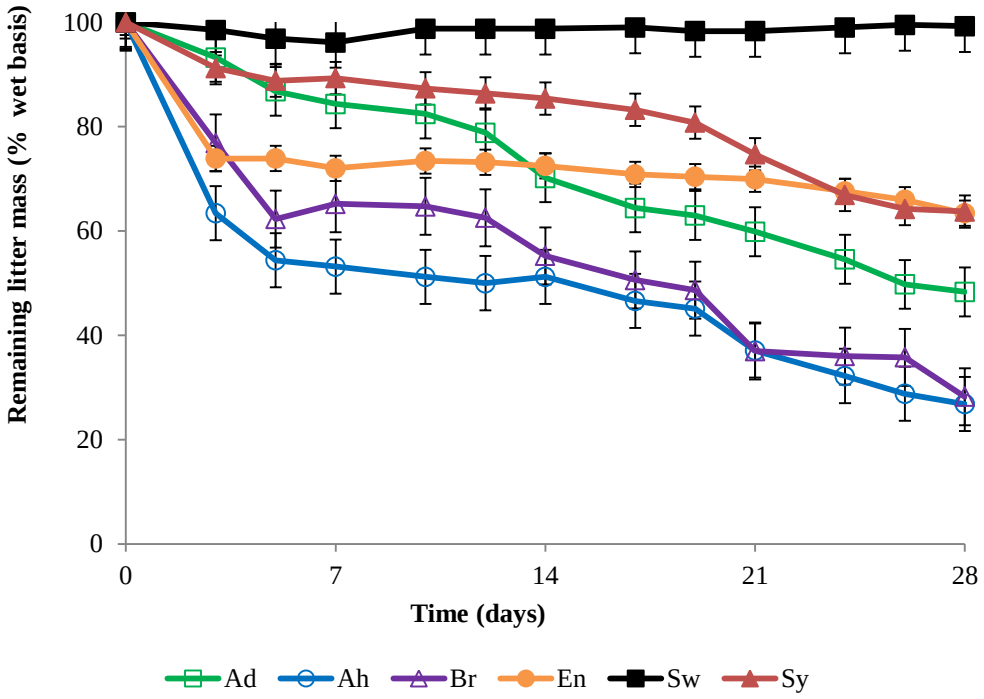



**Figure 5.2.7 Mean (± se) mass (% wet basis) of remaining litter in choice chambers of *A. chlorotica* supplied with six types of SRF litter over a period of 28 days, [Abbreviation of SRF species as for Table 5.2.1].**

Figure 5.2.8 shows the pattern of leaf litter removal from choice chambers by *A. caliginosa* over a period of 28 days. The experiment started with 1.37 - 1.45 g of litter (wet basis) in individual food tubes. The first 100% leaf litter removal was recorded for *F. excelsior* after 21 days. At the end of the experiment, the remaining leaf litter (wet basis) for the rest of the SRF species were recorded as; for both *A. glutinosa* and *B. pendula* (2%), *E. nitens* (48%), *C. sativa* (92%) and *A. pseudoplatanus* (37%), which equated to mean leaf litter removal of 98%, 52%, 8% and 63% respectively (see Table 5.2.3 for statistical analysis).

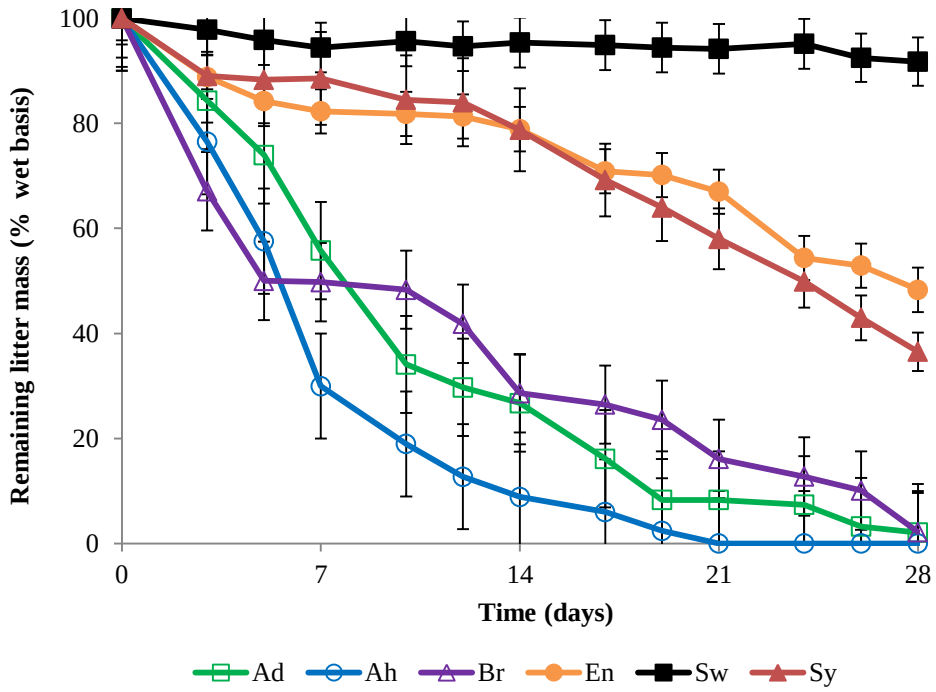



**Figure 5.2.8 Mean (± se) mass (% wet basis) remaining litter in choice chambers of *A. caliginosa* supplied with six types of SRF litter over a period of 28 days, [Abbreviation of SRF species as for Table 5.2.1].**

Figure 5.2.9 illustrates the trend of leaf litter removal from choice chambers by *A. longa* over the experimental period. The experiment started with 1.36 - 1.48 g of litter (wet basis) in individual food tubes. A complete leaf litter removal was recorded for *A. glutinosa* and *F. excelsior* after 7 days, for *B. pendula* after 10 days and for *E. nitens* after 17 days. At the end of the experiment, the mean remaining leaf litter in choice chambers for *C. sativa* was 89% and *A. pseudoplatanus* was 4% which equated to mean leaf litter removal of 11% and 96% respectively (see Table 5.2.3 for statistical analysis).

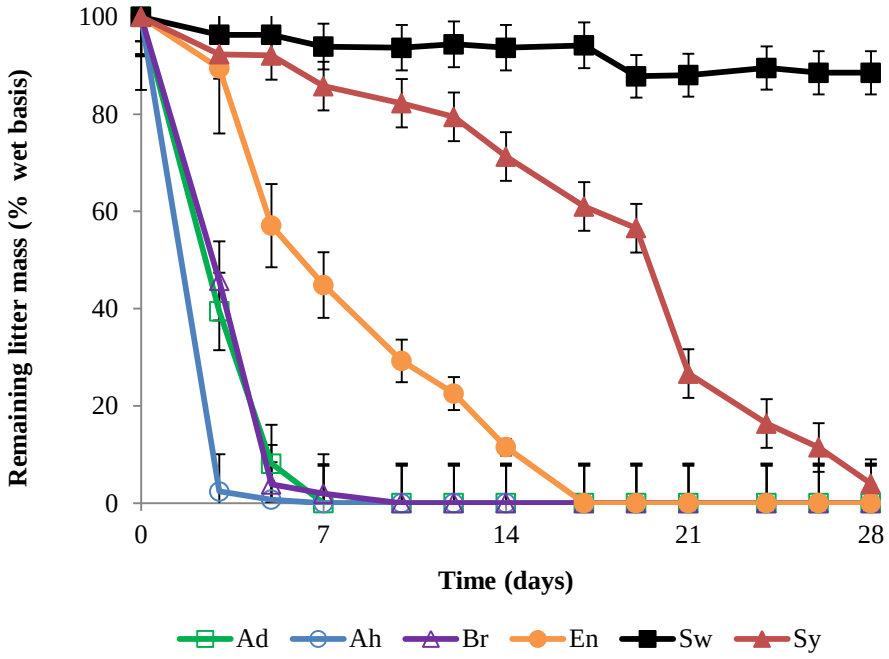



**Figure 5.2.9 Mean (± se) mass (% wet basis) remaining litter in choice chambers of *A. longa* supplied with six types of SRF litter over a period of 28 days, [Abbreviation of SRF species as for Table 5.2.1].**

Figure 5.2.10 shows the pattern of leaf litter removal by *L. terrestris* from choice chambers, over a period of 28 days. The experiment started with 1.37 g - 1.41 g of litter (wet basis) in individual food tubes. A 100% leaf litter removal was recorded for *A. glutinosa* and *B. pendula* after 7 days, for *F. excelsior* after 10 days and for *E. nitens* after 24 days. At the end of the experiment, the remaining leaf litter (wet basis) was recorded as *C. sativa* (93%) and *A. pseudoplatanus* (31%) which equated to the mean litter removal of 7% and 69% respectively (see Table 5.2.3 for statistical analysis). Figure 5.2.11 demonstrates the observable leaf litter removal from choice chambers by *A. longa* (a) and *L. terrestris* (b) in Experiments 1 after ten days.

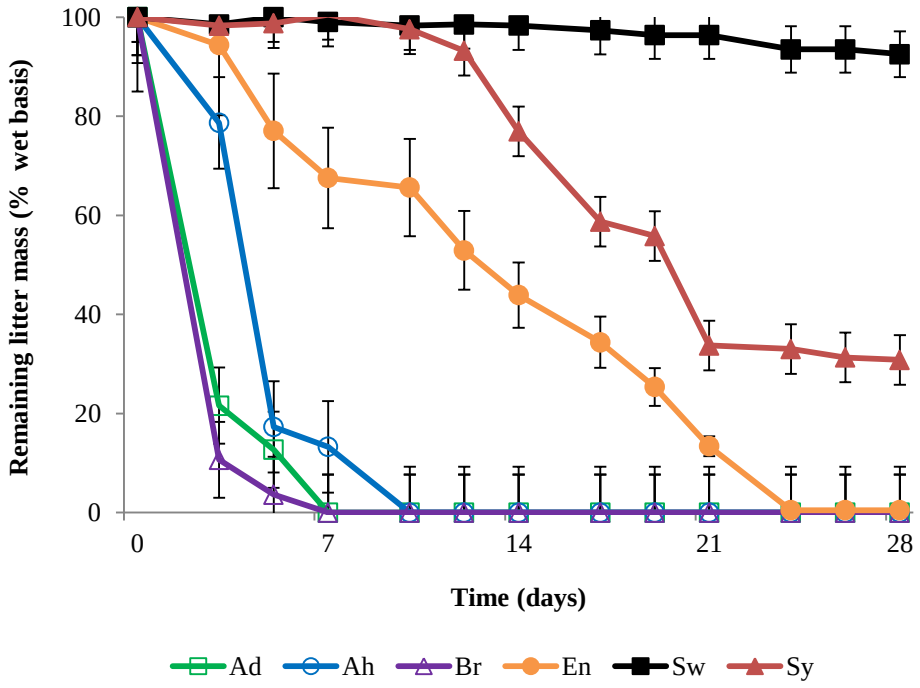
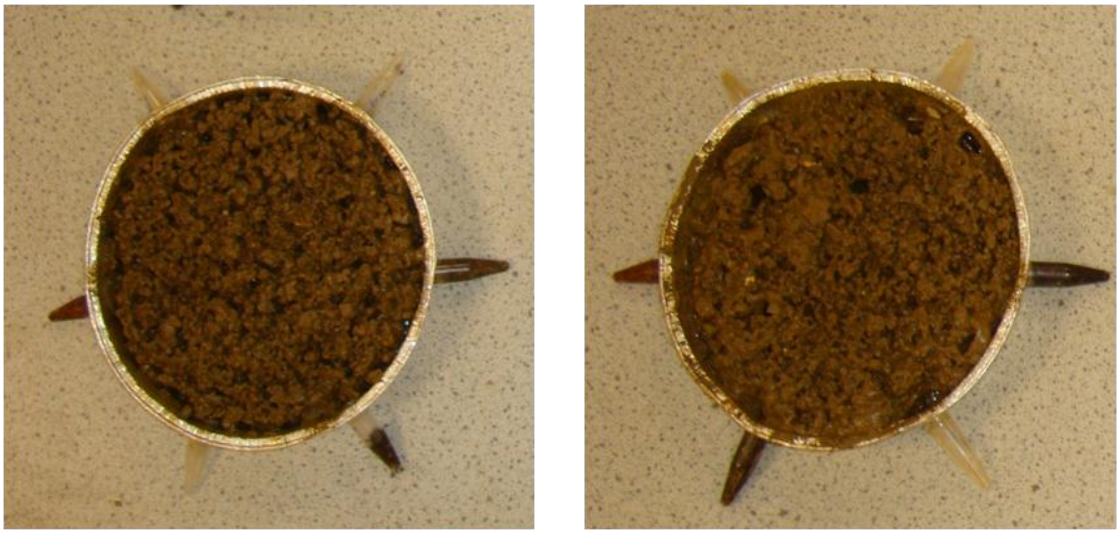



**Figure 5.2.10 Mean (± se) mass (% wet basis) remaining litter in choice chambers of *L. terrestris* supplied with six types of SRF litter over a period of 28 days, [Abbreviation of SRF species as for Table 5.2.1].**



**(a)** **(b)**

**Figure 5.2.11 Leaf litter removal from choice chambers supplied with six types of litter materials; *A glutinosa* (Ad), *F. excelsior* (Ah), *B. pendula* (Br), *E. nitens* (En), *C. sativa* (Sw) and *A. pseudoplatanus* (Sy) after ten days by; (a) *A. longa* and (b) *L. terrestris*; both earthworms showed completely empty food tubes for *A. glutinosa, F. excelsior* and *B. pendula.***

Table 5.2.3 shows the significant differences ($p < 0.05$) in remaining leaf litter (%) in choice chambers of each earthworm species at 50% food removal. The time taken to remove 50% of total litter supplied varied with earthworm species; *A. caliginosa* (17 days), *A. chlorotica* (28 days), *A. longa* (5 days) and *L. terrestris* (7 days). *A. caliginosa* and *A. chlorotica* took a relatively longer period to remove leaf litter from choice chambers than *A. longa* and *L. terrestris*. However, at 50% food removal, all four earthworm species showed a significantly distinct leaf litter selection ($p < 0.05$, Table 5.2.3). For *A. caliginosa, A. longa* and *L. terrestris*, the remaining leaf litter of *A. glutinosa, B. pendula* and *F. excelsior* was significantly lower compared with *A. pseudoplatanus, C. sativa* and *E. nitens* ($p < 0.05$, Table 5.2.2). *A. longa* had a significantly ($p < 0.05$) lower percentage of remaining litter of *E. nitens* compared with

both *A. pseudoplatanus* and *C. sativa*. *L. terrestris* also showed a similar litter preference result to *A. longa* although the result for *E. nitens* was not statistically significant. Endogeic *A. caliginosa* and *A. chlorotica* had a smaller amount of remaining litter of both *E. nitens* and *A. pseudoplatanus* compared with *C. sativa* although these differences were not statistically significant ($p > 0.05$).

**Table 5.2.3 Mean remaining leaf litter (% from original mass) wet basis in choice chambers of different earthworms at 50% of total litter removal, SRF species; *A. glutinosa* (Ad), *F. excelsior* (Ah), *B. pendula* (Br), *E. nitens* (En), *C. sativa* (Sw), and *A. pseudoplatanus* (Sy)**

| **Earthworm species** | **SRF species** | | | | | |
|---|---|---|---|---|---|---|
| | **Ad** | **Ah** | **Br** | **En** | **Sw** | **Sy** |
| ***A. caliginosa*** | 16.0 [b] | 6.1 [b] | 26.3 [b] | 70.9 [a] | 94.8 [a] | 69.4 [a] |
| ***A. chlorotica*** | 48.4 [b] | 26.9 [b] | 28.5 [b] | 64.2 [ab] | 99.3 [a] | 63.6 [ab] |
| ***A. longa*** | 7.8 [c] | 0.7 [c] | 3.8 [c] | 57.0 [b] | 96.3 [a] | 92.2 [a] |
| ***L. terrestris*** | 0.0 [b] | 13.4 [b] | 0.0 [b] | 67.1 [a] | 99.1 [a] | 100 [a] |

Different letters in a row indicate significant differences, ($p < 0.05$, $n = 3$) ANOVA, Tukey-Kramer.

Based on the results presented in Table 5.2.3, the SRF litter preference order by selected species of earthworm are summarised in Table 5.2.4 by taking the highest amount of remaining litter (%) recorded as least preferred SRF species, least amount of remaining litter (%) recorded as most preferred SRF species.

**Table 5.2.4 Selected SRF litter preference shown by selected species of earthworm**

| Earthworm species | SRF litter preference order |
|---|---|
| *A. chlorotica* | Ad, Ah, Br > En, Sy > Sw |
| *A. caliginosa* | Ad, Ah, Br > En, Sy, Sw |
| *A. longa* | Ad, Ah, Br > En > Sy, Sw |
| *L. terrestris* | Ad, Ah, Br > En, Sy, Sw |

*A. glutinosa* (Ad), *F. excelsior* (Ah), *B. pendula* (Br), *E. nitens* (En), *C. sativa* (Sw), and *A. pseudoplatanus* (Sy)

These results led to the selection of two sets of three, from the original six SRF species for Choice Chamber Experiment 2 and 3 with *A. longa* and *L. terrestris*.

### 5.2.3.2 Choice Chamber Experiment 2

Figures 5.2.12 and 5.2.13 illustrate the temporal pattern of leaf litter removal by two species of earthworms supplied with three types of less preferred SRF litter materials. At the termination of the experiment (after 35 days), 100% survival was recorded for both species of earthworms. However, earthworm species recorded a different mass loss as shown in Table 5.2.5.

**Table 5.2.5 Summary of earthworm parameters for Choice Chamber Experiment 2**

| Earthworm attribute | Earthworm species | |
|---|---|---|
| | ***A. longa*** | ***L. terrestris*** |
| Number (ind tray$^{-1}$) | 4 | 2 |
| Initial mean mass (g tray$^{-1}$) | 6.54 | 7.52 |
| Final mean mass (g tray $^{-1}$) | 5.75 | 7.46 |
| Mean mass change (% of original mass) | -12.1 | -0.8 |
| Survival (%) | 100 | 100 |

Figure 5.2.12 shows the trend of leaf litter removal from choice chambers by *A. longa* supplied with three SRF species over the experimental period. The experiment started with mean litter masses (wet basis) in individual food tubes as; *E. nitens* (1.31g), *C sativa* (1.42 g) and *A. pseudoplatanus* (1.34 g). At termination of the experiment, the remaining leaf litter masses in food tubes were recorded as *E. nitens* (3%), *C. sativa* (91%) and *A. pseudoplatanus* (30%) which equated to mean leaf litter removal of 97%, 9% and 70% respectively (see Table 5.2.6 for statistical analysis).

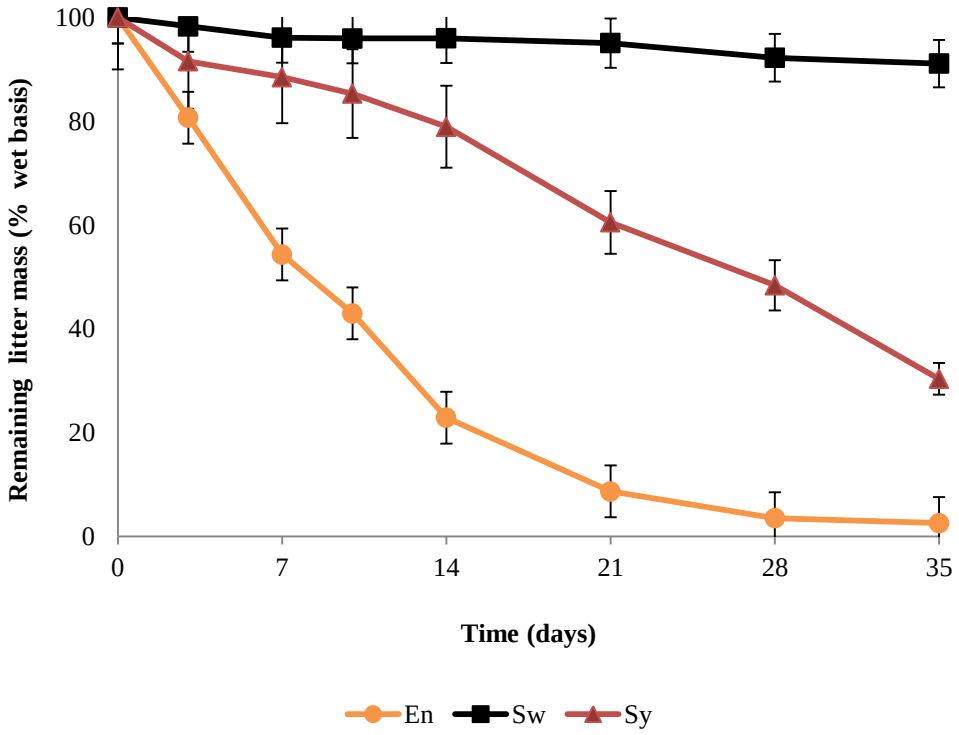



**Figure 5.2.12 Mean (± se) mass (% wet basis) remaining litter in choice chambers of *A. longa* supplied with three types of SRF litter; *E. nitens* (En), *C. sativa* (Sw) and *A. pseudoplatanus* (Sy) over a period of 35 days.**

Figure 5.2.13 shows the pattern of leaf litter removal from choice chambers by *L. terrestris* supplied with three SRF species over a period of 35 days. The experiment started with mean litter mass (wet basis) in individual food tubes as; *E. nitens* (1.32g), *C. sativa* (1.41 g) and *A. pseudoplatanus* (1.32 g). At termination of the experiment, the mean remaining leaf litter in individual food tubes were recorded as; *E. nitens* (9%), *C. sativa* (87%) and *A. pseudoplatanus* (15%) which equated to mean leaf litter removal of 91%, 13% and 85% respectively (see Table 5.2.6 for statistical analysis). Figure 5.2.14 demonstrates leaf litter removal by *L. terrestris* in choice chamber experiment 2 after 10 and 35 days.

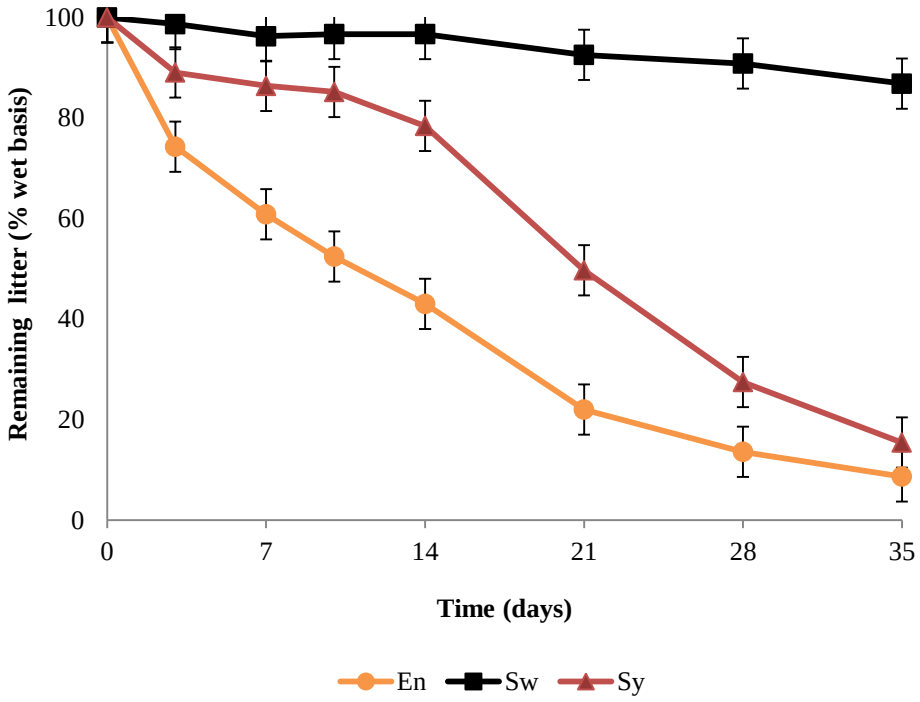
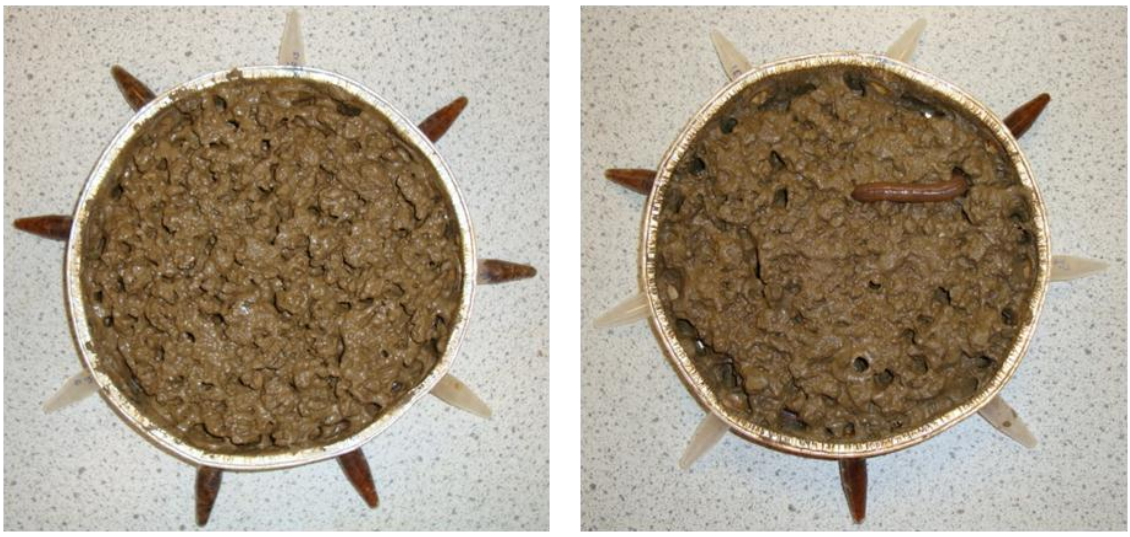



**Figure 5.2.13 Mean (± se) mass (% wet basis) remaining litter in choice chambers of *L. terrestris* supplied with three types of SRF litter over a period of 35 days [SRF species abbreviation as for Figure 5.2.12].**



(a) (b)

**Figure 5.2.14 Leaf litter removal from food tubes by *L. terrestris* supplied with three types of litter materials; *A. pseudoplatanus, C. sativa* and *E. nitens*; In (a) all three *E. nitens* litter tubes emptied after 10 days, and in (b) all three *C. sativa* food tubes remain full even after 35 days (tail of one worm visible at the surface).**

Table 5.2.6 shows the significant differences ($p < 0.05$) of remaining leaf litter (%) in choice chambers of each earthworm species at 50% food removal. The time taken to remove 50% of total litter supplied was 28 days for *A. longa* and 21 days for *L. terrestris*.

**Table 5.2.6 Mean remaining leaf litter (% wet basis) in choice chambers at 50% of total litter removal [Abbreviation of SRF species as for Figure 5.2.12]**

| Earthworm spp. | SRF spp. | | |
|---|---|---|---|
| | **En** | **Sw** | **Sy** |
| *A. longa* | $3.7^{c}$ | $92.3^{a}$ | $48.5^{b}$ |
| *L. terrestris* | $22.1^{c}$ | $92.8^{a}$ | $49.3^{b}$ |

Different letters in the same row indicate significant differences ($p < 0.05$, $n = 12$), ANOVA, Tukey-Kramer.

Based on the results presented in Table 5.2.6, the SRF litter preference order by *A. longa* and *L. terrestris* are given in Table 5.2.7.

**Table 5.2.7 Selected SRF litter preference by selected species of earthworms**

| Earthworm species | SRF litter preference order |
|---|---|
| *A. longa* | En > Sy > Sw |
| *L. terrestris* | En > Sy > Sw |

*E. nitens* (En), *A. pseudoplatanus* (Sy), and *C. sativa* (Sw).

### 5.2.3.3 Choice Chamber Experiment 3

Figures 5.2.15 and 5.2.16 illustrate the temporal pattern of leaf litter removal by two species of earthworms supplied with three types of initially most preferred SRF litter materials (from experiment 1). At the termination of the experiment (after 35 days for *A. longa* and 28 days for *L. terrestris*), 100% survival was recorded for both species. Earthworm species recorded a mass gain for *A. longa* (52%) and *L. terrestris* (13%) by the end of the experiment (see Table 5.2.8).

**Table 5.2.8 Summary of earthworm parameters for Choice Chamber Experiment 3**

| **Earthworm attribute** | ***A. longa*** | ***L. terrestris*** |
|---|---|---|
| Number (Ind tray$^{-1}$) | 1 | 1 |
| Initial mean mass (g tray$^{-1}$) | 1.52 | 4.11 |
| Final mean mass (g tray $^{-1}$) | 2.30 | 4.64 |
| Individual mean mass change (% of starting mass) | 52 | 13 |
| Survivorship (%) | 100 | 100 |

Figure 5.2.15 shows the pattern of leaf litter removal from choice chambers by *A. longa* over a period of 35 days. The experiment started with mean (wet basis) leaf litter of *A. glutinosa* (1.43 g), *B. pendula* (1.38 g), and *F. excelsior* (1.39 g) in individual food tubes. At termination of the experiment, the mean remaining leaf litter in food tubes was recorded as *F. excelsior* (18%) *A. glutinosa* (22%) and *B. pendula* (24%) which equated to mean leaf litter removal of 82%, 78% and 76% respectively (see Table 5.2.9 for statistical analysis).

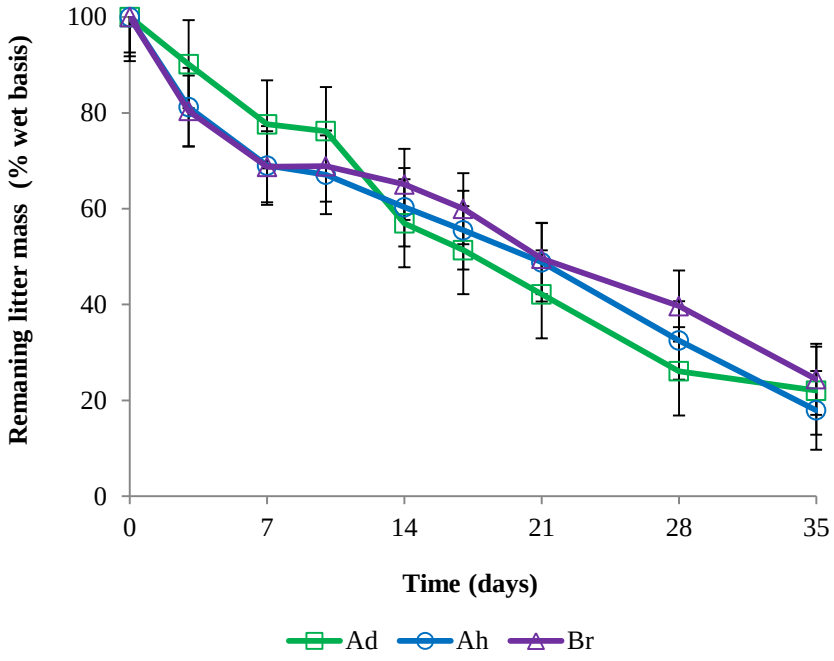



**Figure 5.2.15 Mean (± se) mass (% wet basis) remaining litter in choice chambers of *A. longa* supplied with three types of SRF litter; *A. glutinosa* (Ad), *F. excelsior* (Ah) and *B. pendula* (Br) over a period of 35 days.**

Figure 5.2.16 shows the pattern of leaf litter removal by *L. terrestris* from choice chambers over a period of 28 days. The experiment started with mean (wet basis) leaf litter of *A. glutinosa* (1.44 g), *B. pendula* (1.39 g), and *F. excelsior* (1.38 g) in individual food tubes. Total leaf litter removal was recorded for *B. pendula* after 21 days and for *F. excelsior* after 28 days. At the end of the experiment, the remaining leaf litter for *A. glutinosa* was recorded (6%), which equated to the mean litter removal of 94% (see Table 5.2.9 for statistical analysis).

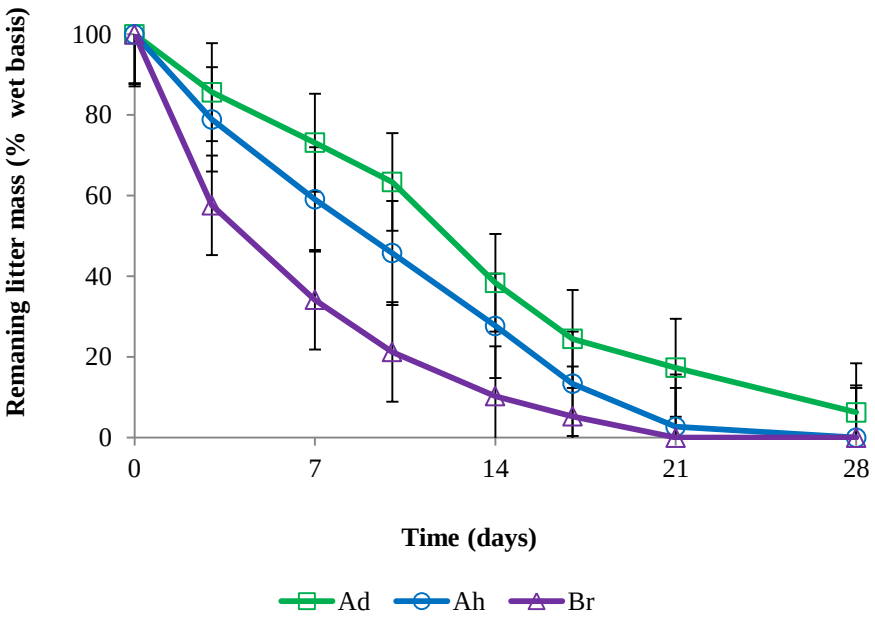



**Figure 5.2.16 Mean (± se) mass (% wet basis) remaining litter in choice chambers of *L. terrestris* supplied with three types of SRF litter; *A. glutinosa* (Ad), *F. excelsior* (Ah) and *B. pendula* (Br) over a period of 28 days.**

Table 5.2.9 shows the significant differences ($p < 0.05$) in remaining leaf litter (%) in choice chambers for both earthworm species at 50% food removal. The time taken to remove 50% of total litter supplied was recorded as; *A. longa* (21 days) and *L. terrestris* (10 days). The remaining litter material showed no significant difference ($p > 0.05$) for *A. longa* at the 50% removal point. However, for *L. terrestris*, the remaining leaf litter was significantly lower ($p < 0.05$) for *B. pendula*, i.e. more birch was removed.

**Table 5.2.9 Mean remaining leaf litter (% wet basis) in choice chambers of earthworms at 50% litter removal [Abbreviation of SRF species as for Figure 5.2.16]**

| Earthworm species | SRF species | | |
|---|---|---|---|
| | **Ad** | **Ah** | **Br** |
| *A. longa* | 41.9[a] | 48.7[a] | 49.6[a] |
| *L. terrestris* | 63.4[a] | 45.8[a] | 21.4[b] |

Different letters in a row indicate significant differences ($p < 0.05$, $n = 12$) ANOVA, Tukey-Kramer.

Based on the results presented in Table 5.2.9, the SRF litter preference order by selected species of earthworms, are given in Table 5.2.10.

**Table 5.2.10 Selected SRF litter preference by selected species of earthworms**

| Earthworm species | SRF litter preference order |
|---|---|
| *A. longa* | Ad = Ah = Br |
| *L. terrestris* | Br > Ah = Ad |

*A. glutinosa* (Ad), *F. excelsior* (Ah), and *B. pendula* (Br)

The appropriate controls prepared without earthworms showed no significant moisture variation throughout the duration of the experiments.

### 5.2.4 Discussion

#### 5.2.4.1 Choice Chamber Experiment 1

The results from this experiment suggested that native British earthworms; *A. chlorotica, A. caliginosa, A. longa* and *L. terrestris* had a clear leaf litter selection and they preferred leaf litter of *A. glutinosa, B. pendula, F. excelsior* over *A. pseudoplatanus, C. sativa* and *E. nitens.* Anecic *L. terrestris* and *A. longa,* which feed directly on litter materials, demonstrated a rapid and clear pattern of leaf litter removal from choice chambers. Endogeic *A. caliginosa,* which feeds on mineral soils, showed a relatively slow, but clear pattern of litter removal from choice chambers. Endogeic *A. chlorotica,* which is predominantly geophagous, recorded the slowest litter removal from choice chambers. However, both endogeic earthworm species indicated a similar pattern of litter removal to experimental anecic earthworms. The differences in rate of litter removal by different species of earthworms could be associated with feeding behaviour of different ecological groupings and their physical size.

Doube *et al*. (1997a) used a similar type of choice chamber approach to study organic matter selection behaviour of four species of earthworms; *A. caliginosa, A. longa, L. rubellus* and *L. terrestris*. Each species of earthworm was provided with nine potential food types; four types of organic matter (cow dung, sheep dung, decomposed leaf litter, sewage sludge) either alone or mixed with sandy loam soil (1:4 organic matter: soil on a dry weight basis), soil alone was also used as a food source. All earthworm species demonstrated a clear preference for pure mineral soil over pure organic matter. Also, soil-organic mixtures were clearly preferred over pure organic sources, with leaf litter plus soil being the preferred mixture for all four earthworm species tested. *L. terrestris*

showed an equal preference for soil, and a litter plus soil treatment where litter was mainly *A. pseudoplatanus*. However, this study did not use a soil-mediated environment for earthworms and instead, authours introduced earthworms to damp filter papers. As a result, the first choice of earthworms was soil, which was a potential food source, but may also have simply been a place of refuge for these soil dwelling animals.

Neilson and Boag (2003) used a feeding chamber approach and offered soils and seven species of plant materials from (Scottish upland pasture) to earthworms to determine if any dietary preference existed. The food sources used were foliage of *Agrostis capillaris, Cerastium fontanum, Holcus lanatus, Lolium perenne, Poa annua, Ranunculus repens* and *Trifolium repens.* The six earthworm species used were; *A. chlorotica*, *A. caliginosa*, *A. longa, L. rubellus*, *L. terrestris* and *Octolasion cyaneum.* Comparable to the current study, it also showed that earthworms with a low biomass (*A. chlorotica*) removed the least amount of food while bigger earthworm (*A. longa* and *L. terrestris*) removed most food. This study suggested that the amount of food removal was positively correlated with body size of earthworm species. It also recorded that *L. terrestris* preferred *P. annua* and rejected soils compared to other available food types. *A. longa* had no obvious food preference, but rejected *R. repens* compared with *L. perenne* and soil. *A caliginosa* had no single preferred food choice while *A. chlorotica* removed noticeably less soil than other available food. The study recorded an unclear reason for selective feeding of available plant material as all materials were very similar in C:N ratio and nutritional quality. However, chemical analyses for other possible chemical compounds which might affect earthworm food selection were not conducted.

The N content, C: N, lignin content, phenolic compounds (tannins), calcium content and protein content in plant materials have been considered to influence selective feeding by

earthworms (Edward and Heath, 1963; Satchell and Lowe, 1967; Hendriksen, 1990). The hardness, hairiness, water content even shape and colour of the litter material are also known to influence earthworm food selection (Darwin, 1881; Satchell and Lowe, 1967). Of the six SRF species considered in the current experiment, *C. sativa* litter was the least preferred food for all four earthworms. Litter chemistry results suggested that particularly low total N, Ca content and highest C: N in *C. sativa* litter material can be associated with its lesser preference by earthworms (Satchell and Lowe, 1967; Hendriksen, 1990; Reich *et al.*, 2005; Hobbie *et al.*, 2006). However, the differences in selection of other SRF litter materials cannot be explained only by the above litter quality parameters particularly, low earthworm preference for *A. pseudoplatanus* litter which had a high level of N and Ca similar to other preferred species. Other chemical analyses for lignin content and phenolic compounds (tannins), which have a potential influence on earthworm food selection might have assisted here, but were not performed in the current study. Satchell and Lowe (1967) and Hendriksen (1990) further suggested that earthworm litter selection can be influenced by the relative state of decomposition/weathering of litter materials. This cannot have influenced litter selection in the current experiment, as freshly fallen litter was used. Food particle size can influence earthworm food intake, hence their growth and reproduction (Boström and Lofs-Holmin, 1986; Boyle, 1990). Lowe and Butt (2003) suggested that the influence of food particle size on earthworm growth is both species and life stage specific. This study further recorded that smaller earthworms benefit more from reduced particle size. Boyle (1990) recorded that food particle size was not important for *L. terrestris,* but it influenced *A. caliginosa* growth. This study recorded that *A. caliginosa* fed with food particles < 0.2 mm, doubled in weight after 150 days compared with animals fed with food particles between 0.2 – 1.0 mm. The possible effect of food particle size in the current experiment was minimised by using a uniform size of litter particles (1 - 2 mm).

However, this size could have negatively influenced the rate of litter removal from choice chambers by small earthworms such as *A. caliginosa* and *A. chlorotica*, even though adult animals were used.

#### 5.2.4.2 Choice Chamber Experiment 2

The results from this experiment suggest that of the three less preferred SRF species, the first choice for both *L. terrestris* and *A. longa* was *E. nitens*. The second choice was *A. pseudoplatanus* whilst final and least preferred was *C. sativa.* These results confirm that earthworm leaf litter selection is non-random as it provided three food tubes for each SRF species to each choice chamber which was not applied in experiment 1. This also authenticates the less preferred SRF litter selection sequence as it had no influence from preferred SRF species which existed in Experiment 1. The least preference for *C. sativa* can be equated with litter chemistry analyses, as explained in section 5.2.3.1., but, preference for *E. nitens* over *A. pseudoplatanus* cannot be explained with reference to N, Ca content or C:N ratio difference. Further chemical analyses for lignin, phenolic compounds (tannins), protein and carbohydrate content in leaf litter are recommended to identify the reason for the given preferential sequence of the selected earthworms for SRF litter.

#### 5.2.4.3 Choice Chamber Experiment 3

The results suggest that *A. longa* has an equal preference for native *A. glutinosa, B. pendula* and *F. excelsior*. However, *L. terrestris* preferred *B. pendula* over *A. glutinosa,* and *F. excelsior*. In the first experiment, both earthworm species removed all three species of native SRF litter rapidly (within 5 - 7 days) where earthworm numbers were

four and three per choice chamber for *A. longa* and *L. terrestris*, respectively, so did not permit a preferential sequence to be established. To slow down the litter removal rate and the potential competition between individuals, the earthworm number was reduced to one per choice chamber for both species in Experiment 3. Therefore, in this experiment, *A. longa* took 35 days to remove 84% of litter and *L. terrestris* took 28 days to remove 98% litter from the total supply. Most of the previous earthworm-tree studies (e.g Satchell and Lowe, 1967; Hendriksen, 1990; Muys *et al*., 1992) have shown that *Alnus* and *Fraxinus* litter was highly palatable to earthworms. Butt (2011), in laboratory experiments, suggested that *B. pendula* was a highly palatable source of food for *L. terrestris* which is in line with current findings.

The novel leaf litter choice chamber approach which recorded 100% earthworm survival throughout the series of experiments was successful in determining earthworm food selection behaviour. This was the first choice chamber experiment which provided a soil-mediated environment for experimental earthworms. Further, this was the first record of using a choice chamber approach to compare earthworm preference for forest tree litter.

This study demonstrated that a choice chamber approach was more useful for larger, litter feeding earthworms such as *L. terrestris* and *A. longa* than small geophagous earthworms such as *A. caliginosa* and *A. chlorotica*. However, the experimental system was associated with a few practical difficulties. Sometimes soil particles entered the food tubes (through earthworm activities). Also small earthworms (especially *A. chlorotica*) sometimes took up residence within food tubes. On some occasions earthworm casts were also observed within food tubes. These practical issues were mostly associated with the smaller earthworms. Because of this, a slight mass increment

of food tubes/remaining litter within the experimental period was recorded for *A. chlorotica* (see Figure 5.2.8) which was unexpected, but could therefore be explained.

Summary of the major findings of the leaf litter choice chamber experiments:

- This work suggested that native British earthworms preferred native *A. glutinosa B. pendula* and *F. excelsior* litter over non-native *A. pseudoplatanus, C. sativa* and *E. nitens* litter.
- All selected earthworms showed least preference for poor quality (e. g. low in N, Ca and high in C:N) *C. sativa* litter.
- Litter-feeding British earthworms preferred *E. nitens* more than *A. pseudoplatanus* and *C. sativa.*
- Litter-feeding British earthworms equally preferred *A. glutinosa, B. pendula* and *F. excelsior* litter.

## 5.3 A webcam technique to observe SRF litter choice by *L. terrestris*

### 5.3.1 Introduction

A relatively inexpensive webcam recording technique was used to observe SRF litter selection behaviour of *L. terrestris*. This particular earthworm species was purposely selected as these animals are capable of using their mouth to carry leaves and stones from the surrounding environment to their burrow. A webcam technique permitted recording and observation of night time foraging behaviour of individual earthworms at the soil surface. Similar types of techniques have been used in the past for different earthworm behavioural studies. Video recording was successfully used by Nuutinen and Butt (1997) to observe *L. terrestris* mating behaviour. Butt *et al*. (2005) used infrared video recording to observe night time foraging behaviour of the same earthworm species supplied with three types of organic materials; board-mill sludge, barley straw and board-mill sludge plus chicken manure. More recently, a webcam technique was used by Valckx *et al*. (2010) to observe the night time surface activity and dispersal behaviour of *L. terrestris*. In the current study, a series of infrared webcam recordings were made with the following objectives:

a) To observe the surface leaf litter selection behaviour of *L. terrestris* in darkness.
b) To provide direct evidence that SRF litter selection by *L. terrestris* is non-random.
c) To compare the direct observation results with indirect choice chamber results of *L. terrestris*.

### 5.3.2 Materials and methods

Circular plastic vessels of 20 L (depth 0.4 m, diameter 0.3 m) were half-filled with 10 L of moist (25%), sterilised Kettering loam (Butt *et al*., 1994; Butt, 2011) and compacted manually to have a smooth surface and similar bulk density (approx. soil depth was 0.2 m). Then, using a standard pencil, an artificial burrow was created from soil surface to the base, in the centre of the each soil-filled vessel. Individual, field collected adult *L. terrestris* (mean mass 5.05 g) were introduced into the previously made artificial burrows (one individual per vessel, i.e. tail inserted first to encourage settlement in the burrow). Then, dry barley straw *(Hordeum vulgare)* was cut into 0.02- 0.04 m pieces and sprinkled on to the soil surface (1 g vessel$^{-1}$) and moistened by water sprayed on to the surface of each vessel. Before the experiment, the adult *L. terrestris* (n = 48) had been acclimated to laboratory conditions as described in section 4.2.3.4.

**Figure 5.3.1 Initial earthworm (20 L) vessel preparation for the webcam experiment; an adult *L. terrestris* was introduced to the artificial burrow made at the centre of each vessel with barley straw sprinkled on the surface for potential midden formation.**

Earthworm vessels were closed with lids (ventilation was facilated by tiny holes made with mounted needle) and kept undisturbed for about 2 weeks to allow permanent burrow and midden formation. Straw particles were provided to encourage midden formation to prevent collection of leaf litter for this purpose during the experiment. Vessels were observed daily and supplied, as necessary, with further moisture. Any burrow made at the side of the vessels was filled using additional soil and pressed down manually. This procedure encouraged each earthworm to make its permanent burrow and midden at the centre of the experimental vessel. After 2 weeks, most of the experimental earthworms (Approx. 70%) made their permanent burrow and midden at the centre of the vessel. Those vessels, which had a clear central midden (see Figure 5.3.2) were used for webcam recording. Fluorescent lights provided a 12 hour daylight period (6.30 - 18.30) to experimental vessels. Experimental vessels were adapted to this 12:12 hour day/night cycle at least 2 days before experimental recording.

**Figure 5.3.2 A clear *L. terrestris* midden constructed with straw particles (observed in the centre of an earthworm vessel after 2 weeks) (Scale 1:1).**

To reduce any bias associated with leaf shape or size, homogeneous leaf shapes were offered to the earthworms in this experiment. Previously collected, air-dried leaves of selected SRF species were water-soaked for 30 minutes. Then using a metal cork borer (Fisher Scientific, UK), 10 mm diameter leaf disks were cut (see Figure 5.3.3). This was a similar practice used by Satchell and Lowe (1967). When cutting leaf disks, the central main vein of the leaf was avoided.

**Figure 5.3.3 The 10 mm diameter leaf circles cut with metal cork borer (from *C. sativa*) for use in webcam behaviour experiment.**

Before recording, any remaining straw particles in experimental vessels, not used for midden formation, were removed. The surrounding clear area was then divided into 4 zones (see Figure 5.3.4). These zones were identified with four straw pieces placed at the edge of the vessel, but had no physical barrier. The selected species of leaf disks were randomly arranged in each zone as required for each experiment. The experimental vessels were sprayed with sufficient water to prevent surface drying, before webcam recording began, over a period of 12 hours in darkness.

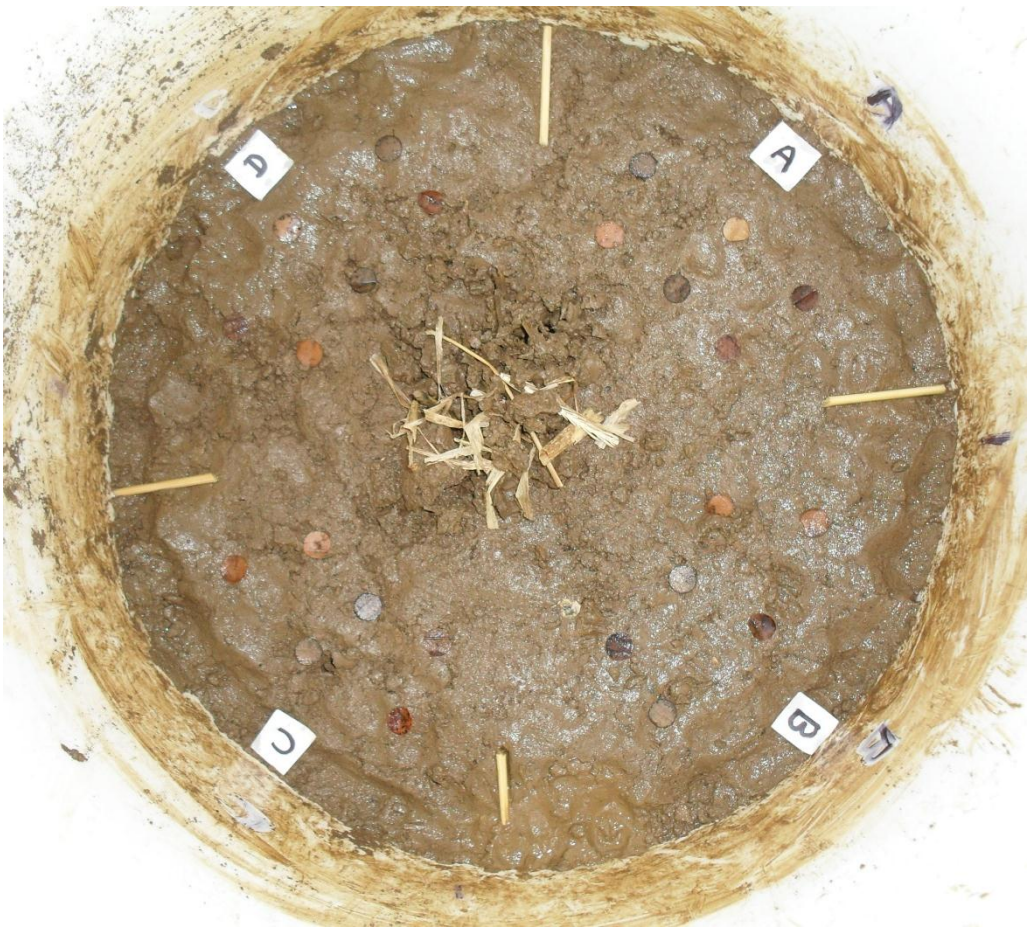



**Figure 5.3.4 Leaf disks arrangement within four zones (A, B, C and D; labels removed before webcam recording) of the earthworm vessel. An earthworm burrow and midden made with previously introduced straw at the centre of the vessel.**

The night time foraging/leaf litter selection behaviour was studied in a room where ambient temperature varied from 15 - 18 °C. Infrared webcam recording of two earthworm vessels were simultaneously carried out with two individual IP webcams (VSTARCAM$^{TM}$ Model: F6836W equipped with 640 x 480 resolutions-pixels) mounted on laboratory retort stands as shown in Figure 5.3.5. Camera height was adjusted so that each captured the surface of a single vessel with a sufficient level of detail. Figure 5.3.6 shows an example of infrared night vision of the surface of an experimental

earthworm vessel through the webcam.The software provided with the camera was installed to a laptop computer to record images of each session.


**Figure 5.3.5 Webcam setting for infrared recording of *L. terrestris* surface leaf litter selection behaviour at the soil surface.**

The 12 hours of darkness (18.30 – 6.30) in a 12:12 hour light/dark cycle were used for infrared webcam recording. Two consecutive nights per each active individual were recorded. Any inactive earthworm vessels were removed after two nights recording. Some of the inactive vessels were re-examined after a few days, but if still inactive for a second time, they were permanently removed from the experiment.

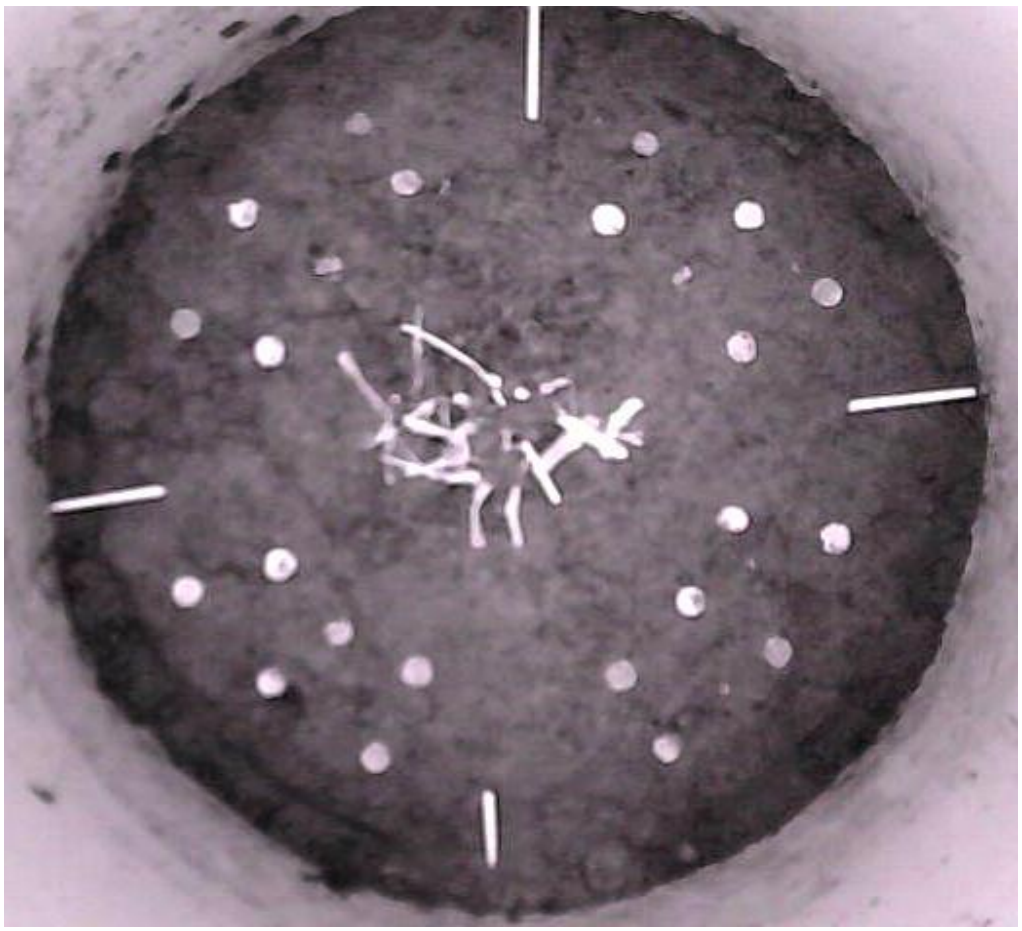


**Figure 5.3.6 An example of infrared night vision view of the surface of an experimental earthworm vessel through the webcam.**

Three sets of webcam recording experiments were conducted to assess SRF litter selection behaviour and allow comparison with previous choice chamber results for *L. terrestris*.

### 5.3.2.1 Webcam Recording Experiment 1

This experiment was conducted to observe and record litter selection behaviour by *L. terrestris* supplied with six types of SRF litter materials; alder (*A. glutinosa*), ash (*F. excelsior*), birch (*B. pendula*), eucalyptus (*E. nitens*), sweet chestnut (*C. sativa*), and sycamore (*A. pseudoplatanus*). Sixteen earthworm vessels were initially prepared as

described above. Of these, only eleven made a burrow and midden at the centre which could be used for webcam recording. One disk from each of the six SRF species were randomly arranged within each of the four zones around the midden, in known initial positions. Each vessel therefore had a total of 24 litter disks. Infrared webcam recording was undertaken using two webcams as described above. In this series, eleven earthworm vessels had to be recorded to obtain five satisfactory replicates of two consecutive night activity.

#### 5.3.2.2 Webcam Recording Experiment 2

This experiment was conducted to observe and record litter selection behaviour by *L. terrestris* supplied with three types of SRF litter materials; eucalyptus (*E. nitens*), sweet chestnut (*C. sativa*), and sycamore (*A. pseudoplatanu*). A new set of 14 earthworm vessels were prepared as described in section 5.3.2. Ten earthworms made a burrow and midden at the centre of the vessel which could be used for webcam recording. The litter disks from three SRF species were randomly arranged within each of the four zones around the midden (each zone receiving a total of six litter disks: two from each SRF species). The 12 hours infrared webcam recording was undertaken at night as described in section 5.3.2. A total of ten earthworm vessels were recorded to obtain five satisfactory replicates.

#### 5.3.2.3 Webcam Recording Experiment 3

This experiment was conducted to observe and record litter selection behaviour by *L. terrestris* supplied with three types of SRF litter materials; alder (*A. glutinosa*) ash (*F. excelsior*), and birch (*B. pendula*). A new set of 12 earthworm vessels were prepared as described above. Eight of theses made a burrow and midden in the centre which could

be used for recording. The litter disks (n = 2) from the three different SRF species were randomly arranged within each zone around the midden. Each vessel received a total of 24 litter disks (8 from each SRF species). The 12 hour infrared webcam recording was continued in darkness as described in section 5.3.2. A total of eight vessels were recorded to obtain five satisfactory replicates.

#### 5.3.2.4 Statistical analysis

SRF preference was assessed by observing the webcam recording of leaf litter selection behaviour. The earthworm vessels which had more than 50% of the initially provided leaf disks removed after two nights were selected for data collection. Earthworm night time foraging activity on SRF leaf disks was divided into three major categories/incidents: (1) Taken into Burrow (TB); (2) Moved and Abandoned (MA) and (3) Rejection (RJ). TB included carrying leaf disks to the burrow mouth and disappearing within it. MA included moving towards the burrow and abandoning. RJ included all other encounters/touches which were not included in either TB or MA categories. TB was considered to be associated with earthworm preference, RJ with non-preference and MA with indecision. Non parametric Kruskal-Wallis tests were used to compare medians of incidents, to test whether there was a significant difference in terms of SRF species.

### 5.3.3 Results

#### 5.3.3.1 Webcam Recording Experiment 1

Figure 5.3.7 demonstrates the number of total incidents recorded by *L. terrestris* throughout Webcam Recording 1, when six types of SRF litter disk were provided. A total of 92 incidents were recorded during the experiment as; Taken into Burrow - TB (39), Moved and Abandoned - MA (21) and Rejection - RJ (32). TB incidents for different SRF species recorded as *A. glutinosa* (9), *B. pendula* (9), *F. excelsior* (8), *A. pseudoplatanus* (5) *C. sativa* (5) and *E. nitens* (3). RJ incidents were recorded as *C. sativa* (8), *A. pseudoplatanus* (7), *E. nitens* (7), *A. glutinosa* (4), *B. pendula* (4), and *F. excelsior* (2).

Figure 5.3.7 and 5.3.8 clearly demonstrate that TB incidents were relatively high for *A. glutinosa, B. pendula, F. excelsior* compared with *A. pseudoplatanus, C. sativa* and *E. nitens.* Conversely, similar figures show that RJ was high for *A. pseudoplatanus, C. sativa* and *E. nitens* compared with *A. glutinosa, B. pendula,* and *F. excelsior*. As shown in Figure 5.3.7, MA had high representation for *B. pendula* and low for *C. sativa*. Table 5.3.1 shows that there was no significant difference ($p > 0.05$) in median values of each incident with respect to different SRF species. However, recorded median value for TB high for *A. glutinosa, B. pendua, F. excelsior* compared with *A. pseudoplatanus, C. sativa* and *E. nitens.* Figure 5.3.9 shows night foraging behaviour of *L. terrestris* viewed via an infrared web camera.

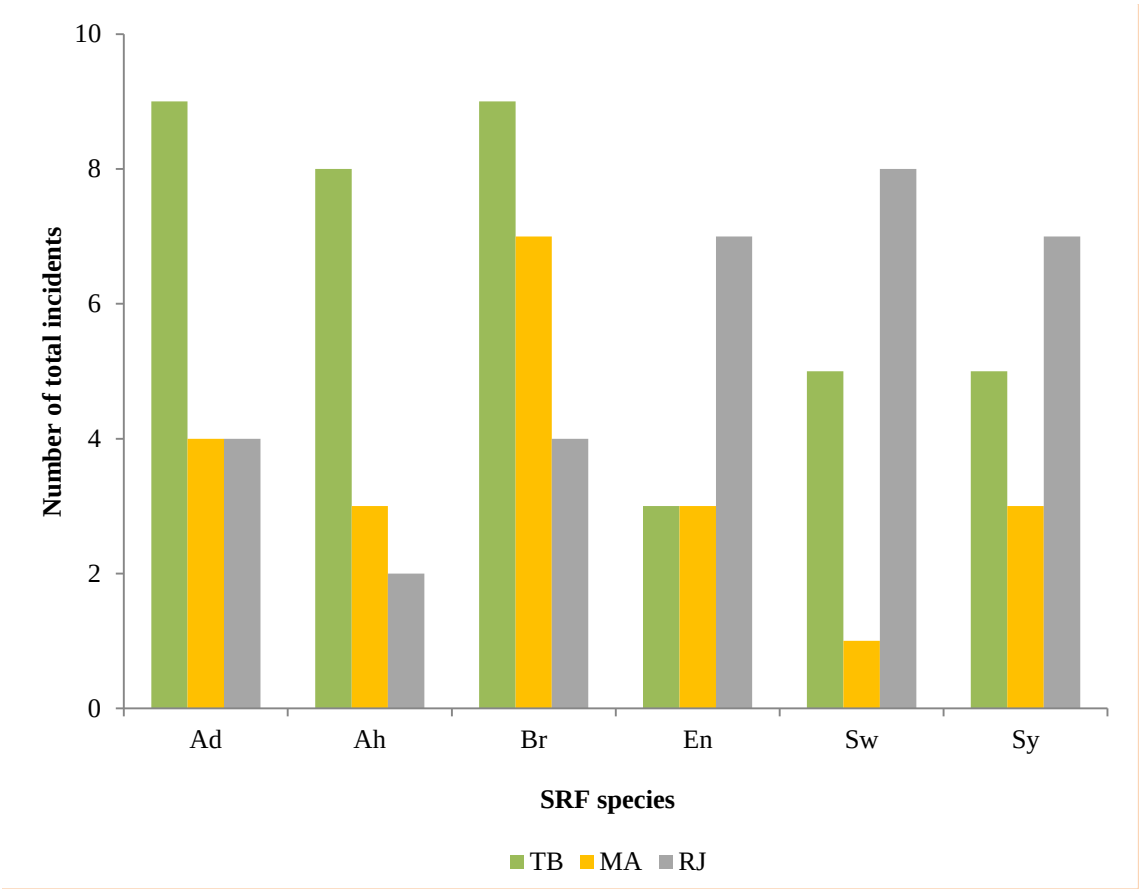
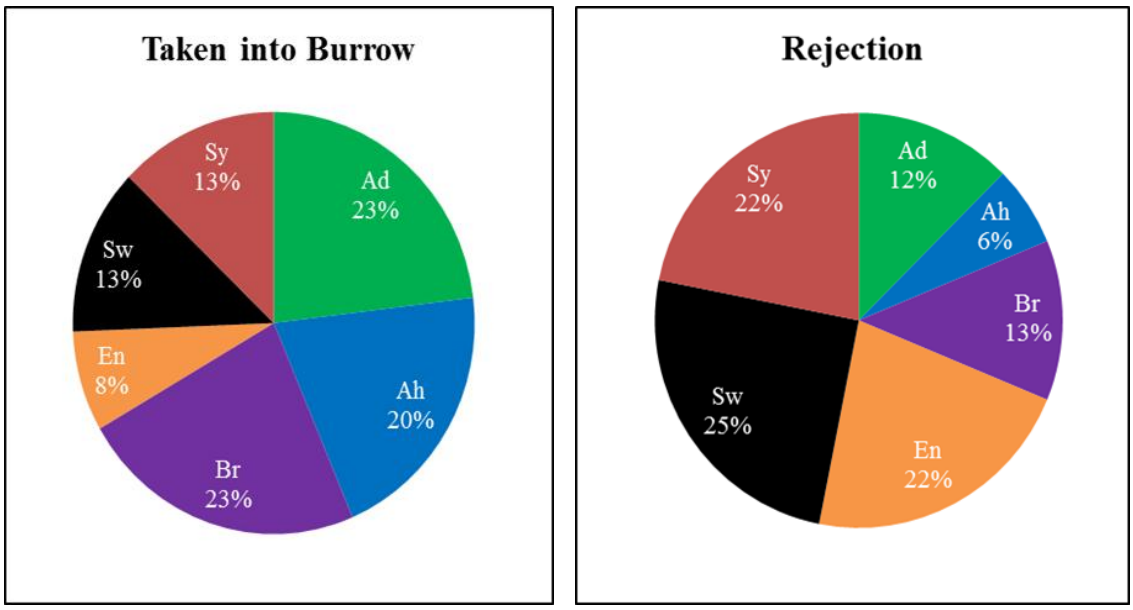



**Figure 5.3.7 Number of total incidents; Taken into Burrow (TB), Moved and Abandoned (MA), Rejection (RJ) recorded by *L. terrestris* throughout webcam experiment 1 - with six types of SRF litter; Ad (*A. glutinosa*), Ah (*F. excelsior*), Br (*B. pendula*), En (*E. nitens*), Sw (*C. sativa*), and Sy (*A. pseudoplatanus*), a total of 120 leaf disks provided as 20 from each SRF species for five active earthworm vessels.**




(a) (b)

**Figure 5.3.8 Percentage contribution of SRF species for each separate incident; (a) Taken into Burrow - total number of incidents = 39, (b) Rejection – total number of incidents = 32, [Abbreviation of SRF species as for Figure 5.3.7].**

**Table 5.3.1 Median of incidents recorded by *L. terrestris* supplied with six types of litter materials [Abbreviation of SRF species as for Figure 5.3.7]**

| **Incident** | **Ad** | **Ah** | **Br** | **En** | **Sw** | **Sy** | ***P*** | **Significance** |
|---|---|---|---|---|---|---|---|---|
| Taken into Burrow | 2 | 2 | 2 | 0 | 1 | 1 | 0.184 | ns |
| Moved and Abandoned | 0 | 0 | 1 | 0 | 1 | 0 | 0.767 | ns |
| Rejection | 0 | 0 | 1 | 1 | 2 | 1 | 0.635 | ns |

(Kruskal-Wallis Test, $p < 0.05$, $n = 5$).

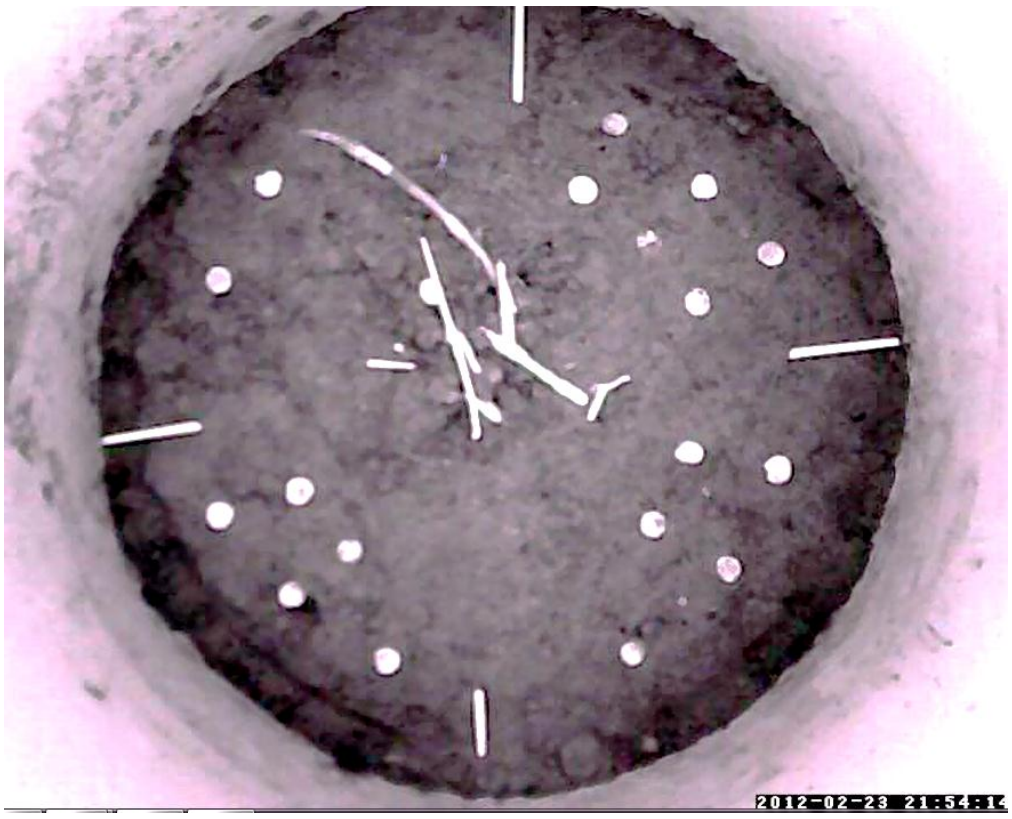



**Figure 5.3.9 Night foraging behaviour of *L. terrestris* viewed via an infrared web camera. The earthworm can be seen emerging from its burrow in the "11 o' clock" position.**

### 5.3.3.2 Webcam Recording Experiment 2

Figure 5.3.10 demonstrates the number of total incidents recorded by *L. terrestris* throughout Webcam Recording 2 supplied with three types of less preferred SRF litter disks (from Webcam Recording 1)**.** A total of 94 incidents were recorded throughout the experiment; Taken into Burrow- TB (50), Moved and Abandoned - MA (10) and Rejection - RJ (34). TB for each SRF species recorded as *E. nitens* (24), *A. pseudoplatanus* (20) and *C. sativa* (6). RJ recorded as *C. sativa* (19), *A. pseudoplatanus* (11), and *E. nitens* (4).

Figures 5.3.10 and 5.3.11 show that TB was high for both *E. nitens* and *A. pseudoplatanus* compared with *C. sativa*, that RJ was highest for *C. sativa.*

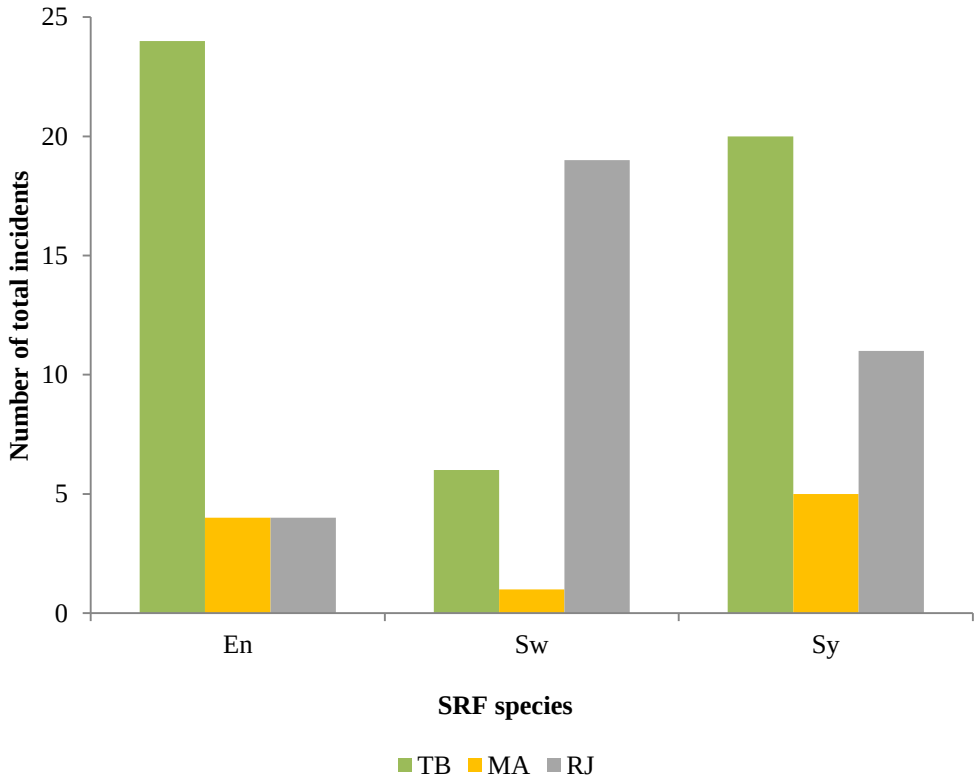
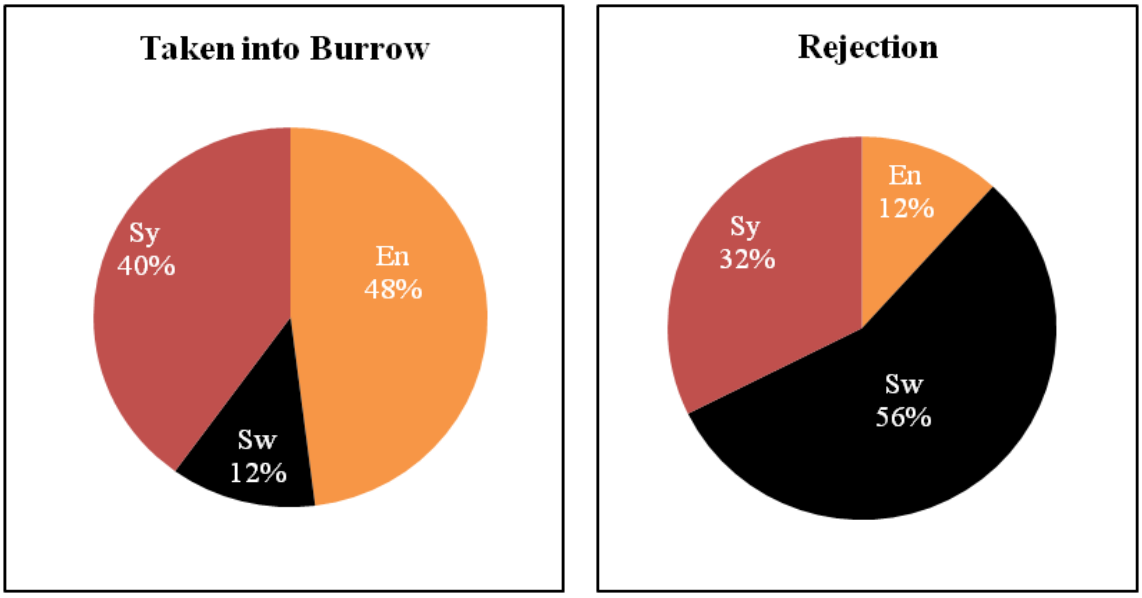



**Figure 5.3.10 Number of total incidents recorded by *L. terrestris* throughout Webcam Recording 2 provided with three types of SRF litter; En (*E. nitens*), Sw (*C. sativa*), and Sy (*A. pseudoplatanu*)s; atotal of 120 leaf disks provided as 40 from each SRF species for five active earthworm vessels [Abbreviation for incidents as for Table 5.3.1].**




**(a)** **(b)**

**Figure 5.3.11 Percentage contribution of SRF species for each separate incident (a) Taken into Burrow - total number of incidents = 50, (b) Rejection - total number of incidents = 34, [Abbreviation of SRF species as for Figure 5.3.10].**

Table 5.3.2 shows comparisons of median values for each incident with respect to SRF species. TB recorded a significant difference ($p < 0.05$) between SRF species. However, RJ and MA did not show any significant differences ($p > 0.05$).

**Table 5.3.2 Median of each incident recorded by *L. terrestris* supplied with three types of SRF litter [Abbreviation of SRF species as for Figure 5.3.10]**

| Incidence | En | Sw | Sy | *p* | Significances |
|---|---|---|---|---|---|
| Taken into Burrow (TB) | 5 | 1 | 4 | 0.018 | * |
| Moved and Abandoned (MA) | 0 | 0 | 1 | 0.391 | ns |
| Rejection (RJ) | 0 | 3 | 1 | 0.068 | ns |

(Kruskal-Wallis Test, $p < 0.05$, $n = 5$).

### 5.3.3.3 Webcam Recording Experiment 3

Figure 5.3.12 demonstrates the number of total incidents recorded by *L. terrestris* throughout Webcam Recording 3, supplied with three types of preferred SRF litter disks (based on results of Webcam Recording 1)**.** A total of 75 incidents were recorded throughout the experiment as Taken into Burrow - TB (53), Moved and Abandoned - MA (8) and Rejection - RJ (14). TB recorded as; *F. excelsior* (19), *B. pendula* (18) and *A. glutinosa* (16). RJ recorded as; *B. pendula* (6) and *A. glutinosa* (5), and as *F. excelsior* (3). Figure 5.3.12 and 5.3.13 show that TB incidents were very similar for these selected SRF species. It is noteworthy that total number of RJ recorded was low throughout this experiment and did not show a clear difference between SRF species.

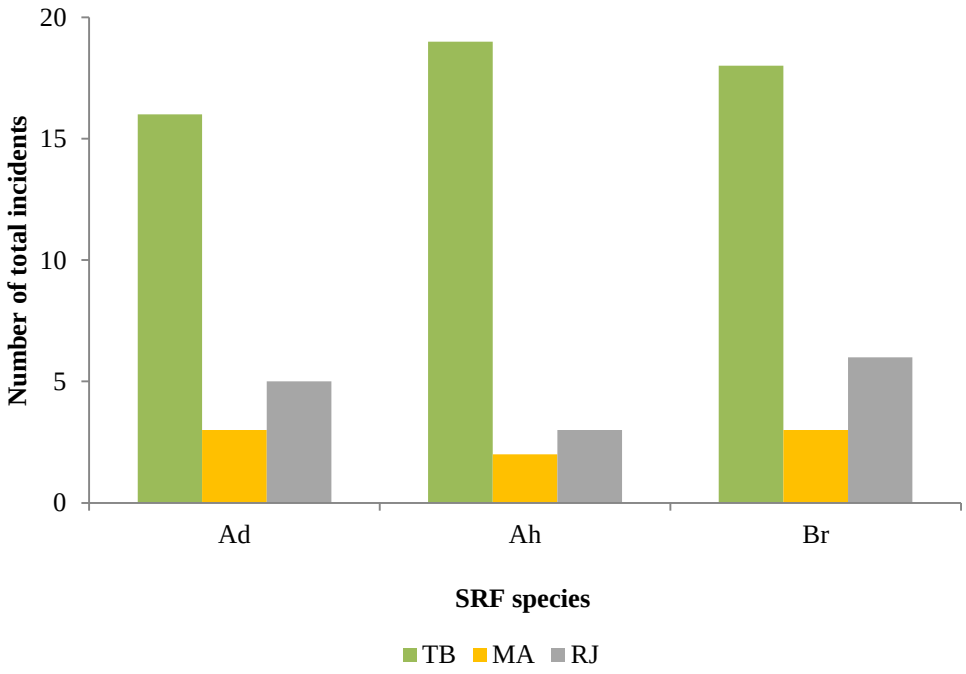



**Figure 5.3.12 Number of total incidents recorded by *L. terrestris* throughout the Webcam Recording 3 provided with three types of SRF litter; *A. glutinosa* (Ad), *F. excelsior* (Ah) *B. pendula* (Br); a total of 120 leaf disks were provided as 40 from each SRF species for five active earthworm vessels [Abbreviation for incidents as for Table 5.3.1].**

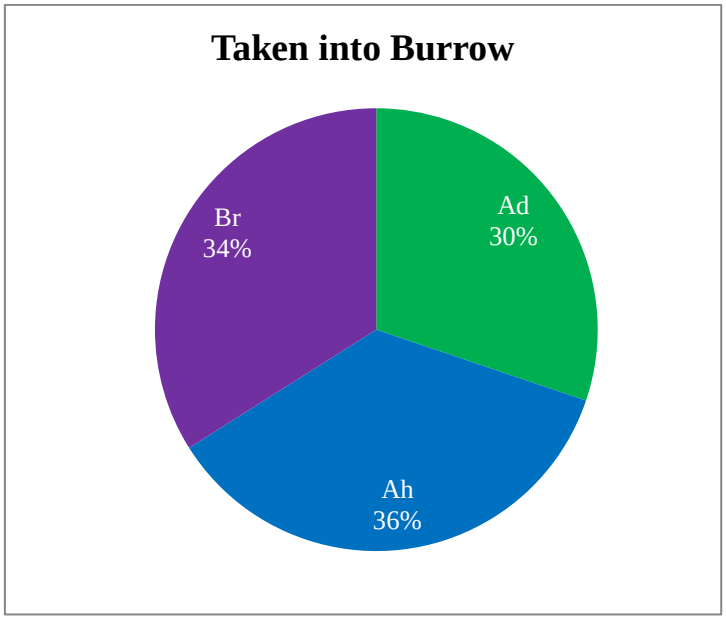



**Figure 5.3.13 Percentage contribution of SRF species for Taken into Burrow- total number of incidents = 53 [Abbreviation of SRF species as for Figure 5.3.12].**

Table 5.3.3 shows that there was no significant difference ($p > 0.05$) in median values for each incident with respect to the SRF species used.

**Table 5.3.3 Median of each incident recorded by *L. terrestris* supplied with three types of SRF litter [Abbreviation of SRF species as for Figure 5.3.12]**

| **Incidences** | **Ad** | **Ah** | **Br** | ***p*** | **Significance** |
|---|---|---|---|---|---|
| Taken into Burrow (TB) | 3 | 4 | 4 | 0.719 | ns |
| Moved and Abandoned (MA) | 0 | 0 | 0 | 0.851 | ns |
| Rejections (RJ) | 0 | 0 | 1 | 0.394 | ns |

(Kruskal-Wallis Test, $p < 0.05$, $n = 5$).

### 5.3.4 Discussion

#### 5.3.4.1 Webcam Recording Experiment 1

The direct observations from this experiment show that *L. terrestris* has clear leaf litter selection behaviour and it is not a random activity. As with the first Choice Chamber Experiment (see Figure 5.2.10), the results from the current experiment suggest that *L. terrestris* preferred leaf litter of *A. glutinosa B. pendula* and *F. excelsior* over *A. pseudoplatanus C. sativa* and *E. nitens.* An interesting phenomenon in this experiment is that both Taken into Burrow (TB) and Rejection (RJ) incidents reflect similar results for earthworm litter preference. Moved and Abandoned (MA) did not reflect a clear trend about the preference, although the records of this incident were highest for *B. pendula* and lowest for *C. sativa.*

Satchell and Lowe (1967) provided uniform disks of leaf material to *L. terrestris* and determined the order of preference by counting the number of litter disks that disappeared in the night. Although, it was not a direct observation method like webcam recording, after a series of experiments, they suggested that *L. terrestris* preferred certain species of leaf litter over others. Similar to current findings, their study suggested that *A. glutinosa* and *F. excelsior* were highly palatable to *L. terrestris*. In contrast, Satchell and Lowe (1967) categorised *A. pseudoplatanus* as highly palatable and *Betula* spp. as a moderately palatable species. The difference in the quality of the litter used in the two sets of experiments could be a major reason for this difference. As an example, the N content of the *A. pseudoplatanus* litter used by Satchell and Lowe (1967) was high (1.99%) compared with 1.45% for the same species used in the current

experiment. The N content in birch (*B. verrucosa)* litter they used was 0.84% compared with 1.59% for the *B. pendula* used in the current experiment.

#### 5.3.4.2 Webcam Recording Experiment 2

The results from the current experiment suggest that *L. terrestris* preferred *E. nitens* more than *A. pseudoplatanus* and *C. sativa*, which coincided with the results of Choice Chamber Experiment 2 (section 5.2.3.2) conducted with the same similar species of earthworm. Confirming the findings from webcam experiment 1, both Taken into Burrow (TB) and Rejection (RJ) incidents reflect a similar result and Moved and Abandoned (MA) did not reflect any preference. However, the percentage of MA in this experiment, which used 3 SRF species, was recorded as low (11%) compared with webcam 1 (23%) which used 6 SRF species. The presence of a lower number of SRF species may, by a reduction in the complexity of the experiment, have influenced earthworm behaviour.

#### 5.3.4.3 Webcam Recording Experiment 3

Equivalent to the third Choice Chamber Experiment, the results from the current experiment suggest that *L. terrestris* has an equal preference for native *A. glutinosa, F. excelsior* and *B. pendula.* Taken into Burrow (TB) incidents were recorded as very high (71%) in this experiment compared with webcam 1 (42%) and 2 (53%). This is almost certainly due to the use of preferred SRF species in this experiment. Moved and abandoned (MA) and Rejection (RJ) records were low (11% and 19% respectively) in this experiment and did not reflect any obvious about preference.

The webcam recording or similar kind of video recording approaches have been successfully used for various earthworm behavioural studies (Nuutinen and Butt, 1997; Butt *et al*., 2005; Valckx *et al*., 2010). But, this was the first time of use for observation of *L. terrestris* tree litter selection behaviour. Further, all other previous studies on earthworm tree litter selection provided information based on indirect techniques such as litterbags or litter disappearance (Edwards and Heath, 1963; Satchell and Lowe, 1967; Hendriksen, 1990). Current webcam recording appears to be the only direct evidence on this aspect of earthworm tree litter selection.

In this series of experiments, construction of a permanent burrow at the centre of the earthworm vessel was encouraged to provide adequate space to arrange leaf disks and prevent any proximity influence on leaf litter selection. Midden formation with straw particles prevented collection of leaf litter disks for the purpose of constructing a midden. This pre-establishment provided a more natural habitat for *L. terrestris* for their night time foraging. Earthworms are known to prefer plant leaves of particular shape (Darwin, 1881). To eliminate that factor, uniform leaf disks (diameter 10 mm) were used throughout the experiment as suggested by Satchell and Lowe (1967). The leaf disk arrangements in four zones around the burrow were set up to cover each part of the vessel with each species of litter. The zonal arrangement made species identification straight forward through the webcam recording and also prevented any influence of earthworm emergence and movement pattern in similar directions (which was observed through pre-trials) on litter selection behaviour.

The use of infrared sensitive web cameras proved to be inexpensive and immediately allowed observation of *L. terrestris* litter selection activity without interfering with the animal's natural behaviour. The camera had continued infrared LED illumination during

recording and it had already been demonstrated that red light and longer wavelength radiation have no effect on earthworm activity (Valckx *et al*., 2010). The requirement of a PC, internet facility and large amounts of storage capacity to save images were some of the limitations of this technique. In addition to technical issues, individual earthworm behaviour influenced the efficiency of the recording. Individual earthworm behaviour was unpredictable; some did not emerge at all, while some emerged from side burrows thus invalidating any true choices. These kinds of incidents need to be allowed for during such experiments. The large human workload associated with viewing and reviewing of recorded material was another factor which influenced the efficiency of this experimental technique.

Summary of the findings of webcam recording experiments:

- *L. terrestris* had clear leaf litter selection behaviour and it was not a random activity.
- This supports the results of choice chamber experiments;
  - *L. terrestris* preferred native *A. glutinosa B. pendula, F. excelsior* over non-native *A. pseudoplatanus, C. sativa* and *E. nitens* and showed the least preference for *C. sativa.*
  - *L. terrestris* preferred *E. nitens* litter more than *A. pseudoplatanus* and *C. sativa* litter, while it demonstrated an equal preference for *A. glutinosa, B. pendula* and *F. excelsior* litter.

# CHAPTER 6: SRF LITTER DECOMPOSITION, CARBON AND NITROGEN RELEASE BY EARTHWORMS

## 6.1 Introduction

Decomposition of plant litter is essential for the transfer of nutrients and energy in any terrestrial ecosystem dominated by plants (Irmler, 2000). Litter decomposition regulates the release of plant nutrients, build-up of soil organic matter and flux of $CO_2$ from soil (Zhang *et al*., 2008). In many forest systems, foliar litter is the main input of organic carbon into soils and its decomposition is the major supply of nutrients for tree growth (Zhang *et al*., 2008). The understanding of litter decomposition processes and governing factors are important for studying nutrient cycling, developing carbon budgets and assessing the implications for climate change (Zhou *et al*., 2008). The key factors regulating decomposition are commonly recognised as litter quality, microclimate and decomposer community (Swift *et al*., 1979; Meentemeyer, 1984).

Earthworms are known to provide major contributions to overall breakdown and incorporation of tree litter within mineral soils in many temperate woodlands (Satchell, 1967; Scheu and Wolters, 1991; Benham *et al*., 2012) (see section 2.3.2.1 for details). These animals greatly contribute to carbon cycling and nutrient mineralisation processes (Raw, 1962; Satchell, 1967; Irmler, 2000) directly by breakdown and digestion of litter materials (Bernier and Ponge, 1994) and also indirectly by the stimulation of micro-organisms (Postma-Blaauw *et al*., 2006). The rate of litter decomposition and nutrient release by earthworms greatly depends on tree litter quality (Edward and Heath, 1963). Although the contribution of earthworms to litter decomposition and nutrient cycling has been widely explored within various British woodlands, it has not been investigated

within SRF systems. Eually the contribution of native British earthworms to decomposition of non-native SRF species litter is almost unknown.

The litterbag technique (section 2.4.2.3) is the method most often used to determine litter decomposition rate in various forest habitats (Bocock and Gilbert, 1957; Edwards and Heath, 1963; Irmler, 2000). This method is simple and inexpensive and allows quantification of decomposition pattern over a period of time. Past researchers used different size meshes to exclude certain groups of soil decomposer fauna and quantify the contribution of earthworms to litter decomposition and nutrient release (e.g. Edwards and Heath, 1963; Heath *et al*., 1964). In addition to the litterbag technique, some researchers have measured available nutrient content (especially N content) in earthworm casts to quantify the nutrient release by these animals (Parle, 1963; Syers *et al.*, 1979; Syers and Springett, 1984; Parkin and Berry, 1994). Parkin and Berry, (1994) suggested that the magnitude of N accumulation in earthworm casts reflected the N content of the organic matter used as a food source. However, the influence of tree litter feeding on earthworm cast nutrient content has not been widely investigated.

The major aim of the present study was to investigate the influence of earthworms on SRF litter decomposition and the temporal pattern of nutrient release under field conditions. A series of long-term (12 month) litterbag experiments were designed to explore this within selected, established forest systems. A parallel litterbag study at an ex-agricultural site was used to compare the decomposition of selected SRF species litter. In addition, a laboratory experiment was conducted with *L. terrestris* to investigate the influence of SRF species litter on earthworm N release through casts over a relatively short period.

This chapter initially presents litterbag experiments undertaken at selected forest sites and at an ex-agricultural site. The latter part of the chapter demonstrates the laboratory experiment which compared the mineral nitrogen content of *L. terrestris* casts fed with three selected SRF species litter.

## 6.2 SRF litter decomposition: Field litterbag studies

### 6.2.1 Introduction

In litterbag studies, a known amount of freshly collected air-dried litter is enclosed in bags with appropriate mesh sizes and laid on the soil surface or buried at different depths of the soil profile. A large number of litterbags are installed at the start and sampled periodically over time. Decomposition rates are determined from the mass loss of litter included in mesh bags. The mesh size is usually selected to optimise the access of all soil organisms to the litter while minimising excessive particle loss (Karberg *et al*., 2008). However, some studies have used different mesh sizes to exclude certain groups of soil decomposer fauna (e.g. Edwards and Heath, 1963, see section 2.4.2.3 for details). In the present study, litterbags were prepared with two different sizes of nylon mesh. A 5 mm mesh was used to facilitate earthworm access, while a 0.5 mm mesh was used to prevent earthworm access (Edwards and Heath, 1963). Two sets of litterbag experiments were set up and maintained at established forest sites and at an ex-agricultural site from January 2011 until January 2012 with the following objectives;

a) To evaluate the contribution of earthworm populations to SRF litter decomposition, C and N release at selected forest sites.

b) To quantify and compare SRF litter decomposition, C and N release by earthworms at an ex-agricultural site.

### 6.2.2 Methodology

The selected SRF species examined were; *A. pseudoplatanus*, *B. pendula*, *C. sativa, E. nitens*, and *F. excelsior*. The litterbags were prepared with 5 mm and 0.5 mm nylon meshes (Figure 6.2.1) bought from Plastok (Meshes and Filtration) Ltd, UK. The mesh bags (0.2*0.2 m) were prepared by stitching with nylon thread leaving one side open. Approximately 10 g of air-dried whole leaf litter (previously collected and prepared) from one SRF species was inserted into each bag as per experimental design. The open end of each litterbag was then covered with duct tape and affixed with staples (a heavy duty, hand-operated stapler was used for this process). The completed set of litterbags were clearly marked for identification purposes and taken to the allocated field sites.

**(a)** **(b)**

**Figure 6.2.1 Litterbags (0.2 m * 0.2 m) containing e. g. *C. sativa*; (a) 0.5 mm mesh (b) 5 mm mesh (scale1: 4).**

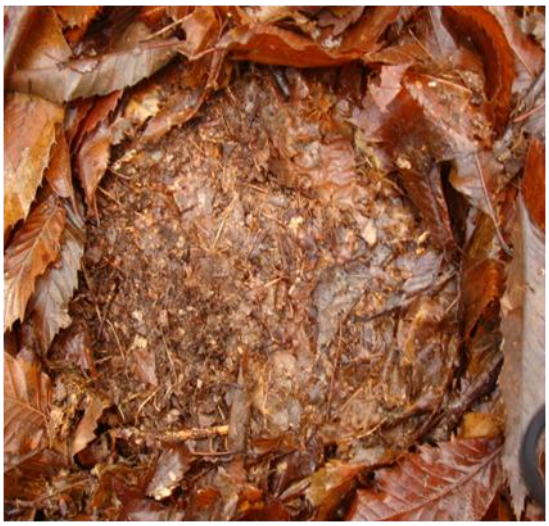
### 6.2.2.1 Experiment 1: Existing forest sites

This litterbag experiment was conducted at existing Forestry Commission sites, to assess the contribution of earthworms to SRF litter decomposition within established forest systems. The forest sites and tree plantations (monoculture) used for this experiment are summarised in Table 6.2.1 (for further site details refer to section 4.2.2.1).

**Table 6.2.1 Selected original forest sites and tree plantations used for litterbag experiment 1**

| Site name | Location | Tree plantation |
|---|---|---|
| 1. Alcan | Northumberland | *E. nitens* |
| 2. Rogate | Hampshire | *C. sativa* |
| 3. Gisburn Forest | Lancashire | a) *A. pseudoplatanus*;<br>b) *B. pendula*;<br>c) *F. excelsior* |

Litterbags with corresponding species of tree litter were established under each tree plantation, where the fresh litter layer was removed; bags placed as appropriate and pinned-down to the soil (with stiff wire) (Figure 6.2.2). The fresh litter layer was then re-spread above the litterbags and locations were marked with red pegs to assist relocation. A total of 24 litterbags (12 of each mesh size) were placed at each forest plantation site. This gave a total of 120 litterbags across the 5 sites. Figures 6.2.3 and 6.2.4 show the initial litterbag set-up at two of the sites. These demonstrate that litterbag configuration in the field was largely determined by the tree planting arrangement.



**(a)** **(b)**

**Figure 6.2.2 Litterbag set-up at Rogate; (a) fresh litter layer removed (b) litterbag (0.5 mm mesh) pinned-down.**

**Figure 6.2.3 Preparations for litterbag experiment 1 at Rogate (*C. sativa* site): January 2011.**

**Figure 6.2.4 Preparations for litterbag experiment 1 at Alcan (*E. nitens* site): January 2011.**

This experiment began in January 2011 and during the subsequent year, three replicates of each mesh size were collected after three, six, nine and twelve months for each SRF species. On collection, litterbags were separately placed into polythene bags, sealed and transported to the laboratory. The content of each bag was removed and adhering debris such as plant roots, soil, and organisms were carefully separated. The remaining litter was air-dried to a constant mass. The litter mass (dry basis) for each SRF species was recorded to estimate the pattern of mass loss over time. Sub-samples from air-dried litter were ground (after drying at 70 °C) and analysed for total C and N following a standard procedure used by the Forest Research laboratory (see section 3.3.3 for details).

### 6.2.2.2 Experiment 2: Carlshead ex-agriculture site

Parallel to experiment 1, using the same species of SRF, this litterbag experiment was conducted at Carlshead, an ex-agricultural (arable) site, to assess and compare SRF litter decomposition. The site (National Grid Reference SE 360468) is located in Yorkshire, where the mean annual temperature is 9.0 $^{\circ}$C and mean annual precipitation is 1,000 mm. The experimental site was generally flat and consisted of a clay loam soil. Earthworm and soil sampling was conducted at this site as described in chapter 4 (see section 4.2.2.2 and 4.2.2.3). This site was recently (May 2010) planted with numerous SRF species, but experimental litterbags were set-up within a control area (unplanted with trees). A total of 120 litterbags (24 of each SRF species; 12 of each mesh size) were placed on site. Litterbags were pinned-down to the soil surface and locations were marked with red pegs (Figure 6.2.5). The experiment commenced in January 2011 and litterbags were collected and processed as described in Experiment 1.

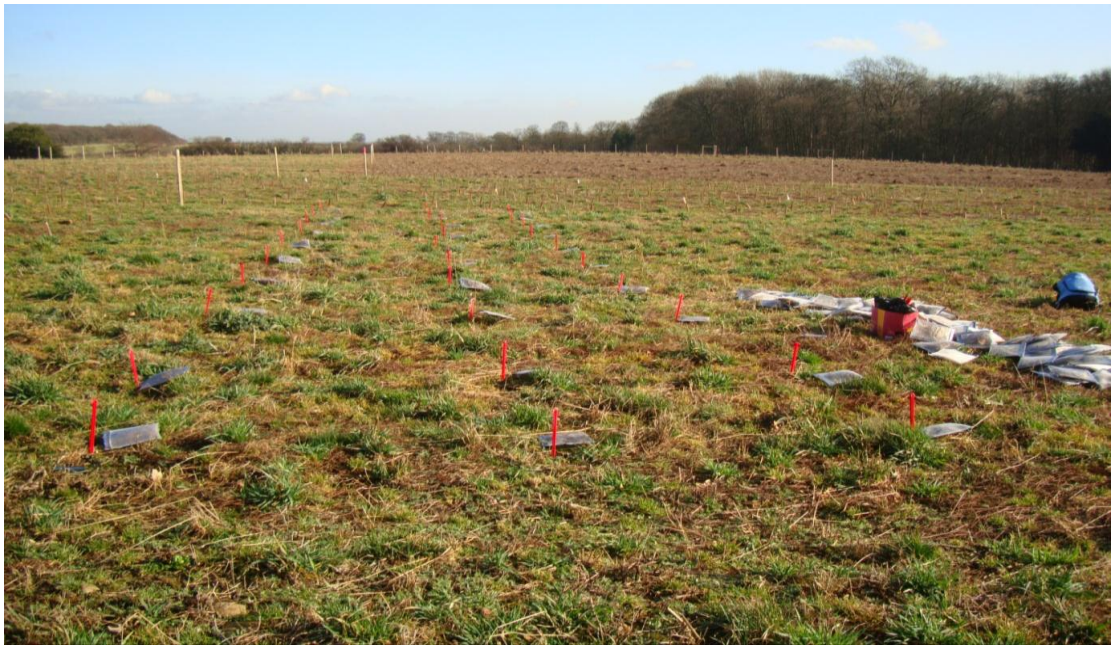


**Figure 6.2.5 Initial litterbag set up at Carlshead ex-agriculture site: January 2011.**

#### 6.2.2.3 Statistical analysis

The percentage mass remaining after twelve months and annual decomposition rate constants were used for statistical analysis. Annual decomposition rate constants ($k$ $yr^{-1}$) were calculated from data on the percentage of remaining litter mass, using an exponential decay model proposed by Olson (1963).

$$X_t = X_0 \, e^{-kt}$$

Where $X_t$ is remaining mass at time t, $X_0$ is initial mass of the litter, $e$ is the base of the natural logarithm, and $k$ is the decomposition rate constant. Since the mass loss curve is exponential, it is not possible to calculate the total disappearance time, so the 99% disappearance time was used for required calculations. Student's t-test was used to compare two different mesh sizes of each SRF species in each site. One way analysis of variance (ANOVA) was used to compare similar sized mesh bags of the five SRF species at the ex-agricultural site. As appropriate, the amount of litter loss was calculated by subtracting the remaining mass from the original mass.

### 6.2.3 Results

#### 6.2.3.1 Experiment 1: Existing forest sites

Figure 6.2.6 shows the pattern of litter loss from the two litterbags sizes for *E. nitens* at the Alcan site, over a period of nine months**.** The difference in remaining mass (%) between the two sizes of mesh, was graphically discernible after three months, and became more obvious thereafter. After nine months, mean mass loss was recorded as

36.5% for 0.5 mm mesh and 85.9% for 5 mm mesh. The litterbag experiment at this site had to be terminated after nine months due to unexpected site disturbances.

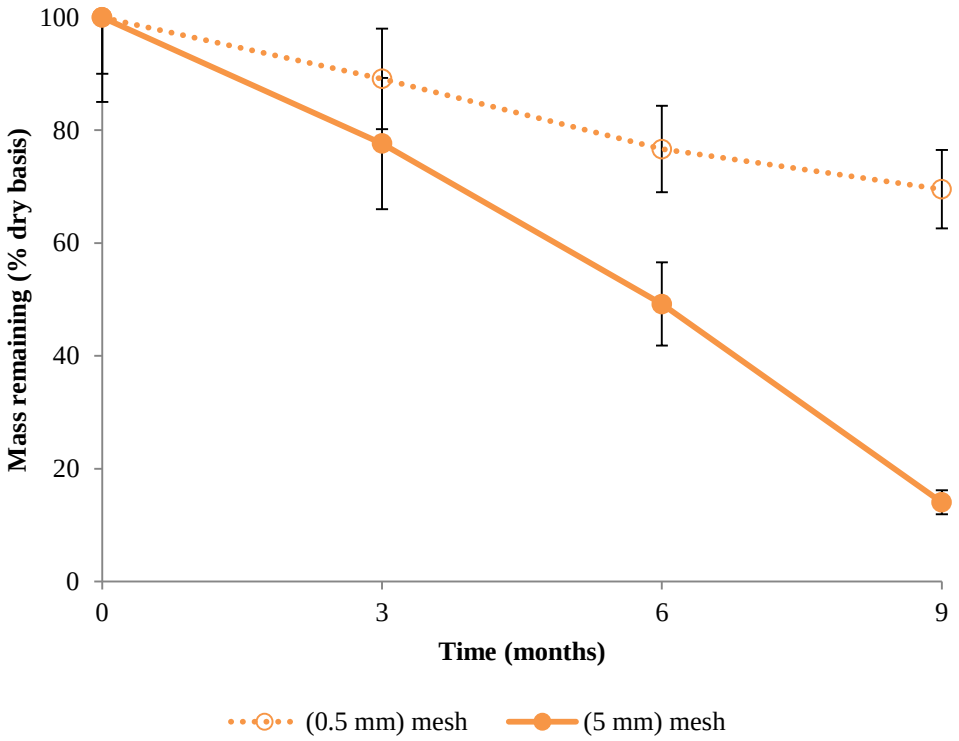
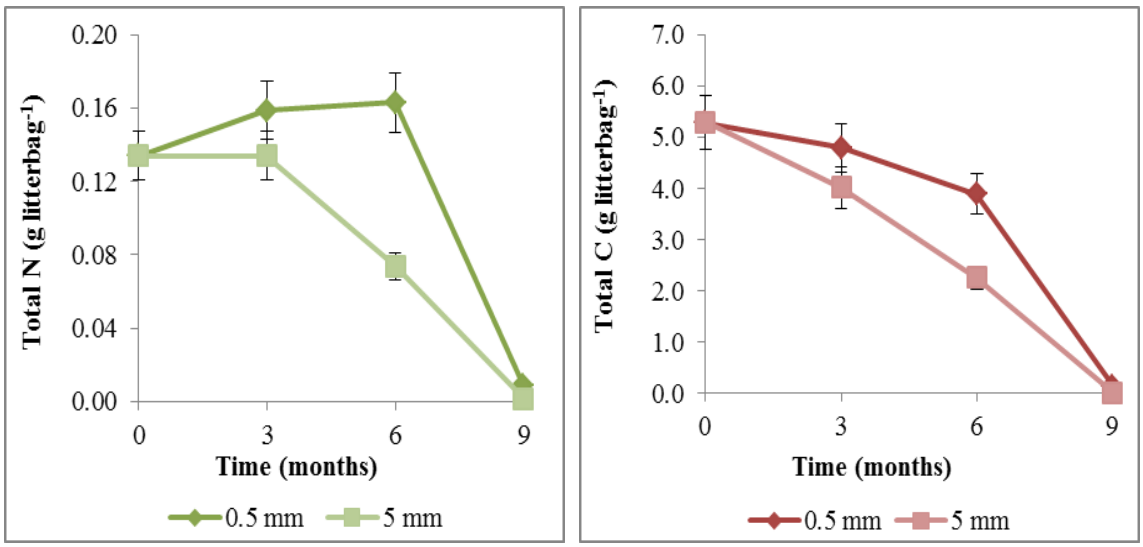



**Figure 6.2.6 Mean (± se) percentage of mass remaining in litterbags of different mesh size containing *E. nitens* at the Alcan site over a period of nine months: Jan 2011 – Oct 2011, (n = 3).**

The changes in N and C content (g) in decomposing litter of *E. nitens* at the Alcan site, with respect to the remaining litter mass, are demonstrated in Figure 6.2.7. N content in 0.5 mm mesh litterbags initially increased and then decreased after six months, while in 5 mm mesh litterbags N content initially remained constant and decreased after three months. C content in both sizes of litterbags decreased throughout the experimental period. However, C content in 5 mm mesh litterbags was lower at month three and six compared with 0.5 mm mesh litterbags and became very similar at month nine (at zero).




**(a)** **(b)**

**Figure 6.2.7 Mean (± se) N and C content (g) in decomposing litter of *E. nitens* in different mesh size litterbags (with respect to remaining litter mass) at the Alcan site over nine months; (a) total N, (b) total C, (n = 3).**

Figure 6.2.8 illustrates the pattern of litter loss from the two sizes of litterbag for *C. sativa* at the Rogate site over a period of twelve months**.** The remaining litter masses (%) in the two types of litterbag were not noticeably different over the experimental period. At the termination of the experiment (after twelve months), mean litter mass loss equated to 51.7% for 0.5 mm mesh and 52.7% for 5 mm mesh.

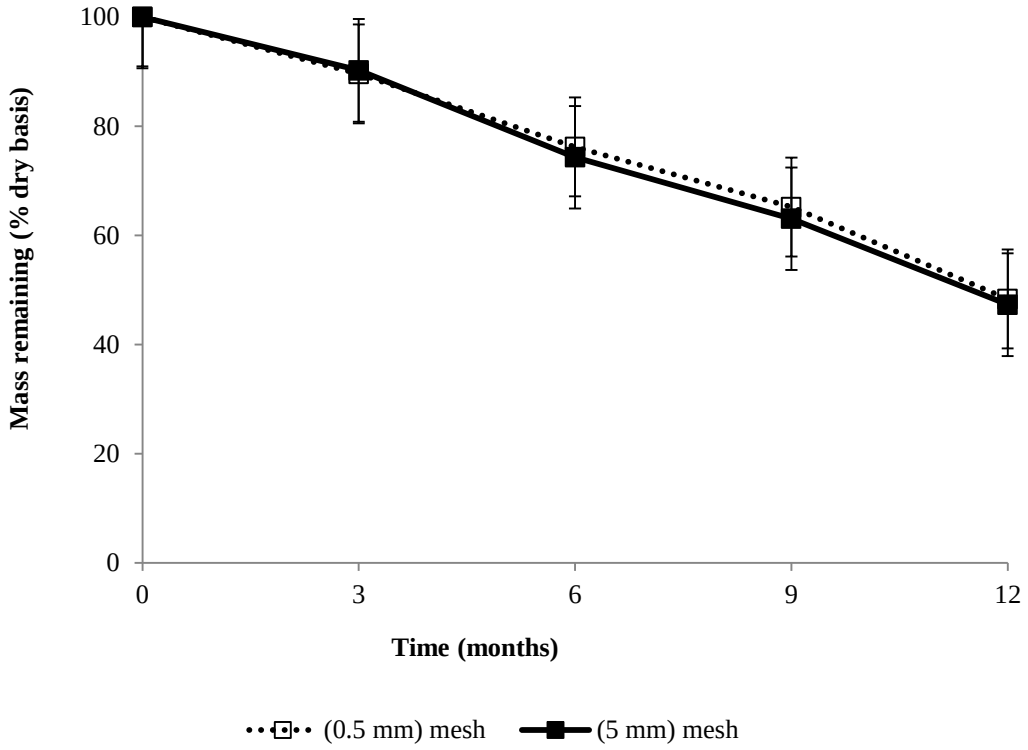
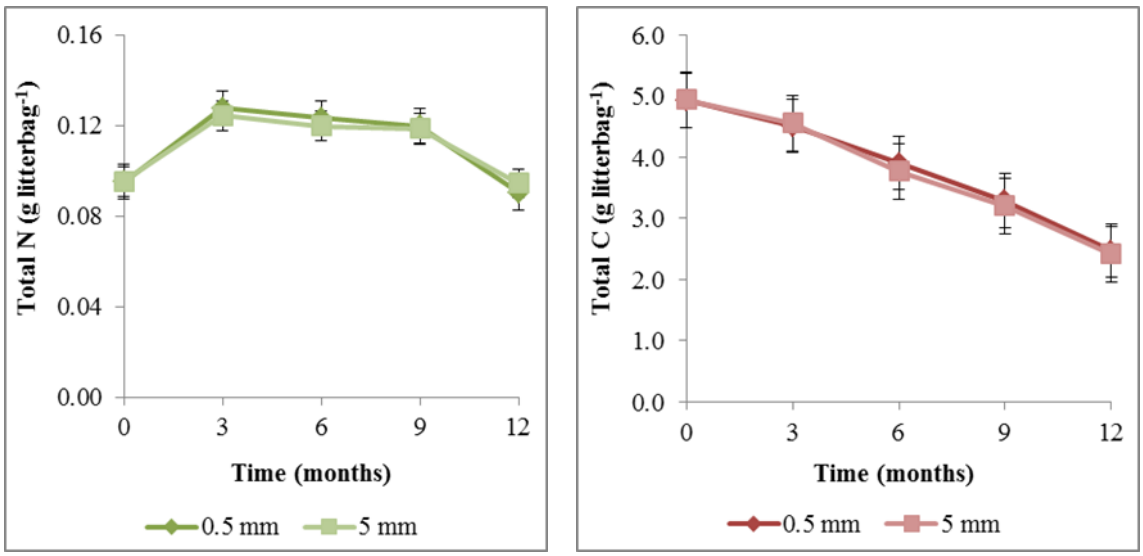



**Figure 6.2.8 Mean (± se) percentage of mass remaining in litterbags of different mesh size containing *C. sativa* at the Rogate site over a period of twelve months: Jan. 2011 – Jan. 2012, (n = 3).**

The changes in N and C content (g) in decomposing *C. sativa* litter at the Rogate site, with respect to remaining litter mass, are shown in Figure 6.2.9. Differences in C or N content in the two types of litterbags were not discernible. N content in both litterbags initially increased and then decreased after three months. However, it did not fall below the initial levels even after twelve months. C content in both litterbag mesh sizes recorded a slight decrease throughout the experimental period.




**(a)** **(b)**

**Figure 6.2.9 Mean (± se) N and C content (g) in decomposing litter of *C. sativa* in different mesh size litterbags (with respect to remaining litter mass) at the Rogate site over twelve months; (a) total N, (b) total C, (n = 3).**

Figure 6.2.10 demonstrates the pattern of litter loss from two sizes of litterbag of *A. pseudoplatanus* at Gisburn Forest over a period of twelve months. Although the 0.5 mm mesh recorded a slightly higher rate of remaining litter over the period, a considerable difference in the two sizes of litterbag was not observed. After twelve months, mean litter mass loss equated to 44.4% for 0.5 mm mesh and 53.2% for 5 mm mesh.

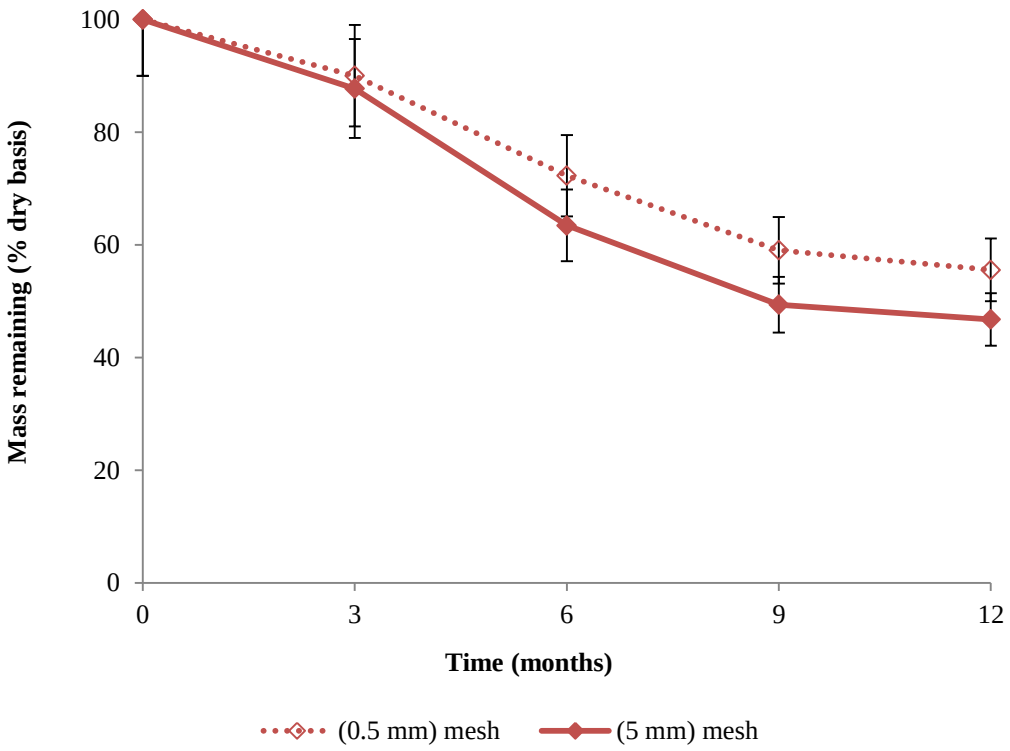
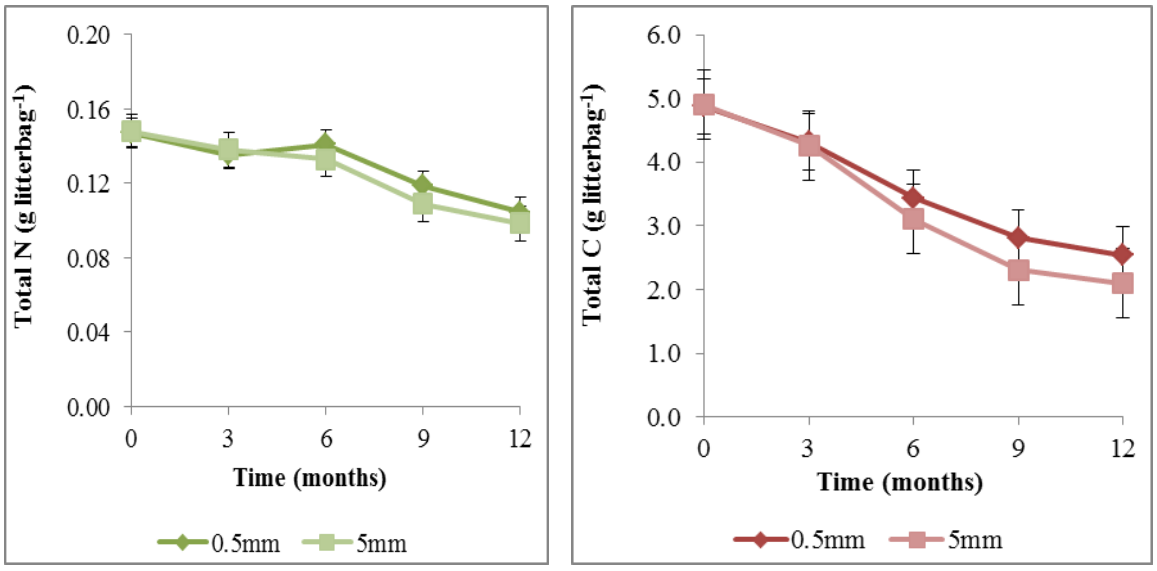



**Figure 6.2.10 Mean (± se) percentage of mass remaining in litterbags of different mesh size containing *A. pseudoplatanus* at Gisburn Forest over a period of twelve months: Jan. 2011 – Jan. 2012, (n = 3).**

Figure 6.2.11 illustrates the changes in N and C content in decomposing litter of *A. pseudoplatanus* at Gisburn Forest over twelve months with respect to the remaining litter mass. Significant differences in C or N content in the two sizes of litterbag were not observed over the experimental period. However, C and N content in both litterbags recorded a slight decrease throughout the experimental period.




**(a)** **(b)**

**Figure 6.2.11 Mean (± se) N and C content (g) in decomposing litter of *A. pseudoplatanus* in different mesh size litterbags (with respect to remaining litter mass) at Gisburn Forest over a period of twelve months (a) total N, (b) total C, (n = 3).**

Figure 6.2.12 shows the pattern of litter loss from two different litterbags of *B. pendula* at Gisburn Forest over a period of twelve months. Although the 0.5 mm mesh had a slightly higher amount of remaining litter over the period, no significant difference ($p > 0.05$) in the two mesh sizes of litterbag was recorded. After twelve months, mean litter mass loss equated to 48.5% for 0.5 mm mesh and 53.4% for 5 mm mesh.

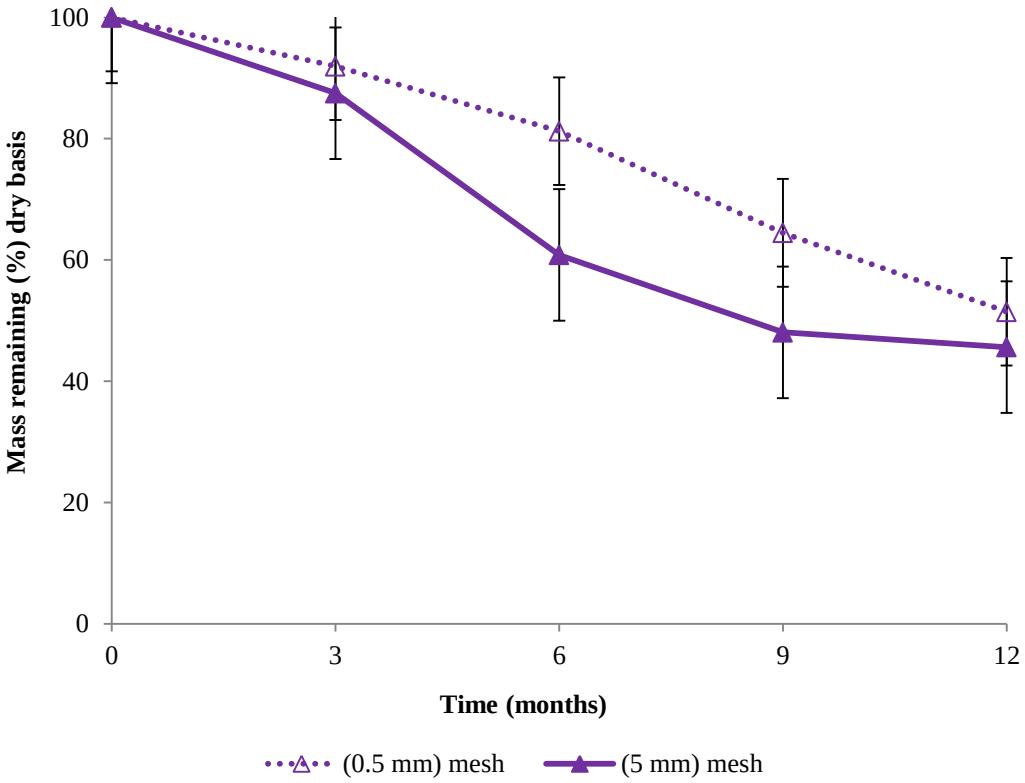
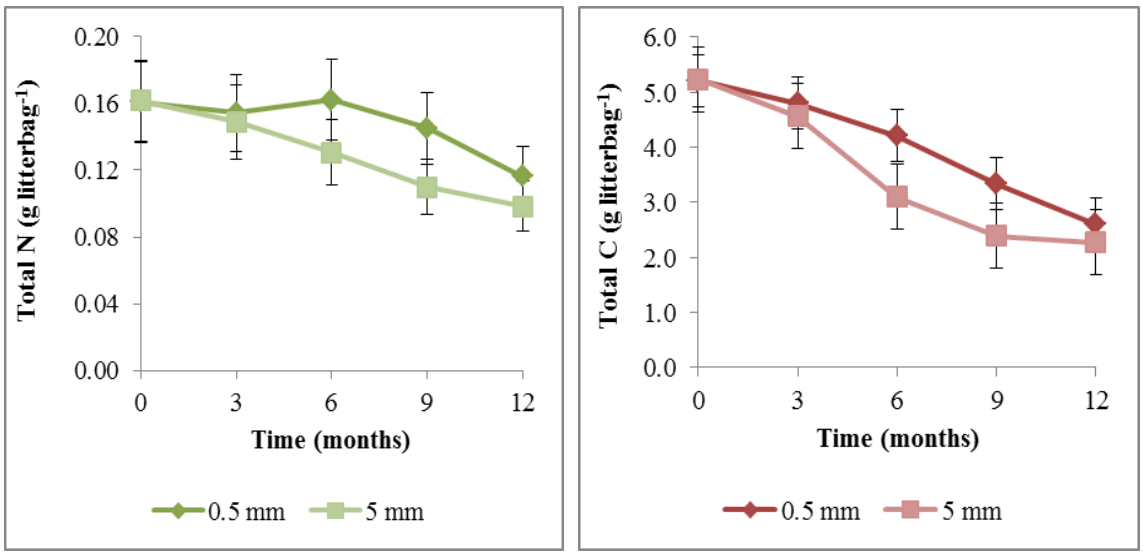



**Figure 6.2.12 Mean (± se) percentage of mass remaining in litterbags of different mesh size containing *B. pendula* at Gisburn Forest over a period of twelve months; Jan. 2011 – Jan. 2012, (n = 3).**

The changes in N and C content (g) in decomposing litter of *B. pendula* at Gisburn forest, with respect to remaining litter mass, are shown in Figure 6.2.13. N content in 5 mm mesh litterbags decreased throughout the experimental period, while in 0.5 mm mesh litterbags N content was initially slightly increased and then decreased after six months. C content in both litterbag mesh sizes recorded a decrease throughout the experiment, but was always lower for 5 mm than for 0.5 mm litterbags.




**(a)** **(b)**

**Figure 6.2.13 Mean ± se N and C content (g) in decomposing litter of *B. pendula* in two different mesh size litterbags (with respect to remaining litter mass) at Gisburn Forest over a period of twelve months; (a) total N, (b) total C, (n = 3).**

Figure 6.2.14 illustrates the pattern of litter loss from two different sizes of litterbag of *F. excelsior* at Gisburn Forest, over a period of twelve months**.** A difference in remaining mass (%) was graphically discernible after three months, and became more obvious thereafter. The 5 mm mesh litterbags recorded a 100% litter mass loss after nine months. The 0.5 mm mesh recorded a mean litter mass loss of 66.2% after twelve months.

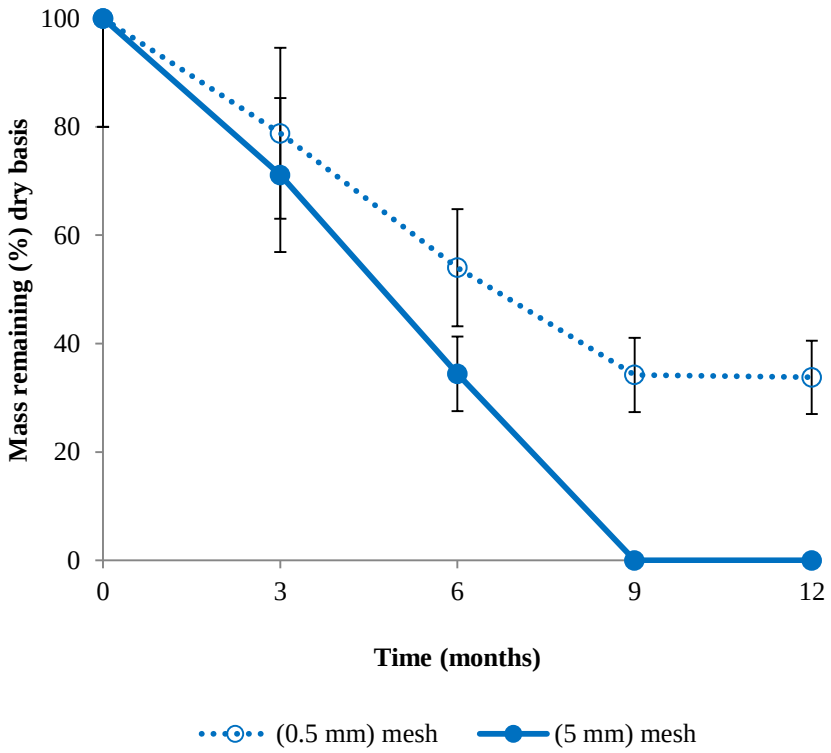
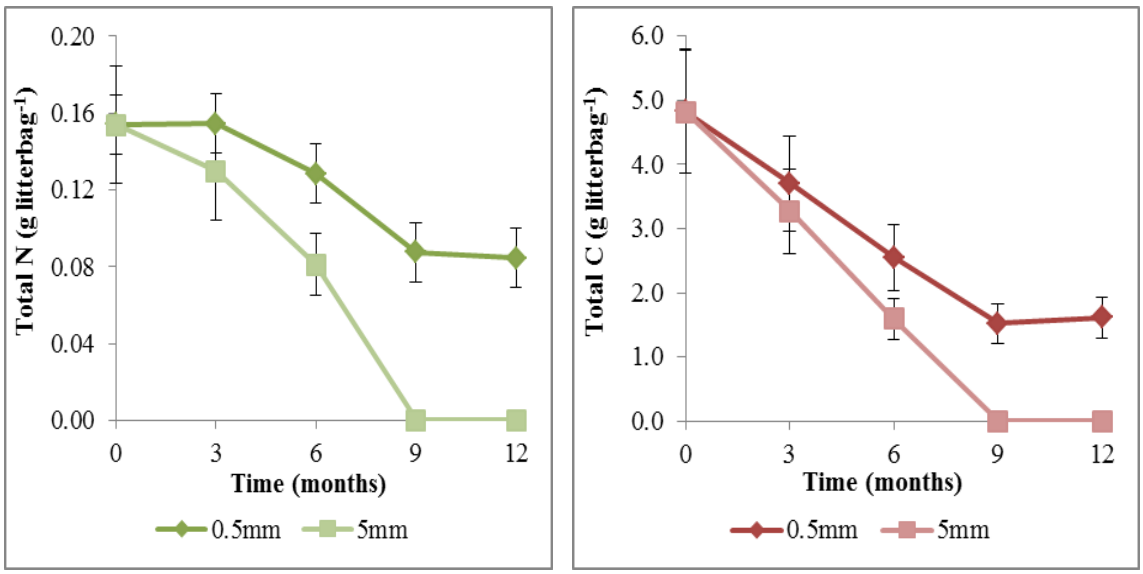



**Figure 6.2.14 Mean (± se) percentage of mass remaining in litterbags of different mesh size containing *F. excelsior* at Gisburn Forest over a period of twelve months; Jan. 2011 – Jan. 2012, (n = 3).**

Figure 6.2.15 shows changes in N and C content (g) in decomposing litter of *F. excelsior* at Gisburn Forest over the experimental period, with respect to the remaining litter mass. The N and C content in both sized mesh bags decreased over the experimental period. However, both N and C content was always lower for 5 mm mesh compared with 0.5 mm mesh litterbags.




**(a)** **(b)**

**Figure 6.2.15 Mean ± se N and C content (g) in decomposing litter of *F. excelsior* in two different mesh mesh size litterbags (with respect to remaining litter mass) at Gisburn Forest over period of a twelve months; (a) total N, (b) total C, (n = 3).**

Table 6.2.2 summarises the significant differences in litter decomposition attributes (% litter remaining and annual decomposition rate constant) between two mesh sizes of litterbag at each original forest system. Alcan (*E. nitens*) and Gisburn (*F. excelsior*) sites recorded significant differences (probability levels are indicated in Table 6.2.2) between two litterbag mesh sizes in terms of both litter remaining (%) and the annual decomposition rate constant ($k$ $yr^{-1}$). However, Rogate (*C. sativa*) and Gisburn Forest (*A. pseudoplatanus* and *B. pendula*) sites showed no significant difference ($p > 0.05$) between the two mesh sizes of litterbag for both litter decomposition parameters.

**Table 6.2.2 Mean earthworm density, biomass and % litter mass remaining at the termination of the experiment (Alcan: *E. nitens* after 9 months, all others after 12 months) and the annual decomposition rate constant (*k* $yr^{-1}$) for two mesh sizes of litterbags in established forests.**

| Forest site: SRF species | Mean earthworm parameters | | Decomposition attribute | Litterbag (mesh size) | | Significance |
|---|---|---|---|---|---|---|
| | Density (No. $m^{-2}$) | Biomass (g $m^{-2}$) | | 0.5 mm | 5 mm | |
| Alcan (*Eucalyptus nitens*) | 152.5 | 89.7 | % litter mass remaining | 69.5 | 14.1 | ** |
| | | | *k* $yr^{-1}$ | 0.21 | 1.14 | ** |
| Rogate (*Castaneas sativa*) | 23.0 | 3.7 | % litter mass remaining | 48.3 | 47.3 | ns |
| | | | *k* $yr^{-1}$ | 0.32 | 0.33 | ns |
| Gisburn (*Acer pseudoplatanus*) | 26.0 | 8.7 | % litter mass remaining | 55.6 | 46.8 | ns |
| | | | *k* $yr^{-1}$ | 0.26 | 0.33 | ns |
| Gisburn (*Betula pendula*) | 6.0 | 0.9 | % litter mass remaining | 51.4 | 45.6 | ns |
| | | | *k* $yr^{-1}$ | 0.30 | 0.34 | ns |
| Gisburn (*Fraxinus excelsior*) | 66.0 | 13.6 | % litter mass remaining | 33.8 | 0.0 | *** |
| | | | *k* $yr^{-1}$ | 0.47 | 2.67 | *** |

ns: not significant, * significant at $p < 0.05$, ** significant at $p < 0.01$, *** significant at $p < 0.001$ ($n = 3$), (refer to chapter 4 for earthworm species composition in each forest system).

### 6.2.3.2 Experiment 2: Carlshead ex-agriculture site

Table 6.2.3 summarises the mean soil and earthworm parameters for the Carlshead ex-agricultural site, where sampling was conducted in October 2011.

**Table 6.2.3 Mean (± se) soil properties of the Carlshead ex-agriculture site (n = 4), plus earthworm community measurements (n = 10)**

| Carlshead ex-agriculture site | |
|---|---|
| **Soil parameters** | |
| Soil moisture (%) | 14.8 ± 0.5 |
| Soil pH ($H_2O$) | 6.4 ± 0.02 |
| Organic matter (%) | 6.6 ± 0.08 |
| Total N (%) | 0.2 ± 0.00 |
| Total C (%) | 2.0 ± 0.03 |
| CEC (cmol+/kg) | 11 ± 0.08 |
| BS (%) | 98.4 ± 0.10 |
| **Earthworm measurements** | |
| Density (No. $m^{-2}$) | 298 ± 24.5 |
| Biomass (g $m^{-2}$) | 56.6 ± 4.9 |

Table 6.2.4 shows the earthworm species composition at the Carlshead site. A total of six earthworm species were recorded; two anecic, three endogeic, and one epigeic. *A. chlorotica* was the most dominant species, while *L. terrestris* records were relatively low.

**Table 6.2.4 Earthworm species and detail of density and biomass at the Carlshead site**

| Earthworm species | Density (No $m^{-2}$) | Biomass (g $m^{-2}$) |
|---|---|---|
| *A. caliginosa* | 40 | 9.14 |
| *A. chlorotica* | 127 | 9.47 |
| *A. longa* | 18 | 17.2 |
| *A. rosea* | 29 | 2.86 |
| *L. castaneus* | 30 | 3.99 |
| *L. terrestris* | 2 | 10.3 |
| *L.* spp. (Immature) | 52 | 3.59 |
| **Total** | **298** | **56.6** |

Figure 6.2.16 shows the litter mass loss of five SRF species from litterbags with two mesh sizes at the Carlshead site over a period of twelve months. The difference in remaining masses (%) between the two mesh sizes was graphically discernible after three months, and became more obvious thereafter. From the 5 mm mesh litterbags, *F. excelsior* showed the highest mass loss throughout and recorded 100% mass loss after nine months. The other four SRF species in 5 mm mesh bags: *B. pendula, E. nitens, C. sativa* and *A. pseudoplatanus,* showed a relatively similar pattern of mass loss throughout the experimental period. From the 0.5 mm mesh litterbags, again *F. excelsior* showed a relatively higher rate of mass loss compared with the other four species.

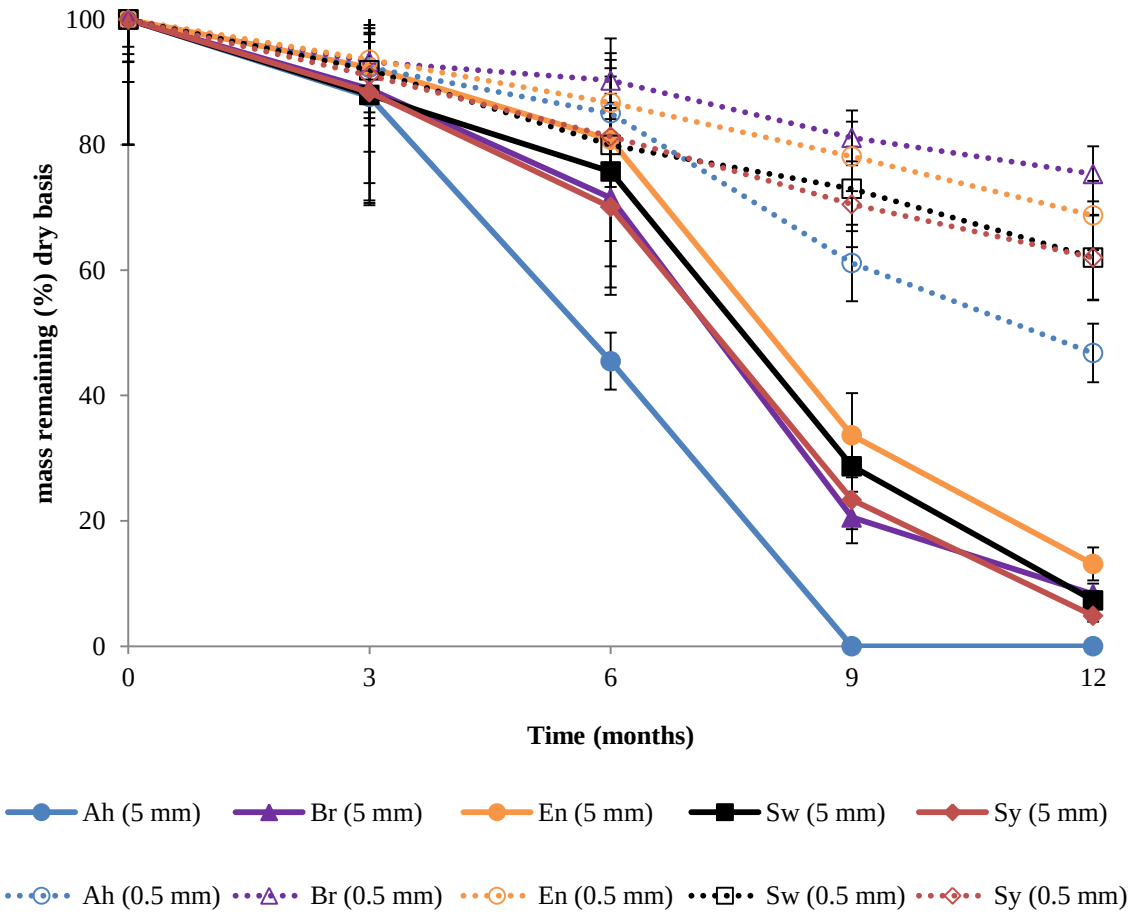
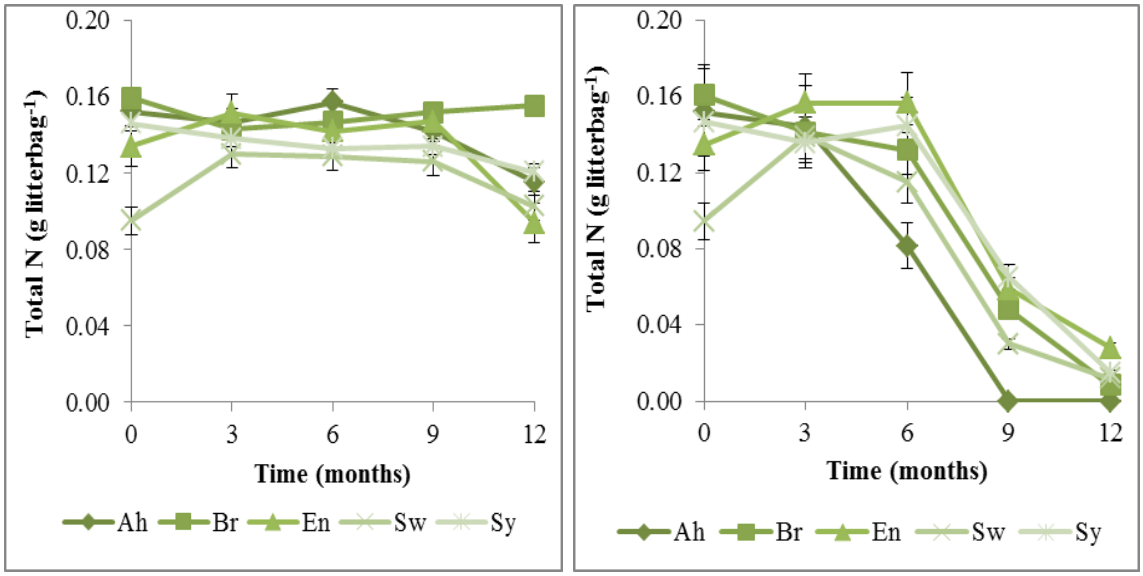



**Figure 6.2.16 Mean (± se) percentage of mass remaining in litterbags of different mesh sizes for five SRF species; *F. excelsior* (Ah), *B. pendula* (Br), *E. nitens* (En), C. *sativa* (Sw) and *A. pseudoplatanus* (Sy) at the Carlshead ex-agriculture site, over a period of twelve months; Jan. 2011 – Jan. 2012, (n = 3).**

The changes in N content (g) in decomposing litter from litterbags with two mesh sizes at the Carlshead site are shown in Figure 6.2.17. N content in 0.5 mm mesh litterbags of *C. sativa* and *E. nitens* was initially increased. In similar mesh bags, N content in all five species of litter stayed constant between three to nine months and then after nine months it was slightly decreased in all species of litter except *B. pendula*. N content in 5 mm mesh litterbags of *F. excelsior* and *B. pendula* was decreased throughout the experimental period, but in similar mesh of *C. sativa* and *E. nitens*, it was initially

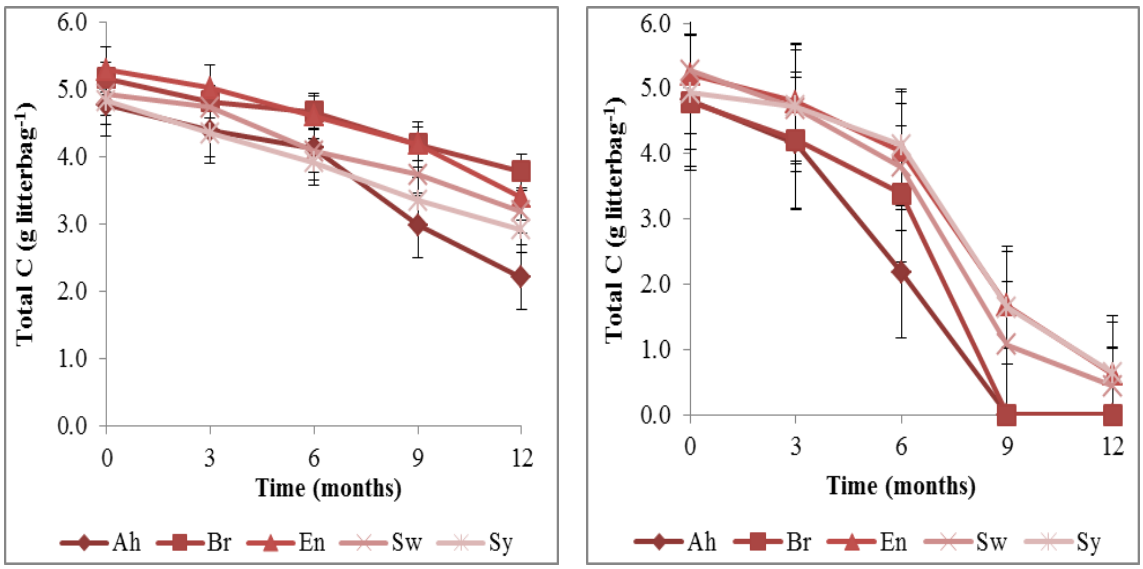
increased. However N content in 5 mm mesh bags decreased after six months for all five SRF species used while it was lowest for *F. excelsior* .




**(a)** **(b)**

**Figure 6.2.17 Mean (± se) N content (g) in decomposing litter of five SRF species (with respect to remaining litter mass) at the Carlshed ex-arable site over a period of twelve months; (a) 0.5 mm mesh, (b) 5 mm mesh, (n = 3), [Abbreviation of SRF species as for Figure 6.2.16].**

Figure 6.2.18 demonstrates the changes in C content in decomposing litter in litterbags with two mesh sizes at the Carlshed site. Total C content in both 0.5 and 5 mm mesh litterbags was decreased for all five SRF species throughout the experimental period. However, rate of decrease was more rapid with 5 mm mesh compared with 0.5 mm mesh litterbags.




**(a)** **(b)**

**Figure 6.2.18 Mean (± se) C content (g) in decomposing litter of five SRF species (with respect to remaining litter mass) at the Carlshead ex-arable site over a period of twelve months; (a) 0.5 mm mesh, (b) 5 mm mesh, (n = 3), [Abbreviation of SRF species as for Figure 6.2.16].**

Table 6.2.5 summarises the significant differences in litter decomposition parameters between litterbags with two mesh sizes at the Carlshed site. At this ex-agricultural site, where mean earthworm density was recorded at 298 $m^{-2}$ (biomass = 56.6 g $m^{-2}$), percentage litter mass remaining after twelve months and the annual decomposition rate constant ($k$ $yr^{-1}$) for all individual SRF species were significantly different for 0.5 and 5 mm mesh litterbags (probability levels for each individual parameter are indicated in Table 6.2.5).

**Table 6.2.5 Mean litter mass (% remaining) after twelve months and annual decomposition rate constant (*k* $yr^{-1}$) for two mesh sizes of litterbags at the Carlshead ex-agriculture site [Abbreviation of SRF species as for Figure 6.2.16]**

| SRF Species | Attribute | Litterbag – mesh size | | Significance |
|---|---|---|---|---|
| | | 0.5 mm | 5 mm | |
| **Ah** | % mass remaining | 46.8 | 0.0 | *** |
| | *k* $yr^{-1}$ | 0.33 | 2.67 | *** |
| **Br** | % mass remaining | 75.3 | 8.3 | *** |
| | *k* $yr^{-1}$ | 0.12 | 1.10 | *** |
| **En** | % mass remaining | 68.7 | 13.1 | ** |
| | *k* $yr^{-1}$ | 0.16 | 0.91 | ** |
| **Sw** | % mass remaining | 62.0 | 7.7 | ** |
| | *k* $yr^{-1}$ | 0.21 | 1.31 | ** |
| **Sy** | % mass remaining | 62.0 | 5.5 | ** |
| | *k* $yr^{-1}$ | 0.21 | 1.61 | ** |

** significant at $p < 0.01$, *** significant at $p < 0.001$ ($n = 3$).

Table 6.2.6 summarises the significant differences in litter decomposition parameters between five SRF species at the Carlshead site. In 5 mm mesh litterbags, % litter masses remaining after 12 months were not significantly different ($p > 0.05$) for five SRF species. However, the annual decomposition rate constant (*k* $yr^{-1}$) in similar mesh size was significantly ($p < 0.05$) higher for *F. excelsior* compared with other species. In 0.5 mm mesh litterbags, both % litter mass remaining after twelve months and annual decomposition rate constant (*k* $yr^{-1}$) were significantly ($p < 0.05$) different as stated in Table 6.2.6.

**Table 6.2.6 Mean litter mass (% remaining) after twelve months and decomposition rate constant (*k* $yr^{-1}$) for five species of litter at the Carlshead ex-agriculture site for two meshsizes of litterbag [Abbreviation of SRF species as for Figure 6.2.16]**

| Litterbag mesh size | Attribute | Ah | Br | En | Sw | Sy |
|---|---|---|---|---|---|---|
| **0.5 mm** | % mass remaining | $46.8^{c}$ | $75.3^{a}$ | $68.7^{ab}$ | $62.0^{b}$ | $62.0^{b}$ |
| | *k* $yr^{-1}$ | $0.33^{a}$ | $0.12^{c}$ | $0.16^{bc}$ | $0.21^{b}$ | $021^{b}$ |
| **5 mm** | % mass remaining | $0.0^{a}$ | $8.3^{a}$ | $13.1^{a}$ | $7.7^{a}$ | $5.5^{a}$ |
| | *k* $yr^{-1}$ | $2.67^{a}$ | $1.10^{b}$ | $0.91^{b}$ | $1.31^{b}$ | $1.61^{b}$ |

Different letters in a row indicate significant differences ($p < 0.05$, $n = 12$), ANOVA, Tukey-Kramer.

### 6.2.4 Discussion

#### 6.2.4.1 Experiment 1: Existing forest sites

The litter decomposition study in original forest sites suggests that earthworms can have a large contribution to SRF litter mass loss plus C and N cycling within these systems when they are present in considerably high numbers. Alcan (*E. nitens*) and Gisburn (*F. excelsior*) sites which had relatively high densities of earthworms (152.5 and 66 $m^{-2}$ respectively) recorded significantly higher rates of mass loss in earthworm-accessible litterbags (5 mm mesh) compared with earthworm-inaccessible controls (0.5 mm mesh). Contrastingly, Rogate (*C. sativa*), Gisburn (*A. pseudoplatanus* and *B. pendula*) which had relatively low earthworm densities (23, 26 and 6 $m^{-2}$ respectively), showed no significant difference in rate of mass loss between earthworm-accessible litterbags and

inaccessible-controls. Earthworms showed a positive influence on N and C release from litter, if they were present in large numbers (e. g. Alcan: *E. nitens* site). An increment of N content (g) in litterbags (especially in smaller mesh bags) at the early stage of decomposition (e.g. after three and six months) was observed in some sites e.g. Alcan: *E. nitens*, Rogate: *C. sativa*, Gisburn: *B. pendula*. This is likely due to early colonisation of litterbags by nitrogen-rich microorganisms (Irmler, 2000), in addition to growth of fungus. Litter decomposition, C and N release can also be influenced by the earthworm species present. Anecic species, such as *L. terrestris* largely contribute to breakdown, and incorporation of surface litter into the soil in many temperate woodlands (Satchell 1967; Scheu and Wolters, 1991; Benham *et al*., 2012). Epigeic earthworms such as *L. rubellus* and *L. castaneus* also consume considerable amounts of plant litter. Endogeic earthworms such as *A. caliginosa* and *A. rosea* feed mainly on fine organic matter mixed with mineral soil and are known to largely contribute to nutrient release (Scheu 1987). Presence of *L. terrestris* certainly accelerates the leaf litter breakdown and incorporation into mineral soil, but increased earthworm diversity can make litter decomposition and nutrient release more efficient. As an example, at Alcan (*E. nitens*) site, which was rich with large number of *L. terrestris* (22 $m^{-2}$) and other epigeic and endogeic species (seven species all together) showed a significant earthworm influence ($p < 0.05$) for litter decomposition, C and N release compared with the control. However, this influence was not significant at the Rogate (*C. sativa*) site which had no *L. terrestris* and less diversity of earthworms (three species) (see Tables 4.2.3, 4.2.5, 4.2.7 and 4.2.9 for earthworm species composition at each site).

Bocock and Gilbert (1957) studied the litter decomposition in three different forest sites in the British Isles using nylon net litterbags. This study recorded that birch (*Betula verrucosa*) and lime (*Tilia cordata*) litter disappearance was greater on mull soils than

two other sites; with moder and peat soil. The authors suggested that this difference was mostly due to the abundance of large invertebrates/earthworms in mull soils. Similarly, Edwards and Heath (1963) studied and compared the rate of disappearance of oak (*Quercus* spp.) and beech (*Fagus* spp.) leaves in selected forest sites at Rothamsted. They used nylon mesh bags of different mesh size to exclude different soil fauna and found that leaf disks of both oak and beech disappeared three times more rapidly in 7 mm mesh bags compared with those in 0.5 mm mesh bags. This experiment showed the overall importance of earthworms in fragmenting leaf materials in forest soils. Heath *et al*. (1964) further investigated the litter decomposition in various forest sites in England and confirmed the importance of earthworms on forest litter fragmentation. Hendriksen (1990) conducted a field litterbag study with major European forest tree species and suggested that there was a positive correlation between the percentage of mass loss and the number/biomass of detritivores below the litterbags. Contrastingly, Irmler (2000) used litterbags of 0.02 mm and 5 mm mesh sizes and investigated the mass loss and N release in a beech (*Fagus* spp.) and mixed forest in northern Germany and suggested that biomass of epigeic Lumbricidae was negatively correlated with litter mass loss, particularly in beech forest. However, this study suggested that Lumbricidae were positively correlated with N release at both sites. Scheu (1987) found a remarkable influence of endogeic earthworms on N mineralisation within beech woods established on lime soils. The current study stands-out from the aforementioned investigations, as this was the first attempt to investigate the influence of earthworms on litter decomposition and nutrient release within SRF systems, which is different from natural forest systems in terms of tree species, tree age, planting density and level of human intervention.

Litterbags with selective mesh sizes have been widely used to estimate the contribution of soil faunal groups to decomposition rates in the field. However, selection of appropriate mesh size is really important to estimate actual contribution. Edwards and Heath (1963) initially suggested using 1 mm mesh to avoid earthworms, but later they noticed that this size did not always exclude smaller earthworms. In the current experiment 0.5 mm mesh was used as a control to exclude earthworm access. However, after nine months, some tiny earthworm hatchlings were even found within 0.5 mm mesh litterbags. In the past, researchers had used 5 mm (e.g. Swift *et al*., 1979; Irmler, 2000) or 7 mm mesh (e.g. Edwards and Heath, 1963, Heath *et al*., 1964, Hendriksen, 1990) to facilitate earthworm access. The 5 mm mesh was selected in the current study to maximise earthworm access while minimising the fragmentation loss. The 20 cm* 20 cm litterbag size was selected for the current study, so that each of the litter species could be inserted without a need for cutting. A major practical problem faced in the current study was missing litterbags at some sites, especially when the experimental site was located close to public walking paths. This could be avoided by selecting the sites away from human and dog access and minimising the above ground visible markings and signs. Soil animals found in 0.5 mm mesh bags included springtails and mites. In addition to microorganisms, these animals largely contribute to mass loss in earthworm-inaccessible litterbags. In addition to earthworms, 5 mm mesh bags contained ants, slugs, woodlice, millipedes, and centipedes. These soil animals could also have directly contributed to litter mass loss in 5 mm mesh bags. In Rogate (*C. sativa*), where earthworm density and diversity was low, a considerable number of woodlice (6 - 8 per bag) were recorded in 5 mm mesh bags in addition to millipedes and centipedes. However, litter mass losses in both 0.5 and 5 mm litterbags were very similar at this site. This suggests that the influence of the above soil animals on *C. sativa* litter mass loss was negligible. Similarly, at Gisburn (*B. pendula*), which had very low numbers of

earthworms, recorded large numbers of ants within its 5 mm mesh bags, but again litter mass loss was not significantly different between 0.5 and 5 mm mesh bags, suggesting that ants had no significant contribution to *B. pendula* litter mass loss at this site.

Summary of the major finding of litterbag Experiment 1:

- The Alcan (*E. nitens*) site showed that native British earthworms had a significant contribution to non-native *E. nitens* litter decomposition, C and N release within this SRF system.
- The Rogate (*C. sativa*) site showed that earthworms had no significant contribution for *C. sativa* litter decomposition, C and N release at this site. Nevertheless, there were few earthworms present.
- The Gisburn Forest work showed that earthworms had a significant contribution to *F. excelsior* litter decomposition, but not for *B. pendula* and *A. pseudoplatanus* litter within this site.
- Overall results at various forest sites showed that the contribution of earthworms to SRF litter decomposition, C and N release depends on earthworm density and diversity present which is a function of tree species, soil type and land-use history.

#### 6.2.4.2 Experiment 2: Carlshead ex-agriculture site

This comparative litter decomposition study at the Carlshead ex-agricultural site suggests that earthworms have a great influence on SRF litter decomposition, C and N release. It also suggests that this influence can vary with SRF species. For all considered SRF species, earthworm-accessible litterbags recorded significantly higher rates of mass loss compared with earthworm-inaccessible controls. In terms of earthworm-accessible

mesh, *F. excelsior* recorded a significantly higher ($p < 0.05$) rate of mass loss whilst all other SRF species; *A. pseudoplatanus, B. pendula, C. sativa* and *E. nitens* had a similar pattern of mass loss. In terms of N and C release from litter, earthworm-accessible litterbags showed a higher rate of release compared with controls. *F. excelsior* demonstrated the highest rate of N and C release from earthworm-accessible litterbags compared with other selected SRF species.

Litter decomposition results for *A. pseudoplatanus, B. pendula,* and *C. sativa* in this experiment were completely different from litterbag Experiment 1, conducted with similar species at original forest sites. The difference between earthworm-accessible and inaccessible litterbags for the above three litter species was significant ($p < 0.01$) at the ex-agriculture site (see Table 6.2.5) where earthworm density was 298 $m^{-2}$. However, this difference was not significant for similar SRF species at Gisburn and Rogate where earthworm densities were lower than 26 $m^{-2}$ (see Table 6.2.2). One reason for these different results could relate to earthworm population size, but other variables such as local climate and soil type could also play an important role as sites were in different locations.

Similar to current findings, former researchers suggested that tree species have a great influence on litter decomposition rates. Bocock and Gilbert (1957) observed litter decomposition in mull soils in Britain with an order of disappearance rate as: *B. verrucosa* (birch) > *T. cordata* (lime) > *Q. petraea* and *Q. robur* (oak). Edwards and Heath (1963) observed that *Quercus* spp. litter disks were consistently fragmented more quickly than *Fagus* spp. The leaf litter from non-deciduous broadleaves such as eucalyptus are generally known to take a relatively longer time to decompose in the soil system compared with native broadleaves (Cornelissen, 1996; Louzada *et al*., 1997).

The leaf litter decomposition rates for some of the SRF species are summarised in Table 2.5.2. The past studies have widely compared the overall litter decomposition of various tree species, but the current experiment was unique as it compared the influence of earthworms for decomposition of selected SRF species litter.

This study recorded a seasonal influence of earthworm activity. In 2011, spring was very dry (soil moisture < 10%) and may not have supported earthworm activity and the expected rate of litter mass loss during the first six months. However, the wet summer (soil moisture > 18%) of 2011 had a positive influence on earthworm activity and accelerated litter decomposition between six to nine months of the experiment. Edwards and Heath (1963) suggested that the rate of leaf disk disappearance had a seasonal fluctuation and these changes were markedly correlated with the moisture condition in the litter. The soil animals found in 0.5 mm mesh bags of this site included springtails and mites. In addition to microorganisms, these animals may largely contribute to mass loss of earthworm-inaccessible litterbags.. Within 5 mm mesh bags, earthworms, slugs, woodlice and millipedes were found. In addition to earthworms, these soil animals could have certainly contributed to litter mass loss in 5 mm mesh bags. However, parallel litterbag studies at Rogate and Gisburn suggested that the contribution of these animals for litter mass loss was not significant, although they were present in relatively large numbers. At the Carlshead site, these other animals were found in lower numbers, suggesting that their contribution to litter mass loss was negligible.

Summary of the major finding of litterbag Experiment 2:

- This showed that earthworms have a major contribution to decomposition of *F. excelsior, B. pendula, E. nitens, C. sativa,* and *A. pseudoplatanus* litter, when they are present at high density and diversity.
- It also showed that the earthworm contribution to SRF litter decomposition varied with tree species; *F. excelsior* recorded a significantly higher decomposition rate while *B. pendula, E. nitens, C. sativa,* and *A. pseudoplatanus* showed a similar rate of decomposition.

However, presence of litterbags on ex-agricultural (arable) site where no trees are present can offer a 'bonanza' of food for soil fauna present. This can increased earthworm activity on any litter available and manipulate true choice in this type of study. The Carlshead ex-arable site was recently planted with a variety of potential SRF species. This habitat change can influence the soil faunal population present and future studies are recommended to investigate longer-term influences.

## 6.3 Influence of SRF species litter on mineral nitrogen content of earthworm casts: A laboratory experiment with *Lumbricus terrestris*

### 6.3.1 Introduction

Earthworms are considered to make a major contribution to the mineralisation process of several elements in the soil system, especially nitrogen (Anderson *et al*., 1985; Scheu, 1987). Earthworm casts are known to contain elevated amounts of plant-available nutrients compared with surrounding soils (Parle, 1963; Syers *et al*., 1979; Syers and Springett, 1984). Most of these studies recorded that freshly deposited casts were high in $NH_4^+$, but with time this decreased and $NO_3^-$ concentration increased. Parkin and Berry (1994) evaluated N transformation in earthworm casts and confirmed the earlier observations that earthworm casts are rich with mineral N. In addition, this study suggested that the amount of N accumulation in earthworm cast can be affected by the organic matter used as a food source by earthworms. However, feeding on different tree litter and influences of these on earthworm cast N content was not the focus in any on the above studies.

The current experiment, under controlled environmental conditions, investigated the influence of SRF species litter on mineral nitrogen content of *L. terrestris* casts over a period of five weeks.

### 6.3.2 Methodology

An experiment was set up that utilised three selected SRF species (*A. pseudoplatanus*, *B. pendula* and *E. nitens* of different origins, as stated in Table 3.3.1) and experimental

earthworms which were field-collected adult *L. terrestris.* All experimental earthworms (n = 48) were acclimated to laboratory conditions for six weeks prior to experiment as described in section 4.2.3.4. At the start of the experiment, plastic vessels of 2 L (depth 0.2 m) were partially filled with moist Kettering loam (1,800 g dry soil, 25% moisture, approx. soil depth 0.16 m). Four earthworms (mean individual mass 5.52 g) were randomly assigned to each individual vessel. A known mass (8 g vessel$^{-1}$) of air-dried whole leaves from single SRF species was water-soaked for 20 minutes and surface applied to a previously labelled experimental vessel (Figure 6.3.1). A control was set up in exactly the same way with earthworms and soil but without any litter. Three replicates per treatment were established (n =12 vessels in total). The vessels were kept in 24 hour darkness, in temperature-controlled incubators at 15 $^{o}$C (Figure 6.3.2). Soil moisture was maintained as 25-30% throughout the experiment by watering on inspection. Earthworm vessels were initially kept undisturbed for two weeks to encourage formation of vertical burrows.

**(a)** **(b)** **(c)**

**Figure 6.3.1 Surface litter supply to 2 L experimental earthworm vessels; (a) *A. pseudoplatanus* (b) *B. pendula* (c) *E. nitens*.**

**Figure 6.3.2 Experimental earthworm vessels (2 L) kept at 15 °C in darkness.**

Fresh earthworm cast collection for mineral nitrogen extraction was begun after two weeks of the experiment set up. To do this, the surface litter layer was carefully removed and 2 g of fresh cast was taken from the soil surface (mainly close to the burrow entrance, see Figure 6.3.3), collected with a small metal spatula. After cast collection, removed litter was replaced in individually coded vessels which were then returned to incubators.

**Figure 6.3.3 Surface view of a 2 L vessel showing fresh *L. terrestris* casts at the soil surface and also burrow entrances.**

A standard procedure provided by the Soil Chemistry Laboratory at the Department of Geography and Environmental Science, University of Reading, was used for potassium chloride (KCl) extraction. Collected fresh casts were immediately transferred into 50 ml polyethylene bottles and 20 ml of 1 M KCl was added (1:10 casts: solution extraction ratio). Bottles were closed tight and shaken for one hour on a mechanical shaker at 200 - 300 rpm. The suspension was filtered using Whatman No. 42 filter paper. The extracted samples were labelled and immediately transferred to (-20 °C) freezers. Cast collection and KCl extraction was conducted weekly for four weeks. After four weeks, the whole set of frozen samples plus blank KCl samples were sent to the University of Reading for mineral N analysis ($NH_4^+$ and $NO_3^-$). At the termination of the experiment, unadulterated surface litter was removed, and earthworm survival and mass changes

were recorded. Mineral N contents in casts were compared across treatments using multi-comparison one way ANOVA.

### 6.3.3 Results

Table 6.3.1 demonstrates the initial C and N content of the soil and SRF litter used. Table 6.3.2 summarises the earthworm production summary for the experiment. At the end of the experiments (after 5 weeks), an earthworm mass reduction was recorded for all 4 treatments, (greatest -28.3% for control which had no litter supply). Of the 3 litter treatments, *B. pendula* recorded the highest mean litter removal (92%). At the termination of the experiment 100% earthworm survival was recorded for all treatments including control.

**Table 6.3.1 Initial C and N content of soil and SRF litter used in an experiment examining mineral N content of *L. terrestris* casts**

| **Materials** | **C (%)** | **N (%)** | **C:N** |
|---|---|---|---|
| Kettering loam soil | 2.5 | 0.2 | 12.5 |
| *A. pseudoplatanus* | 47.9 | 1.45 | 33.0 |
| *B. pendula* | 51.5 | 1.59 | 32.4 |
| *E. nitens* | 52.5 | 1.33 | 39.5 |

**Table 6.3.2 Summary of earthworm attributes (after five weeks) from an experiment examining mineral N content of *L. terrestris* casts**

| Earthworm attribute | SRF species | | | |
|---|---|---|---|---|
| | **Br** | **En** | **Sy** | **Control** |
| Number (ind vessel$^{-1}$) | 4 | 4 | 4 | 4 |
| Initial mean mass (g vessel$^{-1}$) | 22.2 | 22.0 | 22.4 | 21.7 |
| Final mean mass (g vessel$^{-1}$) | 21.1 | 20.5 | 20.3 | 15.6 |
| Mean mass change (% of original mass) | -5.0 | -7.1 | -9.2 | -28.3 |
| Mean Litter removal (%) | 92 | 65.9 | 55.5 | 0 |
| Survivorship (%) | 100 | 100 | 100 | 100 |

*B. pendula* (Br), *E. nitens* (En) and *A. pseudoplatanus* (Sy).

Figure 6.3.4 illustrates the change of $NH_4^+$ - N content in fresh casts of *L. terrestris* fed with three species of litter and with no litter (control) over a period of five weeks. Throughout the experiment, no significant difference in $NH_4^+$ content was observed between treatments. However, up to week 4, $NH_4^+$ content in all treatments had increased. Thereafter, this decreased for *E. nitens* and the control although it continued to increase for *B. pendula* and *A. pseudoplatanus.* Initially, *B. pendula* recorded the lowest amount of $NH_4^+$ while *E. nitens* recorded the highest amount. After five weeks, *B. pendula* recorded the highest amount of $NH_4^+$ while *E. nitens* recorded the lowest amount.

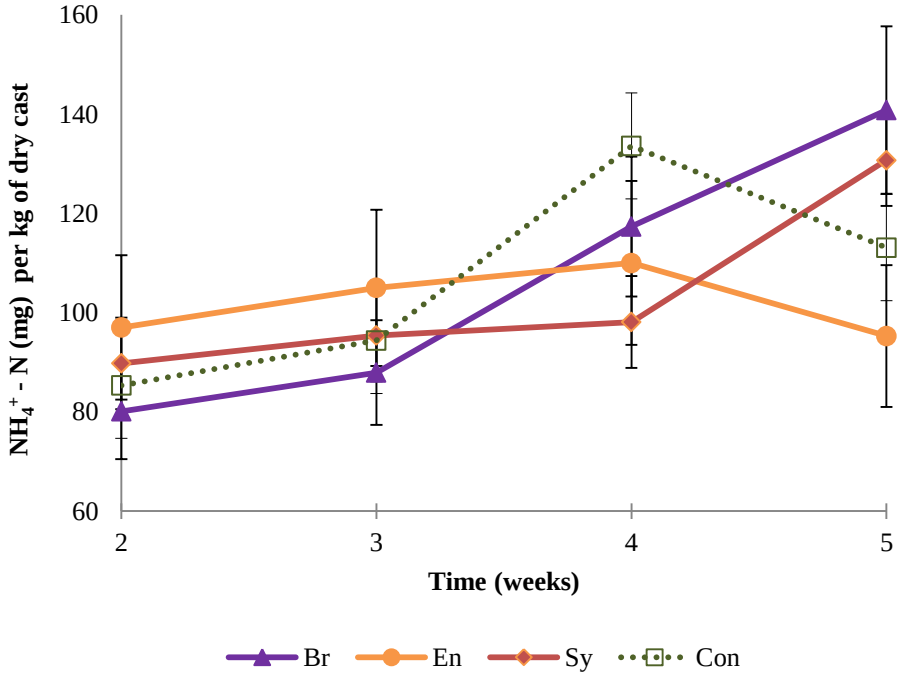



**Figure 6.3.4 Mean (± se) $NH_4^+$ content in casts of *L. terrestris* fed with three SRF species litter; *B. pendula* (Br) *E. nitens* (En) *A. pseudoplatanus* (Sy) and control (Con) over a period of five weeks (n = 3, control with no litter supply).**

Figure 6.3.5 illustrates the change of $NO_3^-$ - N content in fresh casts of *L. terrestris* fed with 3 species of litter and no litter (control) over a period of 5 weeks. Generally, $NO_3^-$ content in fresh casts of all 4 treatments increased with time. Throughout the experiment, a significantly ($p < 0.05$) increased $NO_3^-$ content was observed in the control compared with all litter treatments. However there were no significant differences ($p > 0.05$) between the 3 litter treatments.

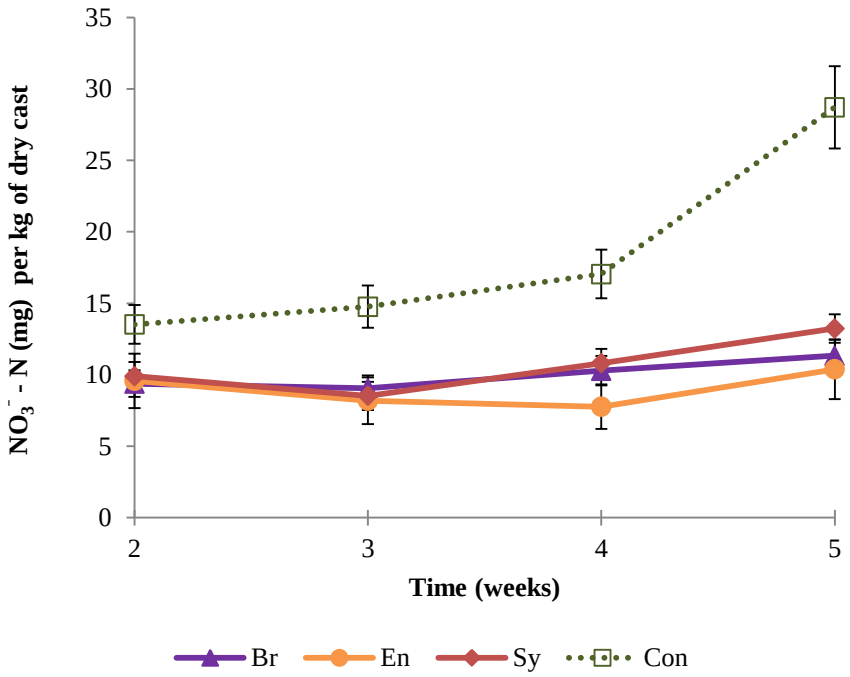



**Figure 6.3.5 Mean (± se) $NO_3^-$ content in casts of *L. terrestris* fed with three SRF species litter and control over a period of five weeks, (n = 3), [Abbreviation of treatments as for Figure 6.3.4].**

### 6.3.4 Discussion

This laboratory study suggests that SRF species litter have an influence on mineral N content of *L. terrestris* casts and its temporal trend. As an example, *B. pendula*-fed earthworms initially recorded a lower amount of $NH_4^+$ in their casts and a higher amount at the end of the experiment. Conversely, *E. nitens*-fed earthworms initially showed a higher amount of $NH_4^+$ in their casts and lower amounts after five weeks.

Buck *et al*. (2000) studied cast production of *L. terrestris* and suggested that mulching materials, as well as soil compaction, influenced earthworm burrowing activity, quantity and nutrient quality of earthworm casts. In the present study, the soil was compacted manually to provide a smooth surface and similar bulk density to avoid a soil

compaction influence on experimental results. *L. terrestris* are known to produce casts at the soil surface as well as within the soil profile. Shipitalo and Protz (1989), who collected surface deposited cast of *L. terrestris,* recorded a 70-180 mg $g^{-1}$ cast production per day while Flegel *et al*. (1998) who also collected the cast from the whole bulk soil recorded 228 – 461 mg $g^{-1}$ cast production per day. In the present study, only fresh surface *L. terrestris* casts were collected for the purpose of chemical analysis and this could have influenced experimental results.

Amounts of mineral N in earthworm casts and their transformations have been widely studied (Parle, 1963; Syers *et al.*, 1979; Syers and Springett, 1984; Parkin and Berry, 1994). Parle (1963) reported that freshly deposited earthworm casts were high in $NH_4^+$, but with time $NH_4^+$ decreased, while $NO_3^-$ increased, indicating high microbial nitrification activity. Similarly, Syers *et al*. (1979) who incubated fresh earthworm casts for 12 days recorded that 87% of the $NH_4^+$ initially present was lost, but increases in the $NO_3^-$ did not match the losses of $NH_4^+$. They suggested that the resulting deficit was due to immobilisation and denitrification. Lavelle *et al*. (1992) suggested that $NO_3^-$ is not an earthworm metabolic product and therefore fresh earthworm cast always recorded low amount of $NO_3^-$ with which current experimental results agree. Further, in the current study, $NO_3^-$ content in earthworm casts significantly increased ($p < 0.05$) in the (no litter) control compared with litter-amended treatments, suggesting that these litter amendments have an influence on nitrification activity. Major factors affecting nitrification in soils include soil moisture, aeration, temperature, and pH (Sahrawat, 2008). In addition, Vernimmen *et al*. (2007) suggested that C:N ratio of the litter and the presence of plant-produced allelochemicals and supply of other nutrients can influence soil nitrification.

Parkin and Berry (1994) evaluated the influence of selected organic residues on microbial N transformations associated with earthworm casting. In this study, they fed two species of earthworms (*Octolasian tyrtaeum* and *Aporrectodea tuberculata*) with chopped, fresh hairy vetch (*Vicia villosa*) (20 g), air-dried vetch (5.5 g) or air-dried horse manure (5.5 g) and measured the $NH_4^+$ and $NO_3^-$ in fresh casts over a period of four weeks. The control was conducted with no litter supply. Similar to the current study, these authors suggested that N- transformation in earthworm casts were affected by the organic residue used as a food by earthworms. The study further indicated that the magnitude of N accumulation in earthworm casts reflected the N content of the organic matter used. Contrasting with the current study, in this experiment $NH_4^+$ content in the casting of all the treatments decreased after 2 weeks, but similarly, $NO_3^-$ content was increased with time. However, the current experiment cannot be compared directly with Parkin and Berry (1994), as soil type, earthworm species, food source, food quantities, and food particle sizes were all different.

The present study was the first recorded attempt to investigate the influence of different tree litter on cast N content of *L. terrestris*. It suggested that mineral N content and its temporal trend in *L terrestris* casts is affected by SRF litter used as a food source. The magnitude of N accumulation in earthworm casts did not reflect the N content of the SRF litter type and the total N content of the initial system. One reason for this may be the similar initial total N content of the selected SRF species litter (see Table 6.3.1). In terms of $NH_4^+$, a significant difference was not observed between litter treatments and control. This may be due to the small amount of added litter (2g per vessel - dry basis) which may not have been enough to have considerably influenced the initial total N content of the system, compared with the (no litter) control. Further, only surface casts were collected in this study, but as previously mentioned, *L. terrestris* also deposits

some casts within the soil. Also, *L. terrestris* are known to have a large contribution to breakdown of, and incorporate of surface litter into the soil (Satchell 1967; Scheu and Wolters, 1991) while endogeic earthworms such as *A. caliginosa* and *A. rosea* feed mainly on fine organic matter mixed with mineral soil and largely contribute to nutrient release (Scheu, 1987). This author found a remarkable influence of endogeic earthworms on N mineralisation within beech woods. Therefore, further experiments with interaction of *L. terrestris* in combination with other earthworm species would be useful to examine the direct influence of individual SRF species on earthworm cast production and N mineralisation activity.

Major findings of this laboratory study:

- *B. pendula*-fed *L. terrestris* initially showed a lower amount of $NH_4^+$ in their casts and a higher amount after five weeks, while *E. nitens*-fed earthworms initially showed a higher amount of $NH_4^+$ in their casts and a lower amount after five weeks.
- Further, $NO_3^-$ content in *L. terrestris* casts increased in the (no litter) control compared with litter-amended treatments.
- SRF species litter have an influence on mineral N content of *L. terrestris* casts and on its temporal trend.

# CHAPTER 7: A FIELD-BASED SRF-EARTHWORM INTERACTION EXPERIMENT USING NATIVE AND NON-NATIVE TREE SPECIES WITH ANECIC AND ENDOGEIC EARTHWORMS

## 7.1 Introduction

Effects of earthworms on soil nutrient dynamics and plant growth have been extensively studied. Scheu (2003) reviewed a total of 67 papers (1947 – 2002), which investigated the influence of earthworms on plant growth. However, most of these studies focused on arable and grassland plant species. Only a very few studies have investigated the influence of earthworms on forest tree species (e.g. Marshall, 1971; Haimi *et al*., 1992; Muys *et al.*, 2003; Welke and Parkinson, 2003). Marshall (1971), in a pot experiment, observed a slight increase in the stem weight of black spruce (*Picea mariana*) seedlings with introduction of earthworms into forest soil. In a laboratory study, Haimi *et al*. (1992) indicated that presence of *Lumbricus rubellus* can increase above-ground biomass of *Betula pendula* seedlings. Welke and Parkinson (2003) indicated that activity of the endogeic earthworm *Aporrectodea trapezoides* increased root biomass of Douglas-fir (*Pseudotsuga menziesii*) seedlings which were grown in a temperature-controlled (15 °C) growth chamber. Most of the aforementioned observed a positive influence of earthworms on forest tree growth. However, almost all of these investigations were pot experiments conducted with very young seedlings (< 1 year) under controlled environmental conditions. The investigation by Muys *et al*. (2003) was the only field study, but these authors did not observe a significant influence of earthworms on growth of *Fraxinus excelsior* established on an acidified sandy loam.

In addition to the effects of earthworms on tree growth, some studies have focused on the effects of tree species on earthworms (Muys *et al*., 1992; Neirynck *et al*., 2000;

Sarlo, 2006) (see chapter 2, section 2.4.2 for more details). These studies, which were mostly based on field surveys, suggested that litter quality and quantity are the determining factors for earthworm community development. However, the direct influences of forest trees and their root systems on earthworms have not been closely investigated in such field surveys.

Some studies which focused on arable and grassland plant species, suggested that effects of earthworms on plant growth can vary with soil type, especially texture, mineral nutrient content and organic matter (Brown *et al*., 2004). Jana *et al*. (2010) suggested that in poor soil with low content of mineral nutrients and organic matter, earthworms increased soil nitrate content significantly and boosted above-ground biomass production of *Arabidopsis thaliana*. Further, plant responses to earthworms can vary with earthworm species or functional group. Laossi *et al.* (2010) suggested that the combined presence of anecic and endogeic earthworms could promote mineralisation and hence plant growth. Besides, the response of plants to earthworms can be influenced by the physiology associated with plant species. As an example, legumes are less responsive to earthworms compared with grasses, since these are less limited by nitrogen (Brown *et al*., 2004). Eisenhauer *et al*. (2009a), who studied the influence of earthworms on regrowth of grassland plant communities, suggested that rapidly growing plant species are promoted more by earthworm activity than slower growing species. However, available evidence concerning the above aspects relevant to forest tree species is very limited.

The aim of the present study was to investigate direct interactions between SRF trees and earthworms under field conditions. Based on a technique used for a tree rooting experiments by Bending and Moffat (1997), a field-based experiment was designed to

provide results within one year. To promote the interaction between tree root systems and earthworms, the trees were grown in tubes buried in the field. This technique allowed removal of the whole experimental system from the ground at the end, permitting very detailed examination of all component parts. The experiment was designed to achieve the following objectives:

a) To investigate the influence of selected SRF species on establishment of introduced earthworms.
b) To explore the effects of earthworm presence on SRF growth and biomass production.
c) To investigate the influence of earthworms on nutrient uptake by SRF trees.
d) To assess the influence of earthworms on SRF litter decomposition and nutrient release.
e) To estimate the influence of earthworms on change of soil properties under selected SRF trees.

## 7.2 Methodology

### 7.2.1 Experimental site

The experiment was established at the Forestry Commission Research Agency, Headley nursery (National Grid Ref. SU 808379), Headley Down, Hampshire. The area, with a mean annual temperature of 11.2 °C, receives an annual average rainfall of 629.8 mm. Local soil type is classified as a sandy humic – ferric podzol (Mackney *et al*., 1983).

### 7.2.2 SRF and earthworm species

The SRF species used were one year old *Betula pendula* (mean above-ground height 0.6 m) and *Eucalyptus nitens* (mean above-ground height 0.4 m). Initial root depth was 0.1 m for both species. These two SRF species, with different origins (native/non-native) were selected for comparative purposes. One year old seedlings were selected, as this is the standard age for field transplantation of these trees. *B. pendula* and its litter has been commonly used for investigations of plant-earthworm interactions (e.g. Satchell and Lowe, 1967; Haimi and Einbork, 1992; Haimi *et al*., 1992; Raty and Huhta, 2004; Butt, 2011). However, investigations on this aspect with eucalyptus are non-existent. The current study permitted comparisons to be drawn with the results of previous studies while providing new information for *E. nitens*.

The earthworm species used were a combination of field-collected *Lumbricus terrestris* (anecic) and *Allolobophora chlorotica* (endogeic) (see Table 7.2.1). *L. terrestris* was selected based on previous earthworm surveys, as it was the most dominant litter-feeding species in most of the SRF sites surveyed (see Chapter 2). *A. chlorotica* was selected with the purpose of testing tree root-earthworm interactions as this endogeic species lives in the upper 0.1 m of the soil and shows a close association with plant root systems (Martinucci *et al*., 1983; Zorn *et al.*, 2005). Combinations of the above species, from different ecological groupings, were used to minimise competition (Lowe and Butt, 1999), but maximise the resource use and influence on soils and plants (Laossi *et al.*, 2010).

### 7.2.3 Tree establishment

Commercially available PVC tubes (0.25 m diameter) were used as tree growing vessels. The tubes were cut into 0.6 m lengths and the base was covered with 1 mm nylon mesh before establishment in the field (Figure 7.2.1).

**Figure 7.2.1 Base of inverted PVC tubes (d=0.25 m, h = 0.6 m) covered with 1 mm mesh before establishment in the field.**

The tubes were buried (mesh covered end at the base) in previously marked positions of an experimental plot, leaving 0.2 m protruding above the soil surface (Figure 7.2.2). The mesh was present to prevent earthworm escape/ingress from the base and the raised height above the soil surface was to deter entry into the top of the tubes during the experiment. Each tube was filled with standard sterilised Kettering loam (Butt *et al*., 1994; Butt, 2011) to the level of the soil surface (approximately 20 kg dry soil per tube)

and moistened to 25 - 30%. The selected SRF species (*B. pendula* and *E. nitens*) were individually planted into the soil-filled PVC tubes (Figure 7.2.3) with 24 trees of each SRF species used. The planting distance was 4 m (between rows) * 2 m (between trees). Each row contained either *B. pendula* or *E. nitens*. A continuous drip irrigation system was allocated to each tube to maintain the required soil moisture level (25%) for optimal tree growth (see Figure 7.2.3). Trees were allowed to equilibrate in the field for two weeks, before earthworm introduction.

**(a)** **(b)**

**Figure 7.2.2 Buried PVC tubes in the experimental plot; (a) 1 mm mesh at the base, (b) 0.2 m gap from soil surface to top of the tube.**

**Figure 7.2.3 One year old *E. nitens* planted in a PVC tube and supplied with drip irrigation (at the start of the experiment).**

### 7.2.4 Earthworm introduction

Two weeks after tree establishment, a combination of adult *L. terrestris* + juvenile *L. terrestris* + adult *A. chlorotica* was introduced to half of the experimental tubes (Table 7.2.1, Figure 7.2.4). The second half of the tubes (12 for each tree species) was kept as a control, with no earthworm addition. A known amount (50 g per tube at the outset) of previously collected air-dried leaf litter from the same tree species, was surface-applied to the tubes as an organic matter source (Figure 7.2.5). The top of each tube was covered with 2 mm mesh to prevent earthworm escape/ingress (Figure 7.2.6). This cover also prevented leaf litter from being blown by the wind and access of predators to the earthworms. Litter addition by the trees to the experimental system was considered as zero due to this upper mesh cover.

**Table 7.2.1 Earthworm inoculum at the beginning of the experiment**

| **Earthworm species** | **Mean individual biomass (g)** | **Density (No. tube$^{-1}$)** | **Biomass (g tube$^{-1}$)** |
|---|---|---|---|
| *L. terrestris* (adult) | 4.27 | 3 | 12.8 |
| *L. terrestris* (juvenile) | 0.66 | 2 | 1.3 |
| *A. chlorotica* (adult) | 0.36 | 10 | 3.6 |
| **Total per tube** | | **15** | **17.7** |

**(a)** **(b)**

**Figure 7.2.4 Earthworm introduction; (a) *L. terrestris* addition (b) Introduced earthworms on the surface of a tube.**

**(a)** **(b)**

**Figure 7.2.5 Surface-applied leaf litter in experimental tubes; (a) *B. pendula*, (b) *E. nitens.***

**Figure7.2.6 Upper end of an experimental tube being covered with 2 mm mesh.**

A total of 150 g of air-dried litter was added per tube throughout the experimental period (on three occasions). No mineral or organic fertilisers were applied. The experimental duration was 12 months, from May 2011 to May 2012.

An electrified (standard) rabbit fence was established around the experimental plot (Figure 7.2.7) to protect trees from small herbivorous mammals.

**Figure 7.2.7 Headley experimental plot in June 2011.**

### 7.2.5 Destructive sampling

After 12 months, any remaining litter on the tube surface was removed to measure litter mass loss. Each expemental tube was carefully lifted from the ground as a complete unit after digging away the surrounding soil (Figure 7.2.8). The lifted tubes were placed on plastic trays and taken into a nearby workshop for processing (Figure 7.2.8 – 7.2.10).

**Figure 7.2.8 Removal of experimental tubes from the ground – May 2012.**

**Figure7.2.9 Ground-lifted *E. nitens* tubes before processing.**

**Figure7.2.10 Ground-lifted *B. pendula* tubes before processing.**

Initially, the above-ground tree section was removed (*B. pendula* from the soil surface and *E. nitens* from the basal node) and processed separately. The above-ground section of *B. pendula* was separated into three sub-samples; leaves, branches and stem (Figure 7.2.11). For *E. nitens*, the above-ground section was divided into four sub-samples; new leaves, old leaves, branches and stem (Figure 7.2.12).

**Figure 7.2.11 *B. pendula* above-ground sub-samples: stem leaves and branches**

**Figure 7.2.12 *E. nitens* above-ground sub-samples: stem, new leaves, old leaves and branches.**

Once the above-ground plant sections had been removed, tubes were laid horizontally on a bench, and cut twice along the vertical axis with an electric saw (Makita BSS611Z 18V LXT Cordless Circular Saw; Figure 7.2.13). This allowed the soil column to be opened undamaged (Figure 7.2.14) and permitted detailed examination of the experimental system including plant roots, earthworm distribution and burrowing below the soil surface.

**Figure 7.2.13 An experimental tube being cut with a circular saw.**

The soil column with plant root system was divided into two sections for sampling; upper (0 - 0.2 m) and lower (0.2 – 0.4 m). Earthworms were hand-sorted from the soil,

washed and species were identified, with earthworm number and live biomass recorded. Non-experimental species were preserved in 4% formaldehyde and taken to the laboratory for identification. Bulk soil and rhizosphere soil (root-attached soil) samples were taken from both upper and lower tube sections for chemical analysis. Rhizosphere soils were separated from roots by shaking the root system directly into a plastic bag. Root samples were divided into 3 sub categories; main root, fine roots (0 – 0.2 m soil depth) and fine roots (0.2 – 0.4 m soil depth). Main root included the stump and all roots bigger than 2 mm in diameter. Fine roots included roots less than 2 mm in diameter. Root samples were jet washed through a sieve (0.5 mm) mesh before oven drying.

Sampling of all experimental tubes was completed within three days. All plant samples were oven-dried at 70 °C for 48 hours. Thereafter, oven dry biomasses were recorded and chemical analyses were performed as described in section 3.3.3. Soil samples were air-dried and chemical analyses were performed as described in section 3.3.5.


**Figure 7.2.14 A cut open experimental tube containing an intact soil column.**

### 7.2.6 Statistical analysis

The direct effects of birch and eucalyptus on earthworms were measured with reference to earthworm density, biomass and population change. The influence of earthworms on the trees was evaluated by recording survival, above and below-ground biomasses and plant nutrient content. The influence of earthworms on litter decomposition was assessed by recording amounts of surface-remaining litter and their nutrient contents. The effect of earthworms on soil was assessed by recording soil moisture, pH, C and N contents. Statistical analyses were performed using Minitab statistical software. A two sample students t-test was used to compare the means of two treatments of each SRF species. Two-way ANOVA was applied to assess the interaction effect of SRF species and earthworms on measured parameters (e.g. tree growth, nutrient stocks, litter decomposition and soil properties).

## 7.3 Results

The experimental plot, which consisted of less fertile sandy soil initially recorded as having no earthworms present. The sterilised Kettering loam (see Table 3.3.5 for soil properties) was used for the experiment to minimise the influence of soil type on earthworm establishment. Further, this provided the opportunity to compare the result with laboratory experiments, as the same soil was used for all the laboratory experiments reported in this thesis.

### 7.3.1 Effects of SRF trees on earthworms

Table 7.3.1 shows the mean earthworm density and live biomass in SRF tubes with earthworms after 12 months. *B. pendula* recorded a significantly higher earthworm density ($p < 0.05$) compared with *E. nitens,* although the same density/live biomass was introduced at the outset of the experiment. However, total earthworm biomass was not significantly different ($p > 0.05$) between the two tree species, even though this was slightly lower under *E. nitens*.

**Table 7.3.1 Mean (± se) parameters after 12 months in earthworm-introduced SRF tubes: *B. pendula* (Br) and *E. nitens* (En)**

| Earthworm parameter | Br | En | Significance |
|---|---|---|---|
| Total density (No tube$^{-1}$) | 15 ± 1.3 | 10 ± 1.3 | * |
| Total biomass (g tube$^{-1}$) | 15.5 ± 1.2 | 13.1 ± 0.8 | ns |

ns: not significant, * significant at $p < 0.05$ (n = 12).

Table 7.3.2 indicates the earthworm species composition in SRF tubes at the end of the experiment. The total number of *L. terrestris* recorded was similar (4) for both tree species. However, total number of *A. chlorotica* was lower for *E. nitens* (4) compared with *B. pendula* (9). In addition to experimental earthworms, a few *L. rubellus* were recorded in both *B. pendula* and *E. nitens* tubes which equated to a mean population of 0.83 and 0.08 per tube respectively. As shown in Table 7.3.3, compared with initial introduced numbers, the *L. terrestris* population decreased (20%) under both tree species after 12 months. The *A. chlorotica* population also decreased by 60% under *E. nitens*, but only by 10% under *B. pendula.*

**Table 7.3.2 Mean earthworm data in earthworm-treated tubes after 12 months [Abbreviations for SRF as for Table 7.3.1)**

| **Earthworm species** | **Density (No. tube$^{-1}$)** | | **Biomass (g tube$^{-1}$)** | |
|---|---|---|---|---|
| | **Br** | **En** | **Br** | **En** |
| *L. terrestris* (mature) | 3 | 2 | 11.3 | 7.81 |
| *L. terrestris* (immature) | 1 | 2 | 1.96 | 3.82 |
| *A. chlorotica* (mature) | 3 | 2 | 0.65 | 0.44 |
| *A. chlorotica* (immature) | 6 | 2 | 0.59 | 0.27 |
| *L. rubellus* (mature) | 0.83 | 0.08 | 0.65 | 0.20 |
| L. spp (immature) | 1 | 2 | 0.36 | 0.74 |
| **Total** | **15** | **10** | **15.5** | **13.1** |

**Table 7.3.3 Population change (%) in earthworm treatments compared with initial numbers**

| **Earthworm species** | **Population change (%)** | |
|---|---|---|
| | ***B. pendula*** | ***E. nitens*** |
| *L. terrestris* | -20 | -20 |
| *A. chlorotica* | -10 | -60 |

In terms of vertical distribution in soil columns, more than 80% of earthworms were recovered from the upper zone (0 – 0.2 m) for both tree species. *L. terrestris* was the only earthworm recorded in the lower zone (0.2 – 0.4 m) from where 50% of this species was recovered.

Although the control tree tubes were expected to be earthworm-free, a few earthworms were recovered. *L. rubellus* was found in both *B. pendula* and *E. nitens*; mean population = 1.25 and 0.92 per tube respectively. In addition, a few *A. caliginosa* were recorded

under the *B. pendula* control (mean population = 0.08). None of the experimental earthworm species were recovered from the control tubes.

Figure 7.3.1 shows an *L. terrestris* burrow opening and middens at the soil surface of an *E. nitens* tube after removal of the surface litter layer at 12 months. Figure 7.3.2 shows an *L. terrestris* burrowing pattern at the edge of the soil column with some burrows extending down to the base (0.4 m).

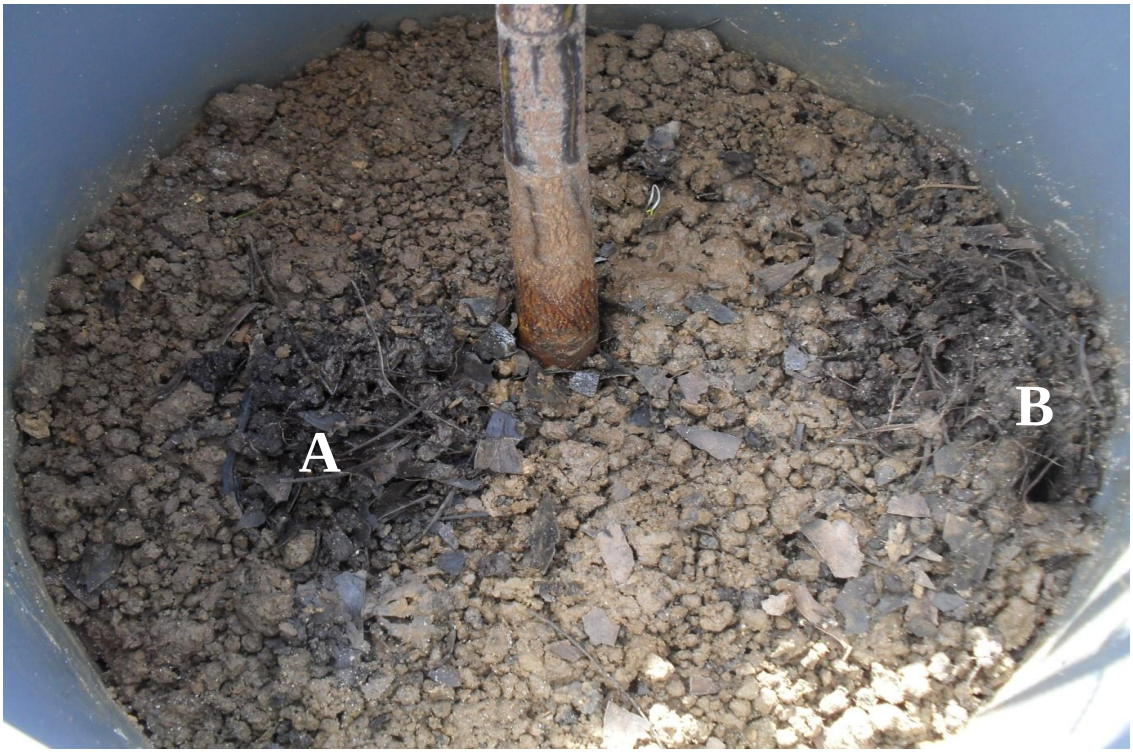



**Figure 7.3.1 *L. terrestris* middens and burrow (A and B respectively) at the soil surface of an *E. nitens* tube after 12 months.**

**Figure 7.3.2 *L. terrestris* burrows at the edge of an *E. nitens* soil column (roots also clearly seen).**

### 7.3.2 Effects of earthworms on SRF growth

A 100% tree survival was recorded for both SRF species at termination of the experiment. Compared with *B. pendula*, *E. nitens* showed pronounced growth throughout the experimental period. Figures 7.3.3 and 7.3.4 demonstrate a visual change of experimental trees during the 12 month period. Differences of root system between tree species (after 12 months) are shown in Figure 7.3.5. Some of the *E. nitens* roots grew through and beyond the basal mesh (Figure 7.3.6).

**(a)** **(b)**

**Figure 7.3.3 Trees at the beginning of the experiment; (a) *B. pendula* (height = 0.6 m), (b) *E. nitens* (height = 0.4 m).**

**(a)** **(b)**

**Figure 7.3.4 Typical trees after 12 months of growth under the given experimental condition; (a) *B. pendula*, (b) *E. nitens* (scale 1:12).**

**Figure 7.3.5 Typical roots after 12 months of growth under the given experimental condition; *B. pendula* (left) *E. nitens* (right) separate into upper (0 – 0.2 m) and lower (0.2 – 0.4) sections (scale 1:8).**

**Figure 7.3.6 An *E. nitens* root which penetrated through the basal mesh.**

Table 7.3.4 shows the mean biomass (oven dry g) of *B. pendula* after 12 months, grown in the presence and absence (control) of earthworms. Introduced earthworms (*L. terrestris* + *A. chlorotica*) showed no significant influence ($p > 0.05$) for below or above-ground biomass of trees compared with the control. However, compared with control, earthworm-treated *B. pendula* trees recorded a slightly lower overall biomass at the termination of the experiment (see Table 7.3.4).

**Table 7.3.4 Mean (± se) below and above-ground biomass (oven dry g) of *B. pendula* after 12 months of growth**

| Attribute | Plant section | Earthworm treatment | Control | Significance |
|---|---|---|---|---|
| **Below-ground biomass** | Main root | 7.0 ± 1.3 | 8.4 ± 1.2 | ns |
| | Fine roots (0 - 0.2 m soil depth) | 2.3 ± 0.1 | 2.6 ± 0.2 | ns |
| | Fine roots (0.2 - 0.4 m soil depth) | 1.6 ± 0.3 | 1.9 ± 0.4 | ns |
| | **Total** | **10.9 ± 1.6** | **12.9 ± 1.8** | **ns** |
| **Above-ground biomass** | Stem | 8.3 ± 1.4 | 10.8 ± 1.0 | ns |
| | Branches | 2.6 ± 0.3 | 3.6 ± 0.5 | ns |
| | Leaves | 1.9 ± 0.2 | 2.2 ± 0.3 | ns |
| | **Total** | **12.9 ± 1.6** | **16.6 ± 1.7** | **ns** |

ns - not significant; (n =12).

Table 7.3.5 demonstrates the mean biomass (oven dry g) of *E. nitens* after 12 months in the presence and absence of earthworms. Earthworm-treated *E. nitens* showed a significantly increased ($p < 0.05$) below-ground, in addition to above-ground biomass compared with the control. In terms of below-ground biomass, earthworms significantly ($p < 0.05$) increased the main root biomass, but showed no significant influence on fine root (< 2 mm) biomass. In terms of above-ground biomass, introduced earthworms

significantly influenced stem ($p < 0.01$) and branch ($p < 0.05$) biomass. Leaf biomass was slightly higher in earthworm-treated *E. nitens* trees compared with control, but it was not significantly different ($p > 0.05$).

**Table 7.3.5 Mean (± se) above and below-ground biomass (oven dry g) of *E. nitens* after 12 months of growth**

| Attribute | Plant section | Earthworm treatment | Control | Significance |
|---|---|---|---|---|
| **Below-ground biomass** | Main root | 64.2 ± 5.0 | 45.1 ± 3.8 | * |
| | Fine roots (0 - 0.2 m soil depth) | 4.9 ± 0.4 | 5.6 ± 0.2 | ns |
| | Fine roots (0.2 - 0.4 m soil depth) | 6.7 ±1.0 | 6.7 ±0.8 | ns |
| | **Total** | **75.9 ± 4.9** | **57.5 ± 3.8** | * |
| **Above-ground biomass** | Stem | 68.9 ± 4.8 | 49.8 ± 3.4 | ** |
| | Branches | 24.9 ± 2.8 | 17.6 ±1.2 | * |
| | Leaves (old + new) | 92.1± 5.5 | 84.5 ±7.4 | ns |
| | **Total** | **186 ± 11.8** | **152 ± 12.2** | * |

ns - not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; (n =12).

Figure 7.3.7 graphically demonstrates differences of the total tree biomass of both SRF species with and without earthworms. The effect of earthworms on total tree biomass was significant in fast growing *E. nitens*, but not in *B. pendula*. Introduced earthworms showed no significant influence on shoot/root ratio (Figure 7.3.8, $p > 0.05$) or basal stem diameter (Figure 7.3.9, $p > 0.05$) of both tree species.

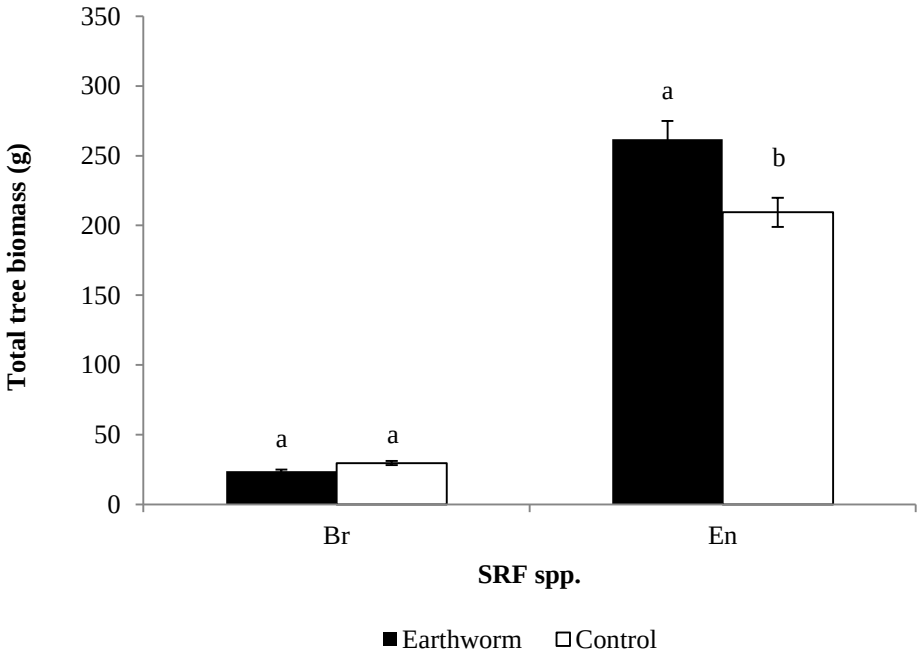



**Figure 7.3.7 Mean (± se) *B. pendula* (Br) and *E. nitens* (En) tree biomass (oven dry grams) at the termination of the experiment (significant differences between earthworm treatment and control for each tree species are indicated by different letters).**

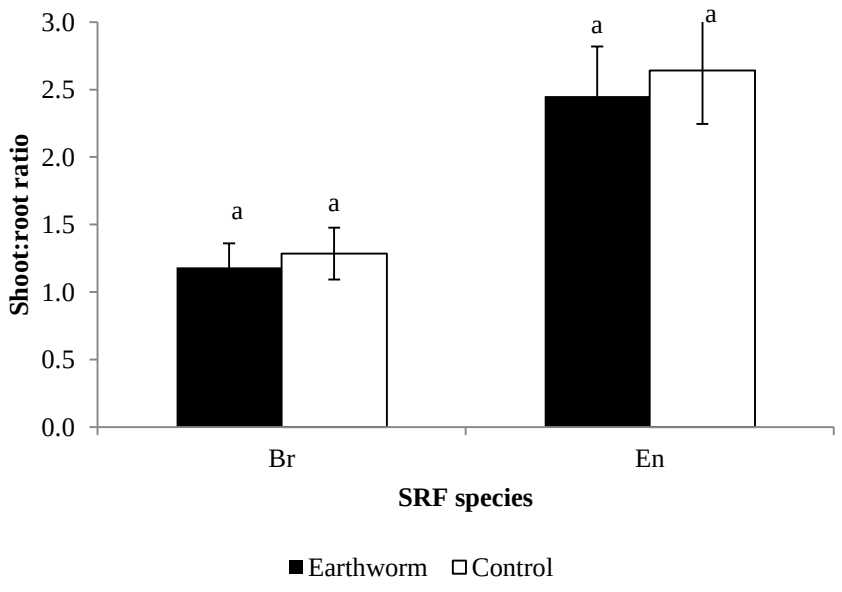



**Figure 7.3.8 Mean (± se) shoot:root ratio of *B. pendula* (Br) and *E. nitens* (En) at the termination of the experiment (significant differences between earthworm treatment and control for each tree species are indicated by different letters).**

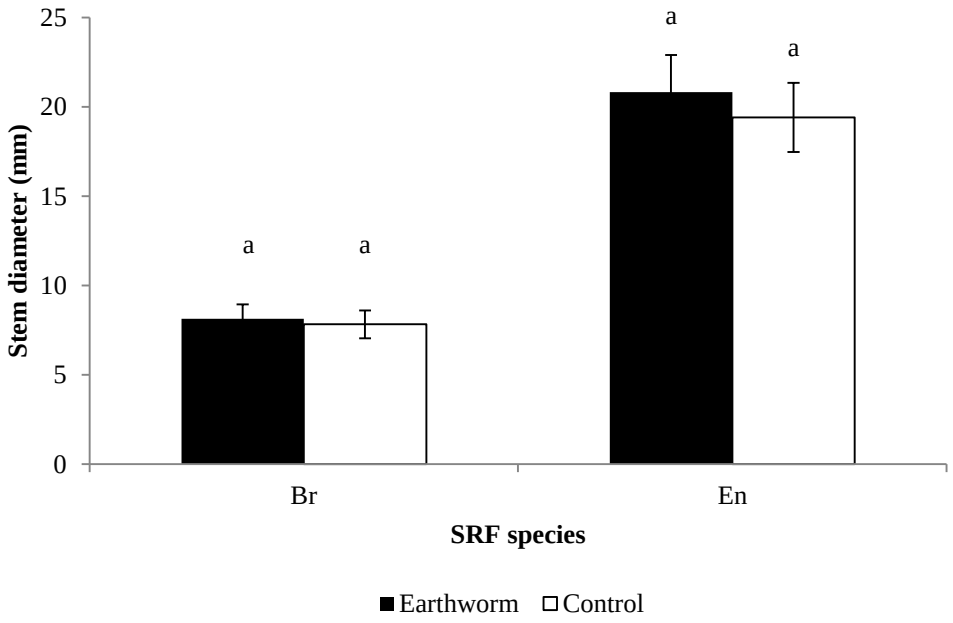



**Figure 7.3.9 Mean (± se) basal stem diameter (mm) of *B. pendula* (Br) and *E. nitens* (En) at the termination of the experiment (significant differences between earthworm treatment and control for each tree species are indicated by different letters).**

The significant interactions in Table 7.3.6 demonstrates that the influence of earthworms on SRF biomass varied with tree species.

**Table 7.3.6 ANOVA Table of F-values showing the effect of SRF species and earthworms on plant oven dry biomass plus stem diameter**

| Source of variation | df | Below-ground biomass | Above-ground biomass | Total tree biomass | Stem diameter |
|---|---|---|---|---|---|
| SRF spp. | 1 | 272 *** | 326 *** | 336*** | 227*** |
| Earthworm | 1 | 6.1 * | 3.2 | 4.2* | 1.1 |
| Interaction | 1 | 9.5 ** | 4.9 * | 6.5* | 0.5 |

* significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$; (n =12).

### 7.3.3 Influence of earthworms on SRF carbon and nutrient stocks and fluxes

Table 7.3.7 demonstrates the mean carbon and nutrient content of different sections of *B. pendula* in the presence and absence of earthworms. Introduced earthworms had no significant influence ($p > 0.05$) on carbon and nutrient stocks of *B. pendula* after 12 months. Table 7.3.8 shows the mean carbon and nutrient content in different sections of *E. nitens* and significant differences in the presence and absence of earthworms. N content in new leaves was significantly increased ($p < 0.05$) in the earthworm treatment compared with control. C, K, Ca, Mg and P content in *E. nitens* branches was significantly increased ($p < 0.05$) in the earthworm treatment compared with control.

**Table 7.3.7 Mean (± se) carbon (g) and nutrient (mg) per plant in different sections of *B. pendula* with respect to oven dry biomass**

| Tree section | Element | Earthworm treatment | Control | Significance |
|---|---|---|---|---|
| **Leaves** | C | 1.01 ± 0.13 | 1.16 ± 0.18 | ns |
| | N | 93.7 ± 9.8 | 114 ± 17.0 | ns |
| | K | 19.0 ± 1.7 | 24.1± 3.3 | ns |
| | Ca | 8.5 ± 1.1. | 8.3 ± 1.1 | ns |
| | Mg | 4.0 ± 0.6 | 4.3 ± 0.7 | ns |
| | P | 10.6 ± 1.0 | 14.0 ± 2.2 | ns |
| **Branches** | C | 1.3 ± 0.2 | 1.8 ± 0.3 | ns |
| | N | 39.9 ± 5.1 | 40.0 ± 7.2 | ns |
| | K | 7.6 ± 1.0 | 10.5 ± 1.6 | ns |
| | Ca | 12.9 ± 1.5 | 17.5 ± 2.6 | ns |
| | Mg | 2.0 ± 0.3 | 2.8 ± 0.4 | ns |
| | P | 3.1 ± 0.4 | 4.3 ± 0.7 | ns |
| **Fine roots (0 – 0.2 m soil depth)** | C | 0.99 ± 0.1 | 1.25 ± 0.1 | ns |
| | N | 55.1 ± 5.9 | 65.6 ± 6.3 | ns |
| | K | 5.1 ± 0.6 | 6.3 ± 0.4 | ns |
| | Ca | 23.0 ± 3.0 | 29.5 ± 3.2 | ns |
| | Mg | 2.3 ± 0.3 | 3.1 ± 0.3 | ns |
| | P | 6.1 ± 0.7 | 8.0 ± 0.8 | ns |
| **Fine roots (0.2 – 0.4 m soil depth)** | C | 0.75 ± 0.14 | 0.92 ± 0.21 | ns |
| | N | 35.4 ± 6.4 | 40.7 ± 8.4 | ns |
| | K | 4.2 ± 0.8 | 4.3 ± 0.6 | ns |
| | Ca | 17.6 ± 3.3 | 24. 0 ± 6.1 | ns |
| | Mg | 1.9 ± 0.4 | 2.1 ± 0.4 | ns |
| | P | 3.7 ± 0.8 | 4.7 ± 1.1 | ns |

ns - not significant at $p > 0.05$; (n =12).

**Table 7.3.8 Mean (± se) carbon (g) and nutrient (mg) per plant in different sections of *E. nitens* with respect to oven dry biomass**

| Tree section | Element | Earthworm treatment | Control | Significance |
|---|---|---|---|---|
| **New leaves** | C | 11.3 ± 1.6 | 9.3 ± 1.0 | ns |
| | N | 265 ± 32.6 | 178 ± 27.9 | * |
| | K | 153 ± 21.0 | 127 ± 13.8 | ns |
| | Ca | 137 ± 21.8 | 108 ± 9.8 | ns |
| | Mg | 25.4 ± 3.7 | 21.7 ± 1.7 | ns |
| | P | 21.5 ± 2.6 | 17.0 ± 3.4 | ns |
| **Old leaves** | C | 35.4 ± 2.3 | 33.1 ± 3.0 | ns |
| | N | 729 ± 76.4 | 571 ± 75.9 | ns |
| | K | 467 ± 37.3 | 433 ± 46.4 | ns |
| | Ca | 724 ± 64.0 | 700 ± 68.4 | ns |
| | Mg | 82.7 ± 6.7 | 83.1 ± 8.9 | ns |
| | P | 64.4 ± 6.3 | 54.4 ± 6.8 | ns |
| **Branches** | C | 11.9 ± 1.3 | 8.4 ± 0.82 | * |
| | N | 153 ± 17.8 | 104 ± 18.7 | ns |
| | K | 174 ±22.7 | 115 ±15.6 | * |
| | Ca | 361 ± 31.8 | 257 ± 21.5 | * |
| | Mg | 23.4 ± 2.2 | 17.3 ±1.7 | * |
| | P | 29.1 ± 2.9 | 19.7 ± 2.7 | * |
| **Fine roots (0 – 0.2 m soil depth)** | C | 2.2 ± 0.21 | 2.5 ± 0.10 | ns |
| | N | 24.1± 1.9 | 30.5 ± 3.6 | ns |
| | K | 25.4 ± 2.6 | 26.6 ± 2.8 | ns |
| | Ca | 80.6 ± 8.3 | 91.6 ± 5.0 | ns |
| | Mg | 6.8 ± 0.7 | 7.9 ± 0.5 | ns |
| | P | 12.2 ± 1.2 | 12.1 ± 1.2 | ns |
| **Fine roots (0.2 – 0.4 m soil depth)** | C | 3.0 ± 0.4 | 3.0 ± 0.4 | ns |
| | N | 32.1 ± 4.0 | 33.5 ±3.1 | ns |
| | K | 38.3 ± 8.2 | 36.8 ± 5.3 | ns |
| | Ca | 109 ± 22.8 | 113 ± 14.6 | ns |
| | Mg | 9.6 ± 1.9 | 10.1 ± 1.4 | ns |
| | P | 24.1 ± 5.2 | 22.5 ± 3.6 | ns |

ns - not significant; * significant at $p < 0.05$; (n =12).

Tables 7.3.9 to 7.3.12 demonstrate that tree species have a significant influence on carbon and nutrient content in major sections of experimental trees in the current work.

**Table 7.3.9 ANOVA Table of F-values showing the effect of SRF species and earthworms on C (g) and nutrient (mg) of leaves per plant with respect to oven dry biomass**

| Source of variation | df | Elements in leaves | | | | | |
|---|---|---|---|---|---|---|---|
| | | C | N | K | Ca | Mg | P |
| SRF spp. | 1 | 347*** | 117*** | 272*** | 265*** | 252*** | 113*** |
| Earthworm | 1 | 0.3 | 1.9 | 0.4 | 0.1 | 0.04 | 0.5 |
| Interaction | 1 | 0.4 | 2.8 | 0.6 | 0.1 | 0.06 | 1.5 |

* significant at $p < 0.05$; *** significant at $p < 0.001$; (n =12).

**Table 7.3.10 ANOVA Table of F-values showing the effect of SRF species and earthworms on C (g) and nutrient (mg) of branches per plant with respect to oven dry biomass**

| Source of variation | df | Elements in branches | | | | | |
|---|---|---|---|---|---|---|---|
| | | C | N | K | Ca | Mg | P |
| SRF spp. | 1 | 103*** | 40.4*** | 93.2*** | 226*** | 159*** | 99*** |
| Earthworm | 1 | 0.3 | 1.0 | 0.3 | 1.2 | 1.2 | 0.4 |
| Interaction | 1 | 1.1 | 2.7 | 0.6 | 1.8 | 2.8 | 1.4 |

*** significant at $p < 0.001$; (n =12).

**Table 7.3.11 ANOVA Table of F-values showing the effect of SRF species and earthworms on C (g) and nutrient (mg) of fine roots (0 – 0.2 m soil depth) per plant with respect to oven dry biomass**

| Source of variation | df | Elements in fine roots (0 – 0.2 m soil depth) | | | | | |
|---|---|---|---|---|---|---|---|
| | | C | N | K | Ca | Mg | P |
| SRF spp. | 1 | 77.3*** | 47.9*** | 152*** | 127*** | 100*** | 24.2*** |
| Earthworm | 1 | 4.4* | 3.1 | 0.5 | 2.7 | 3.9 | 0.8 |
| Interaction | 1 | 0.06 | 0.2 | 0.00 | 0.2 | 0.1 | 0.9 |

*** significant at $p < 0.001$; (n =12).

**Table 7.3.12 ANOVA Table of F-values showing the effect of SRF species and earthworms on C (g) and nutrient (mg) of fine roots (0.2 – 0.4 m soil depth) per plant with respect to oven dry biomass**

| Source of variation | df | Elements in fine roots (0.2 – 0.4 m soil depth) | | | | | |
|---|---|---|---|---|---|---|---|
| | | C | N | K | Ca | Mg | P |
| SRF spp. | 1 | 47.0*** | 0.8 | 45.8*** | 41.8*** | 41.6*** | 34.7*** |
| Earthworm | 1 | 0.1 | 0.3 | 0.02 | 0.1 | 0.1 | 0.01 |
| Interaction | 1 | 0.04 | 0.1 | 0.03 | 0.01 | 0.01 | 0.17 |

*** significant at $p < 0.001$ (n =12).

### 7.3.4 Influence of earthworms on leaf litter decomposition and nutrient release

At the termination of the experiment, surface-remaining litter masses (% dry basis) for both tree species were significantly decreased (Figure 7.3.10, $p < 0.001$) in earthworm treatments compared with controls. Surface litter removal of *B. pendula* was recorded as 61% in the presence of earthworms and 13% without earthworms, while for *E. nitens*, removal was 45% and 17% with and without earthworms respectively.

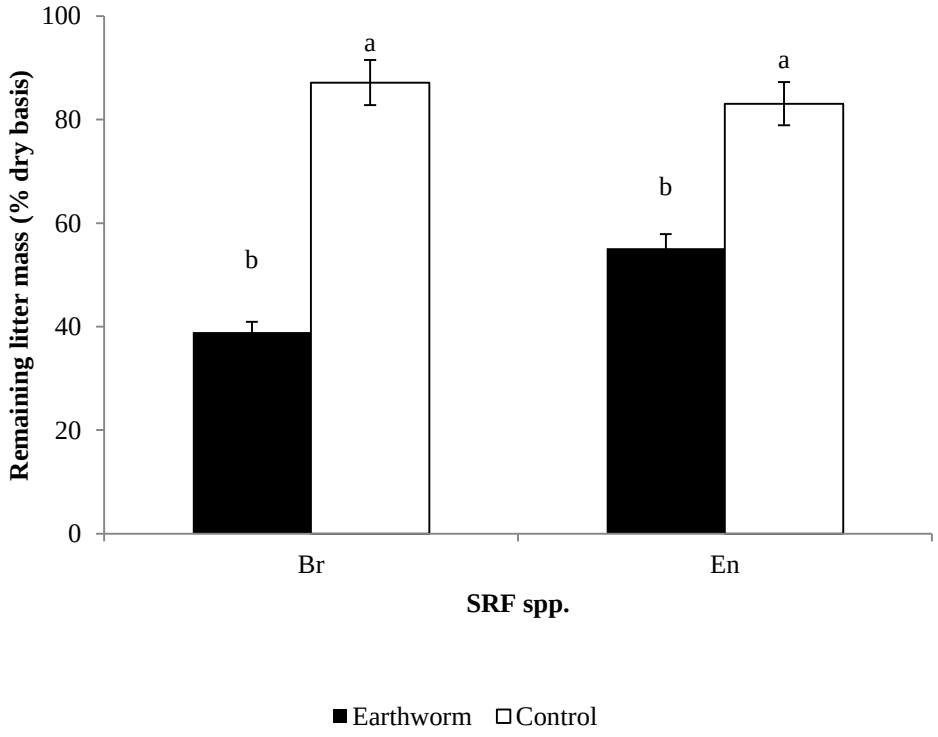



**Figure 7.3.10 Mean (± se) remaining litter mass (%) under *B. pendula* (Br) and *E. nitens* (En) at the termination of the experiment (significant differences between earthworm treatment and control for each tree species are indicated by different letters).**

Table 7.3.13 demonstrates carbon and nutrient content in remaining litter of *B. pendula* with respect to litter mass. After 12 months, carbon and major nutrient content in remaining litter had significantly decreased (probability levels as indicated in Table 7.3.13) in the presence of earthworms compared with no earthworm control.

**Table 7.3.13 Mean (± se) carbon and nutrient content (g) in remaining litter of *B. pendula* at the termination of the experiment with respect to litter mass**

| Element | Earthworm treatment | Control | Significance |
|---|---|---|---|
| C | 22.9 ± 2.0 | 58.8 ± 2.8 | *** |
| N | 0.66 ± 0.05 | 1.97 ± 0.09 | *** |
| K | 0.15 ± 0.02 | 0.22 ± 0.02 | * |
| Ca | 0.65 ± 0.06 | 1.44 ± 0.05 | *** |
| Mg | 0.09 ± 0.01 | 0.23 ± 0.01 | *** |
| P | 0.05 ± 0.00 | 0.13 ± 0.00 | *** |

* significant at $p < 0.05$; *** significant at $p < 0.001$; (n =12).

Table 7.3.14 demonstrates carbon and nutrient content in remaining litter of *E. nitens* with respect to litter mass. At the termination of the experiment, carbon and all other major nutrient in remaining litter had significantly decreased in the earthworm treatment compared with the control.

**Table 7.3.14 Mean (± se) C and nutrient content (g) in remaining litter of *E. nitens* at the termination of the experiment with respect to litter mass**

| Element | Earthworm treatment | Control | Significance |
|---|---|---|---|
| C | 42.7 ± 1.1 | 63.2 ± 1.0 | *** |
| N | 0.84 ± 0.03 | 1.47 ± 0.04 | *** |
| K | 0.08 ± 0.00 | 0.13 ± 0.01 | *** |
| Ca | 1.64 ± 0.05 | 1.99 ± 0.16 | * |
| Mg | 0.12 ± 0.00 | 0.19 ± 0.00 | *** |
| P | 0.04 ± 0.00 | 0.08 ± 0.00 | *** |

* significant at $p < 0.05$; *** significant at $p < 0.001$ (n =12).

The significant interactions in Table 7.3.15 indicate that the influence of earthworms on SRF litter decomposition and nutrient release varied with tree species.

**Table 7.3.15 ANOVA Table of F-values showing the effect of SRF species and earthworms on remaining litter mass (%) and element content (g) after 12 months with respect to litter mass**

| Source of variation | df | Remaining litter (%) | C | N | K | Ca | P |
|---|---|---|---|---|---|---|---|
| SRF spp. | 1 | 7.9** | 42.5*** | 7.2* | 27.2*** | 68.2*** | 57.9*** |
| Earthworm | 1 | 313*** | 231*** | 260*** | 14.8*** | 37.3*** | 225*** |
| Interaction | 1 | 22.2*** | 17.1*** | 32.4*** | 0.5 | 5.5* | 29.4*** |

* significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$; (n =12).

Figure 7.3.11 demonstrates the N loading to soil (g $tube^{-1}$ $year^{-1}$) through litter decomposition. In the presence of earthworms, N loading was four times greater under *B. pendula* compared with the control. The same was double under *E. nitens* in the presence of earthworms compared with the control.

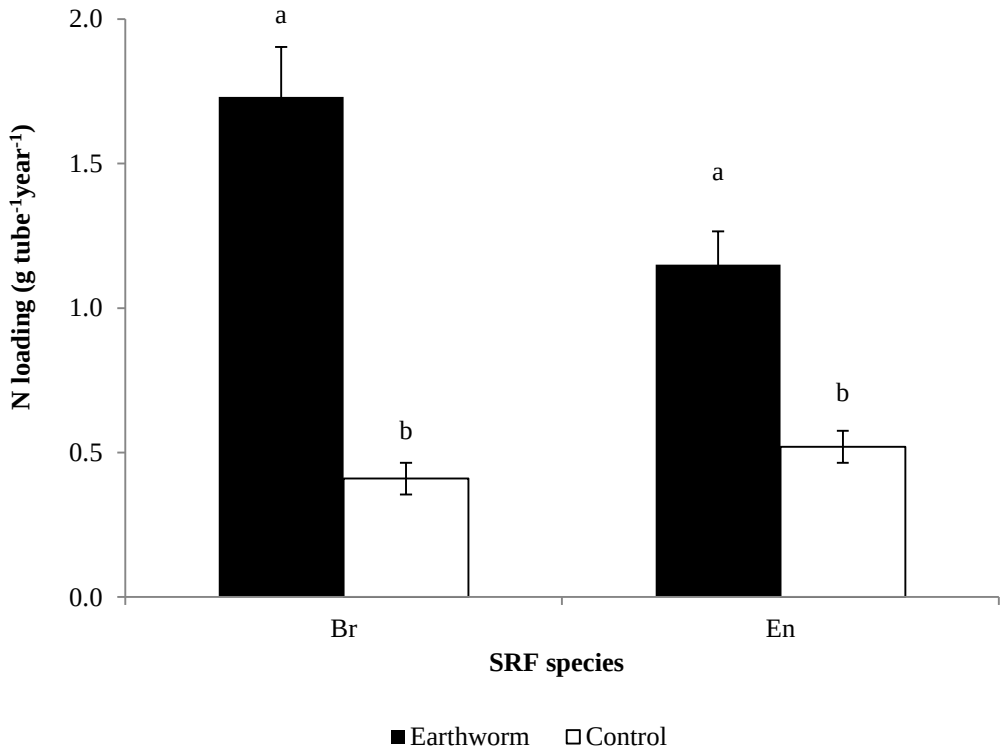



**Figure 7.3.11 Mean (± se) N loading to the soil system (g tube$^{-1}$ year$^{-1}$) through litter decomposition under *B. pendula* (Br) and *E. nitens* (En) (significant differences between earthworm treatment and control for each tree species are indicated by different letters).**

Figure 7.3.12 shows the C loading to the experimental system (g tube$^{-1}$ year$^{-1}$) through litter decomposition. In the presence of earthworms, it was nearly three times greater for *B. pendula* compared with the control. Carbon loading under *E. nitens* was almost double in the presence of earthworms compared with the control.

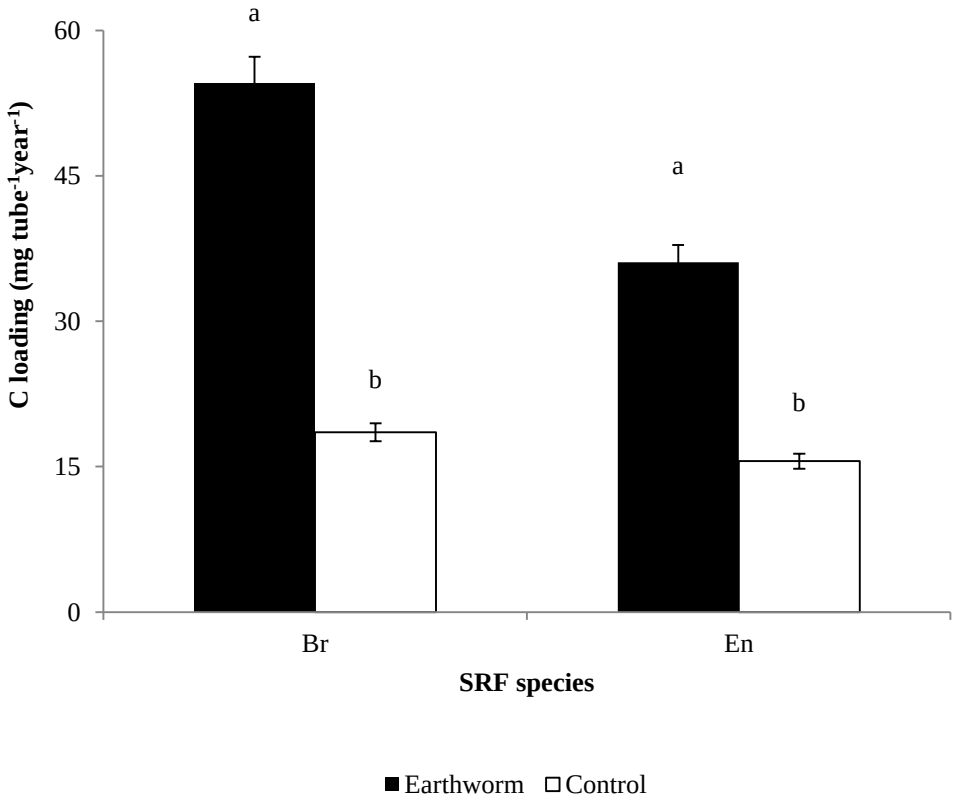



**Figure 7.3.12 Mean (± se) C loading to the soil system (g tube$^{-1}$ year$^{-1}$) through litter decomposition under *B. pendula* (Br) and *E. nitens* (En) (significant differences between earthworm treatment and control for each tree species are indicated by different letters).**

Figure 7.3.13 shows the K loading (g tube$^{-1}$ year$^{-1}$) through litter decomposition. For both tree species, K loading was not significantly different ($p > 0.05$) between the earthworm treatment and control. However, K loading was greater under *E. nitens* compared with *B. pendula*.

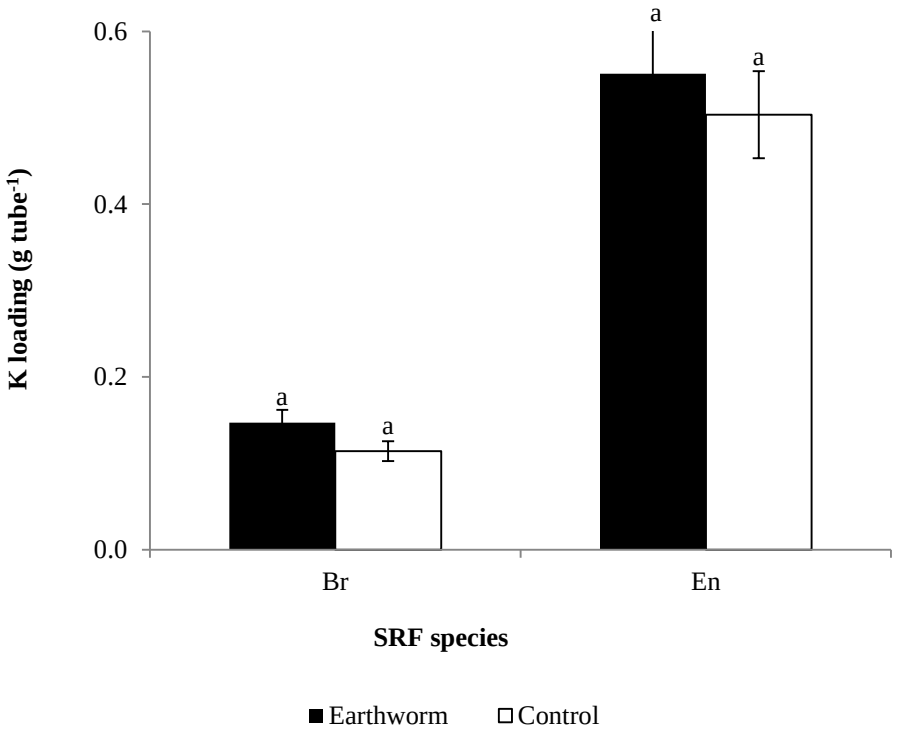



**Figure 7.3.13 Mean (± se) K loading to the soil system (g tube$^{-1}$ year$^{-1}$) through litter decomposition under *B. pendula* (Br) and *E. nitens* (En) (significant differences between earthworm treatment and control for each tree species are indicated by different letters).**

Ca loading (g tube$^{-1}$ year$^{-1}$) through litter decomposition is demonstrated in Figure 7.3.14. In the presence of earthworms, it was three times greater for *B. pendula* compared with the control. Ca loading under *E. nitens* was also significantly greater ($p < 0.05$) in the presence of earthworms compared with control.

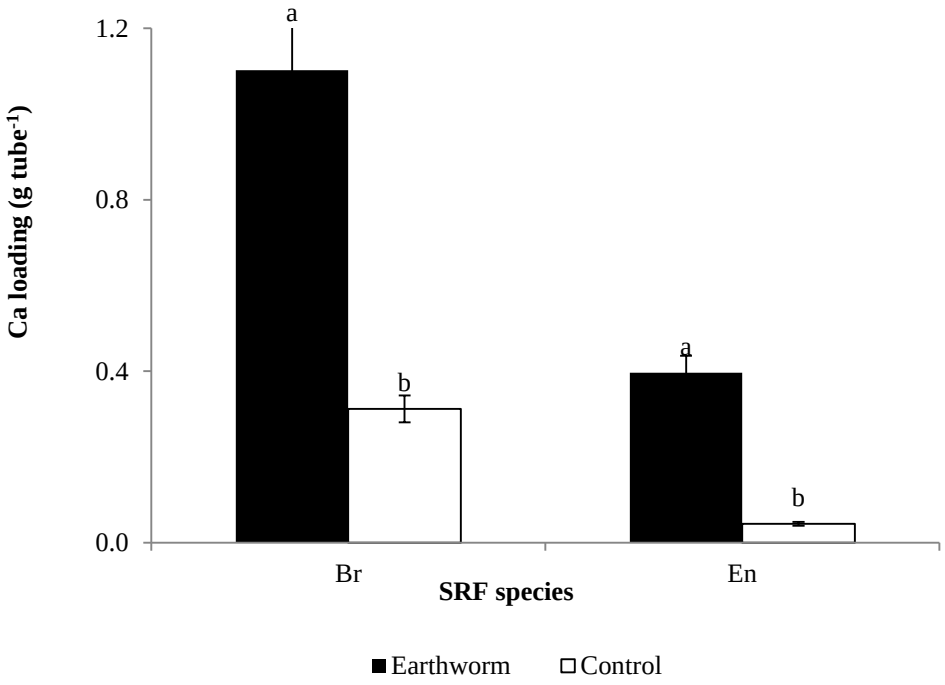



**Figure 7.3.14 Mean (± se) Ca loading to the soil system (g tube$^{-1}$ year$^{-1}$) through litter decomposition under *B. pendula* (Br) and *E. nitens* (En) (significant differences between earthworm treatment and control for each tree species are indicated by different letters).**

Mg loading (g tube$^{-1}$ year$^{-1}$) in the experiment through litter decomposition is shown in Figure 7.3.15 In the presence of earthworms, it was significantly greater ($p < 0.05$) for *B. pendula* compared with the control while under *E. nitens*, there was no significant difference ($p > 0.05$) between the earthworm treatment and the control.

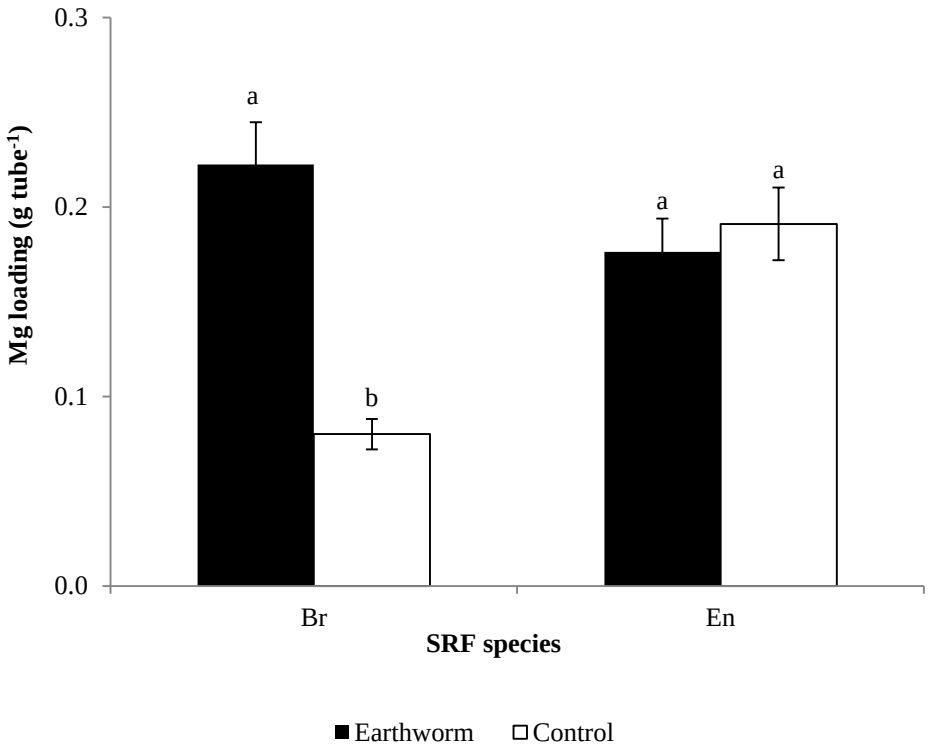



**Figure 7.3.15 Mean (± se) Mg loading to thesoil system (g tube$^{-1}$ year$^{-1}$) through litter decomposition under *B. pendula* (Br) and *E. nitens* (En) (significant differences between earthworm treatment and control for each tree species are indicated by different letters).**

Figure 7.3.16 shows the P loading (g tube$^{-1}$ year$^{-1}$) through litter decomposition. In the presence of earthworms, it was significantly greater ($p < 0.05$) for *B. pendula* compared with the control. P loading under *E. nitens* was not significantly different ($p > 0.05$) between the earthworm treatment and control.

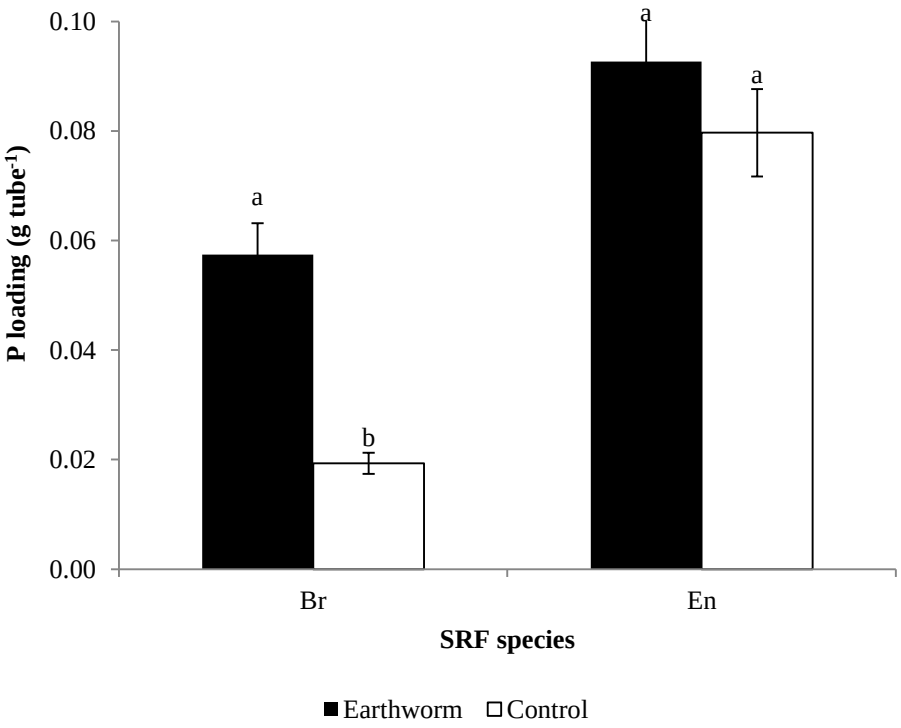



**Figure 7.3.16 Mean (± se) P loading to the soil system (g tube$^{-1}$ year$^{-1}$) through litter decomposition under *B. pendula* (Br) and *E. nitens* (En) (significant differences between earthworm treatment and control for each tree species are indicated by different letters).**

Table 7.3.16 demonstrates the average C and nutrient loading (kg ha$^{-1}$ yr$^{-1}$) through decomposition of *B. pendula* and *E. nitens* litter in the presence and absence of earthworms.

**Table 7.3.16 Carbon and nutrient loading (kg $ha^{-1}$ $yr^{-1}$) to soil system through decomposition of SRF species litter in the presence and absence of earthworms**

| Element | *B. pendula* | | *E. nitens* | |
|---|---|---|---|---|
| | **Earthworm** | **No earthworm** | **Earthworm** | **No earthworm** |
| N | 350.3 | 83.5 | 234.2 | 105.9 |
| C | 11067 | 3766 | 7348 | 3173 |
| K | 30.5 | 22.4 | 112.0 | 101.8 |
| Ca | 224.0 | 63.1 | 81.5 | 8.1 |
| Mg | 44.8 | 16.3 | 36.7 | 38.7 |
| P | 12.2 | 4.1 | 18.3 | 16.3 |

### 7.3.5 Effects of earthworms on soil properties

Table 7.3.17 demonstrates the mean soil parameters under *B. pendula*, and significant differences between earthworm treatment and control after 12 months. The C content (%) in bulk soil (0.2 – 0.4 m) was the only parameter to show a significant influence ($p < 0.05$) produced by earthworms. Soil moisture, pH, C or N content (%) in bulk soil (0 – 0.2 m depth), rhizosphere soil (0 – 0.2 m depth) and rhizosphere soil (0.2 – 0.4 m depth) were not influenced by earthworms after 12 months.

**Table 7.3.17 Mean soil parameters under the two treatments of *B. pendula* at the termination of the experiment**

| Sample type | Soil parameter | Earthworm treatment | Control | Significance |
|---|---|---|---|---|
| Bulk soil (0 – 0.2 m) | Soil moisture (%) | 30.3 ± 0.37 | 30.7 ± 0.34 | ns |
| | Soil pH ($H_2O$) | 8.18 ± 0.00 | 8.19 ± 0.00 | ns |
| | N (%) | 0.18 ± 0.00 | 0.18 ± 0.00 | ns |
| | C (%) | 2.27 ± 0.02 | 2.27 ± 0.02 | ns |
| Bulk soil (0.2 – 0.4 m) | Soil moisture (%) | 32.8 ± 0.1 | 32.5 ± 0.2 | ns |
| | Soil pH ($H_2O$) | 8.18 ± 0.01 | 8.19 ± 0.01 | ns |
| | N (%) | 0.19 ± 0.00 | 0.18 ± 0.00 | ns |
| | C (%) | 2.28 ± 0.02 | 2.18 ± 0.02 | * |
| Rhizosphere soil (0 – 0.2 m) | Soil moisture (%) | 33.7 ± 0.8 | 33.3± 1.5 | ns |
| | Soil pH ($H_2O$) | 8.10 ± 0.01 | 8.13 ± 0.01 | ns |
| | N (%) | 0.20 ± 0.00 | 0.20 ± 0.00 | ns |
| | C (%) | 2.68 ± 0.1 | 2.65 ± 0.1 | ns |
| Rhizosphere soil (0.2 – 0.4 m) | Soil moisture (%) | 31.8 ± 0.6 | 31.9 ± 0.8 | ns |
| | Soil pH ($H_2O$) | 8.14 ± 0.02 | 8.16 ± 0.02 | ns |
| | N (%) | 0.19 ± 0.00 | 0.19 ± 0.00 | ns |
| | C (%) | 2.24 ± 0.04 | 2.23 ± 0.04 | ns |

ns – not significant; * significant at $p < 0.05$; bulk soil (n = 6) and rhizosphere soil (n = 12)

Table 7.3.18 indicates that earthworms had no significant influence ($p > 0.05$) on any of the measured soil parameters under *E. nitens.*

**Table 7.3.18 Mean soil parameters under the two treatments of *E. nitens* at the termination of the experiment**

| Sample type | Soil parameter | Earthworm treatment | Control | Significance |
|---|---|---|---|---|
| Bulk soil (0 – 0.2 m) | Soil moisture (%) | 30.4 ± 0.5 | 30.4± 0.2 | ns |
| | Soil pH ($H_2O$) | 8.20 ± 0.01 | 8.19 ± 0.00 | ns |
| | N (%) | 0.19 ± 0.00 | 0.19 ± 0.00 | ns |
| | C (%) | 2.35 ± 0.04 | 2.30 ± 0.02 | ns |
| Bulk soil (0.2 – 0.4 m) | Soil moisture (%) | 31.2 ± 0.5 | 30.5 ± 0.3 | ns |
| | Soil pH ($H_2O$) | 8.18 ± 0.01 | 8.20 ± 0.01 | ns |
| | N (%) | 0.18 ± 0.00 | 0.18 ± 0.00 | ns |
| | C (%) | 2.22 ± 0.01 | 2.21 ± 0.02 | ns |
| Rhizosphere soil (0 – 0.2 m) | Soil moisture (%) | 33.3 ± 2.1 | 33.6 ± 1.3 | ns |
| | Soil pH ($H_2O$) | 8.05 ± 0.02 | 8.05 ± 0.01 | ns |
| | N (%) | 0.20 ± 0.00 | 0.19 ± 0.00 | ns |
| | C (%) | 3.04 ± 0.3 | 2.91 ± 0.2 | ns |
| Rhizosphere soil (0.2 – 0.4 m) | Soil moisture (%) | 28.2 ± 0.6 | 29.2 ± 0.6 | ns |
| | Soil pH ($H_2O$) | 8.17 ± 0.01 | 8.17 ± 0.01 | ns |
| | N (%) | 0.19 ± 0.00 | 0.19 ± 0.00 | ns |
| | C (%) | 2.24 ± 0.03 | 2.23 ± 0.04 | ns |

ns – not significant; bulk soil (n = 6) and rhizosphere soil (n =12).

The significant interactions in Tables 7.3.19 and 7.3.20 indicated that the influence of earthworms on soil properties varied with tree species.

**Table 7.3.19 ANOVA Table of F-values showing the effect of SRF species and earthworms on bulk soil properties**

| Source of variation | df | Bulk soil (0 – 0.2 m) | | | | Bulk soil (0.2 – 0.4 m) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Moisture | pH | N | C | Moisture | pH | N | C |
| SRF spp. | 1 | 0.05 | 5.3* | 5.3* | 3.3 | 26.4*** | 0.9 | 3.1 | 0.8 |
| Earthworm | 1 | 0.2 | 1.0 | 0.06 | 0.8 | 1.7 | 2.3 | 0.4 | 5.6* |
| Interaction | 1 | 0.4 | 1.7 | 0.02 | 0.5 | 0.3 | 0.1 | 6.9* | 4.8* |

* significant at $p < 0.05$; *** significant at $p < 0.001$; (n = 6).

**Table 7.3.20 ANOVA Table of F-values showing the effect of SRF species and earthworms on rhizosphere soil properties**

| Source of variation | df | Rhizosphere soil (0 – 0.2 m) | | | | Rhizosphere soil (0.2 – 0.4) m) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Moisture | pH | N | C | Moisture | pH | N | C |
| SRF spp. | 1 | 0.0 | 18.2*** | 1.1 | 2.7 | 18.7*** | 2.1 | 4.0* | 4.0* |
| Earthworm | 1 | 0.03 | 1.39 | 0.01 | 0.2 | 0.6 | 0.3 | 0.9 | 0.9 |
| Interaction | 1 | 0.03 | 1.0 | 0.1 | 0.1 | 0.4 | 0.1 | 1.7 | 1.7 |

* significant at $p < 0.05$*** significant at $p < 0.001$; (n =12).

## 7.4 Discussion

### 7.4.1 Effects of SRF species on earthworms

Results suggest that individual SRF species differ in their influence on establishment of introduced earthworms. Compared with *B. pendula*, *E. nitens* offered less support for overall earthworm establishment. This study further suggests that influence of tree species on earthworms can vary with earthworm species. Both *B. pendula* and *E. nitens* had a positive influence on an *L. terrestris* population, however, *E. nitens* had a

negative influence on an *A. chlorotica* population, although this earthworm species was well established under *B. pendula*.

Previous research has suggested that individual tree species have differing effects on earthworm community development, depending on litter quality and quantity (e.g. Hendriksen, 1990; Tian *et al*., 1993; Zou, 1993; Neirynck *et al*., 2000; Sarlo, 2006). In the current experiment, litter quantity was kept constant, but litter quality differed (see Table 3.3.3 for litter chemistry). Lower quality *E. nitens* litter, with a relatively low N content and high aluminium (Al) content, may negatively influence earthworm population development. However, if the litter quality was such a determinant factor in this experiment, it should have had the greatest influence on the litter-feeding species *L. terrestris*. Nevertheless, *L. terrestris* population establishment did not significantly differ between experimental SRF species. The major difference was observed with the endogeic *A. chlorotica*, which feeds on mineral soil (Bouché, 1977) and lives in close proximity of plant root systems (Zorn *et al.*, 2005). Table 7.4.1 indicates the root chemistry of *B. pendula* and *E. nitens* (at 0 - 0.2 m soil depth) where this earthworm species has normally colonised. This suggests that significantly lower N concentration ($p < 0.001$) and significantly higher Al content ($p < 0.05$) in *E. nitens* roots (compared with *B. pendula*) may negatively influence *A. chlorotica* establishment under *E. nitens*.

**Table 7.4.1 Root chemistry of *B. pendula* and *E. nitens* at 0 - 0.2 m soil depth**

| Element | *B. pendula* | *E. nitens* | Significance |
|---|---|---|---|
| N (%) | 2.49 ± 0.1 | 0.54 ± 0.1 | *** |
| C (%) | 47.6 ± 0.2 | 45.1± 0.2 | *** |
| K (%) | 0.24 ± 0.03 | 0.47 ± 0.03 | *** |
| Ca (%) | 1.10 ± 0.06 | 1.62 ± 0.06 | *** |
| Mg (%) | 0.11± 0.00 | 0.14 ± 0.00 | * |
| P (%) | 0.30 ± 0.01 | 0.21 ± 0.02 | * |
| Na (mg/kg) | 163 ± 16 | 2126 ± 92 | *** |
| Al (mg/kg) | 1369 ± 107 | 2032 ± 212 | * |

* significant at $p < 0.05$;*** significant at $p < 0.00l$; (n =12)

A significantly lower pH in rhizosphere soil (0 – 0.2 m depth) was observed below *E. nitens* compared with *B. pendula* (Table 7.4.2, $p < 0.001$). The decreased pH under *E. nitens* might be associated with root exudes which may influence earthworm development.

**Table 7.4.2 Chemistry of rhizosphere soil of two SRF species at 0 – 0.2 m depth**

| Soil parameter | *B. pendula* | *E. nitens* | Significance |
|---|---|---|---|
| Soil moisture (%) | 33.3 ± 1.5 | 33.4 ± 1.3 | ns |
| pH | 8.13 ± 0.01 | 8.05 ± 0.01 | *** |
| C (%) | 2.61 ± 0.17 | 2.91± 0.15 | ns |
| N (%) | 0.20 ± 0.00 | 0.19 ± 0.00 | ns |

ns - not significant* significant at $p < 0.05$; (n =12)

Leaf litter quality can have a certain influence on *A. chlorotica* development as *L. terrestris* mix surface leaf litter with mineral soil. In the current experiment, the

negative influence of *E. nitens* on *A. chlorotica* is likely to be a combined effect of root chemistry and litter quality.

### 7.4.2 Effects of earthworms on SRF growth

In the presence of earthworms, *E. nitens* growth was significantly increased. However, growth of similarly treated *B. pendula* was not significantly influenced by earthworms after 12 months. This suggests that fast growing tree species such as *E. nitens* benefit more from earthworm activity than relatively slower growing SRF species such as *B. pendula*.

With *E. nitens*, *L. terrestris* was considered to be the most responsible species for increasing plant growth, as *A. chlorotica* was not well-established under this tree (only 40% of the initial population was recovered after 12 months). However, there was a possibility that dead *A. chlorotica* may have acted as a nutrient source, especially as a N source for *E. nitens* and this could have increased tree growth. Haimi *et al*. (1992) recorded such an influence for *B. pendula* seedling growth caused by dead *L. rubellus*. However, this influence in the present study is suggested to be negligible as dead *A. chlorotica* cannot provide such an amount of N as its biomass was very small (individual mean mass = 0.36 g) compared with the whole experimental system.

In the present experiment, both below and above-ground tree biomasses (oven dry grams) of *E. nitens* were enhanced by introduced earthworms. In terms of below-ground biomass, earthworm influence was significant for main root production but not for the fine roots (< 2 mm in diameter). For above-ground biomass, earthworm influence was significant for stem and branches but not for the leaves. Both fine roots and leaves are

the plant feeders from soil and air respectively and the most active part of the plant. These behave differently from the other parts such as stems, branches and main root which act as translocators and storage places for nutrients. This may be the reason for the above results when evaluating longer term earthworm influence on biomass of different sections of *E. nitens*. However, all of the above tree growth parameters of *B. pendula* were not significantly influenced by earthworms. Since *B. pendula* was slow growing compared with *E. nitens*, effects on growth as a result of earthworm influence may not be observed within 12 months, especially when exposed to field conditions. Although not statistically significant, results for *B. pendula* in the current experiment showed a slight decrease in both below and above-ground biomasses in the presence of earthworms. Contrasting with current findings, Haimi *et al*. (1992) recorded a significant increase in above-ground biomass of *B. pendula* seedlings in the presence of *L. rubellus* in a laboratory experiment. This 51 week experiment grew *B. pendula* seedling (height = 90 mm) from seeds on a reconstructed forest soil (which included mineral, humus and litter horizons). These authors suggested that enhanced decomposition and nutrient mineralisation by earthworms could be the most important factors which increased seedling growth. The plant growth stage, soil type, the manipulated environmental conditions and the earthworm species can be reasons for completely different results in this study compared with current experimental results. However, similar to current findings, Haimi *et al*. (1992) also recorded a slight decrease in *B. pendula* root biomass in the presence of earthworms.

Welke and Parkinson (2003) found significant increase in root biomass of Douglas-fir (*Pseudotsuga menziesii*) seedlings grown in *Aporrectodea trapezoides*-worked soil. The experiment, conducted in temperature controlled (15 °C) growth chambers, recorded no significant increase in shoot biomass following earthworm activity. However, in

addition to differences in environmental conditions, plant species and earthworm species, Welke and Parkinson's work differed from the current work as they grew seedlings on previously earthworm-worked soil, not in the presence of earthworms. To date, this was the only study which suggested a positive influence of earthworms on forest tree root biomass, but it did not measure the direct influence of earthworm on tree roots.

Jana *et al*. (2010) studied the influence of *A. caliginosa* on growth of a model plant, *Arabidopsis thaliana,* in growth chambers. Although this was not a forest tree species, this laboratory experiment is worthy of comparison with the current study as it investigated the influence of earthworms on plant root growth in depth. The authors used two types of soil and concluded that in poor soil (with low mineral nutrients and organic matter content), earthworms increased soil nitrate content significantly and boosted above-ground biomass production. This study recorded a slight decrease in plant root biomass and length in the presence of earthworms in both types of soils. The authors further indicated that in poor soil, earthworms influenced the architecture of the root system. The biomass corresponding to fine roots was significantly reduced in the presence of earthworms, whereas biomass allocation to larger roots was increased. The current findings with *E. nitens* concur with this, as a significant increase in main root biomass, but slight reduction in fine root biomass was found. However, this was not the case with *B. pendula* root biomass, as a slight reduction in both main and fine root biomass was found. As most *B. pendula* results (+/- earthworms) are not statistically significant after 12 months, a longer-term experiment would be required to confirm the actual influence of earthworms on slower growing SRF species.

In addition to previously discussed laboratory or greenhouse-based experiments, Muys *et al*. (2003) introduced a combination of anecic (*L. terrestris* and *Nicodrilus longus*) and endogeic (*A. caliginosa, A. rosea* and *A. limicola*) earthworms to field planting pits of *F. excelsior*, established on acidified sandy soil with P, K, Ca and Mg fertilisers. In this long-term experiment, tree growth was monitored annually. Control trees with no fertiliser or earthworms failed to grow and died after two years. Fertilised trees grew more rapidly than fertilised plus earthworm-treated trees during the first two years, but this trend was reversed from four years onwards. However, after 10 years, the difference between fertilised plots with and without earthworms was not significant. These authors observed that endogeic earthworms recolonise more successfully in acidified forest soil, than the less acid-tolerant anecic earthworms. The study suggested that the initial inferior growth of *F. excelsior*, in the presence of earthworms, may reflect N immobilisation caused by development of the earthworm population. This kind of N immobilisation by earthworms had been previously suggested by several authors (Syers and Springett, 1984; Robinson *et al*, 1992). The slightly slow growth of *B. pendula* in the presence of earthworms in the current experiment could well be mostly associated with initial N immobilisation by developing earthworms. The well-established earthworm population under *B. pendula* may create resource competition, especially in this closed system and retard tree growth. However, fast growing *E. nitens* may out-compete earthworms for nutrient resources. Further, less *B. pendula* growth in the presence of a high earthworm population can be associated with root feeding; especially by *A. chlorotica*, which lives in close proximity to plant root systems and could possess this kind of influence. Although various authors have suggested that earthworm have the potential to damage plant roots, only one study has confirmed that earthworms feed on unhealthy plant roots (Cortez and Bouché, 1992). The direct influence of *A. chlorotica* on plant growth could not be confirmed in the current study, as it was used in

combination with *L. terrestris*. Therefore, further investigations would be required for confirmation using individual earthworm species inoculation.

### 7.4.3 Influence of earthworms on SRF carbon and nutrient stocks and fluxes

In the current experiment, carbon and nutrient content in any section of *B. pendula* was not significantly influenced by earthworms after 12 months. However, introduced earthworms had significantly increased carbon and nutrient content of some parts of *E. nitens* e.g. N content in new leaves and C, K, Ca, Mg and P in branches.

Some previous investigations found that foliar nutrient levels were elevated in the presence of earthworms, especially N concentration (Haimi *et al*., 1992; Stephens *et al*., 1994; Doube *et al*., 1997b). Contrasting with current findings of *B. pendula*, Haimi *et al*. (1992), observed significantly increased leaf N content of seedlings grown in the presence of *L. rubellus* which they related to increased mineralisation in the presence of earthworms. However, Marshall (1971) found no effect of earthworm activity on the N content of Black spruce needles, whereas Robinson *et al.* (1996) recorded increased uptake of K and Mg by spruce (*Picea sitchensis*) roots grown in limed peat forest soil in the presence of *A. caliginosa*. Welke and Parkinson (2003) observed enhanced Ca in Douglas-fir needles, grown in earthworm-worked soil but not N levels. All of these previous findings, along with current results suggest that the scale of influence of earthworms on plant nutrient uptake, stocks and fluxes can vary with tree species, earthworm population size, earthworm species, soil type, tree growth stage and environmental conditions.

### 7.4.4 Influence of earthworms on SRF litter decomposition and nutrient release

Results suggest that earthworms have a significant influence on surface litter removal of both experimental SRF species. Litter removal of *B. pendula* by earthworms was more rapid than for *E. nitens*. Previous studies observed that *B. pendula* is a preferred source of food, especially for *L. terrestris* (Satchell and Lowe, 1967; Butt, 2011). Some studies suggest that *L. terrestris* has a major contribution to overall breakdown and incorporation of tree litter into mineral soils in many temperate types of woodland (Satchell, 1967; Scheu and Wolters, 1991). Endogeic earthworms such as *A. chlorotica* feed mainly on mineral soil and do not directly remove leaf litter from the soil surface. However, anecic and endogeic earthworms have synergistic effects on organic matter distribution within the soil system (Lowe and Butt, 2003). In the current experiment, total C, N, Ca, Mg and P loadings to the soil system (g $tube^{-1}year^{-1}$) through litter decomposition was significantly increased for *B. pendula* in the presence of earthworms compared with control. For *E. nitens*, C, N and Ca loading was increased in the presence of earthworms compared with control. Total C and nutrient loading through litter decomposition vary with tree litter species. Generally *B. pendula* showed a higher earthworm-mediated C, N, Ca and Mg loading than *E. nitens.* The current study suggests that litter decomposition and nutrient loading to the soil system by earthworms vary with tree litter species.

### 7.4.5 Effects of earthworms on soil properties

In the present study, a significant influence of earthworms on soil properties was recorded only for total C content in bulk soil (0.2 – 0.4 m) of *B. pendula*. All of the

other soil properties (soil moisture, pH, N or C) under *B. pendula* or *E. nitens* were not significantly influenced by introduced earthworms compared to their control soils.

Positive influences of earthworms on soil physical, chemical and biological properties are widely reported by previous researchers (see section 2.3.2 for details). Zhang and Hendrix (1995) found that the endogeic *A. caliginosa* enriched the upper soil layer with $^{13}C$ from litter through translocation from the humus layer. Welke and Parkinson (2003) recorded less amounts of organic matter in the upper horizon (forest floor) of earthworm-worked soil and significantly higher organic matter content in earthworm-worked lower horizon (mineral soil) compared with counterpart controls.

Many studies have investigated the influence of earthworms on soil N content (Syers and Springett, 1984; Lee, 1985; Haimi and Huhta, 1990; Subler *et al*., 1997). However, this effect varied with earthworm species, soil type, as well as tree species grown. Most of the studies on the effects of earthworms on soil N have focused on available N, specifically $NO_3^-$ and $NH_4^+$ levels. In a laboratory experiment, Haimi and Einbork (1992) found an increase of $NH_4^+$ levels in soils in the presence of *A. caliginosa tuberculata*, but no effect on $NO_3^-$. Welke and Parkinson (2003) observed that $NO_3^-$ levels were significantly increased in earthworm-worked soil compared with control after 5 months. At final sampling (10 months), there was no clear trend, although $NO_3^-$ was significantly lower compared to the first sampling. These authors suggested that the low $NO_3^-$ concentration at the end of experiment was due to microbial immobilisation or uptake by plants. In this study, $NH_4^+$ levels did not differ significantly between earthworm-worked soil and control. Jana *et al*. (2010) reported that in nutrient rich soil, neither earthworms nor the *A. thaliana* plant significantly impacted on $NO_3^-$ or $NH_4^+$ content in soil. However, in nutrient poor soil, they observed increased amounts of soil

$NO_3^-$ content and decreased amounts of $NH_4^+$ content in the presence of earthworms. Compared with earthworms, plants had an opposite impact on N content. However, when both earthworms and plant were present, soil $NO_3^-$ content was increased but $NH_4^+$ was decreased. The current study measured only the total N content of soil, neither the $NO_3^-$ - nor $NH_4^+$ levels. The results suggest that earthworms had no significant influence on soil total N content under *B. pendula* or *E. nitens* after 12 months, but this may be too short a time to identify such effects. However, an observed difference in plant N stocks in *E. nitens* suggests a certain influence on available $NO_3^-$ and $NH_4^+$ by earthworms.

Previous studies of effects of earthworms on soil pH recorded variable results; from remarkable increases to minor or no effects. Muys *et al*. (2003) reported an increase of pH in acidified sandy soil with earthworm activity. Haimi and Einbork (1992) found no significant difference in soil pH value in *A. caliginosa tuberculata*-worked humus or mineral soil compared with control soil. Haimi and Huhta (1990), who used epigeic earthworms, observed significant increases in pH of leachate from their experimental systems. Trigo and Lavelle (1993) detected a slight increase in pH near the drilosphere (lining of an earthworm burrow) of *L. terrestris* and *Allolobophora molleri*. The current study, which used pH neutral loam soil, suggested that earthworms had no significant influence on soil pH after 12 months.

This field-based approach, which recorded 100% tree survival at the termination of the experiment, was successful at determining earthworm-tree interactions. The current experiment stands out from past studies, as it was the first field experiment to investigate the direct interaction between SRF trees and earthworms. Also, to date, this appears to be the only use of eucalyptus for such an interaction study.

However, a few practical problems were noted in the current system, although these did not influence experimental results. A very small number of earthworms from the environment entered the experimental system, although the experimental system was designed to prevent earthworm escape or ingress. Further, the main root of two *E. nitens* grew down beyond the basal mesh. These practicalities could be minimised by increasing the tube depth and by leaving a larger distance between soil surface and tube top and by covering top and base of tubes more securely with very fine mesh (< 1mm). The influence of earthworms on *B. pendula* growth was not obvious in this 12 month experiment. A longer-term experiment, probably with a higher density of earthworms is suggested to confirm the influence of earthworms on *B. pendula* growth. The actual influence of *A. chlorotica* on plant growth, especially on roots was not clear in this experiment, as this species was used in combination with *L. terrestris*. A further investigation would be required to confirm this fact, using *A. chlorotica* inoculation alone.

A summary of the major findings from the field-based SRF-earthworm interaction experiment:

- Non-native *E. nitens* had a negative impact on *A. chlorotica* population establishment compared with native *B. pendula*.
- Earthworms increased nutrient uptake, growth and biomass production of fast growing *E. nitens* compared with control.
- Earthworms did not significantly influence *B. pendula* nutrient uptake or growth.
- Surface litter removal of *E. nitens* was three times greater in the presence of earthworms compared with control.

- *B. pendula* surface litter removal was five times greater in the presence of earthworms compared with control.
- The rate of litter decomposition, carbon and nutrient loading to the soil system by earthworms were greater for *B. pendula* than *E. nitens*.

# CHAPTER 8: DISCUSSION

## 8.1 Introduction

This study set out to investigate the effects of SRF tree species and their litter quality on earthworm communities, diversity and activity. In addition, the effects of earthworms on SRF litter decomposition, carbon and nutrient cycling and tree growth were assessed in order to understand more about the interactions of these two ecosystem components**.** The scientific literature on this area, especially with respect to non-native tree species in the context of the UK is sparse and several vital questions within the area awaited investigation. The investigation sought to answer such research questions while providing valuable information to support sustainable SRF expansion. This thesis began with a number of aims and objectives that have been addressed through field survey, laboratory experiments and a field experiment. Results have provided answers to specific questions and hypotheses and an array of SRF-earthworm interactions have been amassed. This final chapter seeks to utilise these findings (as detailed in Chapters 4 - 7), draw them together and present a unified assessment of how the aims and objectives have been met. In addition the implications of research, limitations and suggestions for future research directions are further discussed.

## 8.2 SRF-Earthworm interactions

### 8.2.1 Effects of SRF growth in different land-use systems on earthworm community development

Baseline earthworm and soil surveys (section 4.2) suggested that conversion of various land-use systems (e.g. ex-arable and reclaimed sites) to SRF can act to support earthworm community development. However, the degree of benefit of such

conversions depends not only on the prior land use but also on the SRF species, tree age and soil type. The survey work at an ex-arable loamy soil where eucalyptus was grown as SRF showed that *E. nitens* had grown well, produced large quantities of litter and this had significantly increased earthworm density and diversity after six years, something was not apparent in an adjacent *E. gunnii* stand. Similar eucalyptus growth in reclaimed sandy soil supported rapid earthworm colonisation and after five years, the earthworm density under both *E. nitens* and *E. gunnii* had reached a similar density to adjacent pasture. These eucalyptus plantations were still young and over the time frames investigated, there is every suggestion that the presence of these trees on the considered SRF sites has positively influenced earthworm community development. However, in time, the trees will impose their real impact on the soil completely and may change soil faunal density and diversity. Only longer term investigations will reveal full eucalyptus effect on soil attribute.

Further investigations on growth of *B. pendula* and *A. pseudoplatanus* on a previously coniferous forest site showed no positive influence on earthworm re-establishment even after 15 years. This suggests that the longer-term negative influence imposed by coniferous trees on earthworm development could not be recovered with short-term SRF development and it may require a few rotations to acquire significant change. Also, the growth of *B. pendula* and *C. sativa* on acidic sandy soils had not significantly increased earthworm density, but had affected the species composition after 20 years. An overall comparison could not be established during the field survey across the entire tree species investigated due to the associated number of variables. However, the survey work at selected sites demonstrated that land-use history, soil type, SRF species and tree age display an interactive effect on earthworm density and diversity. This needs further field investigations, but it does mean that selection of suitable SRF species for

appropriate land-use systems is very important for land improvement and sustainable soil management while ensuring maximum biomass benefit.

### 8.2.2 Effects of SRF litter quality and root chemistry on earthworm community development

Earthworm food selection, feeding, growth and reproduction are vital activities which determine overall earthworm community development. Comparative laboratory-based experiments showed that SRF species litter had a direct influence on earthworm life history parameters and behaviour. Choice chamber experiments conducted with ground litter particles (1 – 2 mm) (section 5.2) demonstrated that native British earthworms (*A. chlorotica, A. caliginosa, A. longa* and *L. terrestris*) preferred native *A. glutinosa, B. pendula* and *F. excelsior* litter over non-native *A. pseudoplatanus, C. sativa,* and *E. nitens* litter. Of the six species used, all earthworm species showed least preference for *C. sativa* litter. Further investigation using the same techniques suggested that litter-feeding *A. longa* and *L. terrestris* equally preferred native tree litter and it is notable that both showed a greater preference for *E. nitens* over other non-native tree species. Webcam recording experiments conducted with 10 mm leaf disks (section 5.3) showed that *L. terrestris* had clear leaf litter selection behaviour and this was not a random activity. These results authenticated the results of choice chamber experiments for the same earthworm species. Growth and reproduction experiments with *L. terrestris* suggested that native *A. glutinosa* litter demonstrated a very positive effect while *C. sativa* showed the most depressed effect on hatchling growth, adult mass maintenance and even cocoon production compared with other selected SRF species. Non-native *E. nitens* litter was as effective as native SRF species such as *B. pendula* and *F. excelsior* in terms of promoting earthworm growth in addition to adult mass maintenance. The litter chemistry results suggested that the SRF litter quality, especially N concentration,

C:N ratio and Ca concentration, was the reason for observed difference between SRF species in the above laboratory experiments. However, in contrast with the laboratory findings, a comparative (litterbag) feeding experiment at an ex-agricultural field (section 6.2) showed that a mixed earthworm community accepted *A. pseudoplatanus*, *B. pendula*, *C. sativa*, and *E. nitens* equally, but favoured *F. excelsior*. A field-based SRF-earthworm interaction experiment (Chapter 7) suggested that *B. pendula* litter was more acceptable than *E. nitens* litter to a mixed earthworm community consisting of *L. terrestris* and *A. chlorotica.* This experiment showed that in addition to litter quality, SRF root chemistry (N and Al content and root exudates) had a direct influence on earthworm populations. As a result of the combined effect of litter and root chemistry, *E. nitens* had a negative influence on *A. chlorotica* development while *B. pendula* demonstrated a greater support for the same species development. In this experiment, both tree species supported *L. terrestris* development similarly suggesting that influence of trees on earthworms may vary with earthworm species. Overall, laboratory and field-based results suggest that SRF litter quality and root chemistry impose a direct influence on earthworm community development.

### 8.2.3 Influence of earthworms on SRF litter decomposition, carbon and nutrient release

Both field and laboratory-based experimental results suggested that earthworms make a great contribution to SRF litter decomposition, carbon and nutrient release. The litterbag studies at various original forest sites (section 6.2) showed that the magnitude of the earthworm contribution to litter decomposition and nutrient release was site-specific; Alcan (*E. nitens*) and Gisburn (*F. excelsior*) sites which recorded a high earthworm density and diversity showed a significant contribution, while Rogate (*C. sativa*) and

Gisburn (*B. pendula* and *A. pseudoplatanus*) sites with low earthworm density and diversity showed no significant contribution. These results suggested that the contribution to SRF litter decomposition, C and N release depends on earthworm density and diversity present which is a function of tree species, soil type and land-use history. The comparative litterbag study at an ex-agricultural site (section 6.2) which was earthworm rich also suggested that earthworms have a major contribution to litter decomposition on both native and non-native SRF species. This comparison revealed that earthworm contribution to SRF litter decomposition varied with tree species; *F. excelsior* recorded a significantly higher decomposition rate while *B. pendula, E. nitens, C. sativa* and *A. pseudoplatanus* showed a similar rate of decomposition in the presence of a mixed earthworm community. The field-based SRF-earthworm interaction experiment (chapter 7) suggested that earthworms contribute to both *B. pendula* and *E. nitens* litter decomposition, carbon and nutrient stocks and fluxes in the soil system. This experiment further indicated that this contribution was greater for *B. pendula* than *E. nitens*. Further**,** a laboratory cast analyses experiment (section 6.3) suggested that *L. terrestris* had a direct influence on SRF litter decomposition and N mineralisation. The temporal trend of earthworm-mediated $NH_4^+$- nitrogen release varied with SRF species. *B. pendula*-fed earthworms initially showed a lower level of $NH_4^+$ in their casts and higher amounts after five weeks, while *E. nitens*-fed earthworms showed the reverse. Further, $NO_3^-$ content in earthworm casts of litter-amended treatments decreased throughout the experiment compared with control casts suggesting that litter amendments have an influence on nitrification activity mediated by earthworms. Overall laboratory and field-based results suggest that earthworms are a major component which determines the SRF litter decomposition, carbon and nutrient cycling within most of these sustainable systems.

### 8.2.4 Influence of earthworm on SRF nutrient uptake and tree growth

The field-based SRF-earthworm interaction experiment with one year-old tree seedlings (chapter 7) suggested that earthworms have a direct influence on SRF nutrient uptake, tree growth and biomass production. However, the degree of influence varied with tree species. Presence of a mixture of endogeic and anecic earthworms (*A. chlorotica* + *L. terrestris*) increased nutrient uptake and biomass production of *E. nitens*, but the same earthworms showed no significant influence on *B. pendula*. This short-term (12 month) experiment suggested that rapidly growing tree species, at least in their initial stage of growth, benefit more from earthworm activity than slow growing species during the time frame investigated.

The findings of this research demonstrated a direct influence of tree species on earthworm and soil development. Additionally, it showed the importance of earthworms for litter decomposition, nutrient cycling and tree growth. In a natural ecosystem, all these effects and responses are inter-linked and existence of affirmative mutual-relationships between trees, soils and soil fauna are very important for stability and health of ecosystems.

## 8.3 Key findings

1. Growth of SRF eucalyptus within marginal-arable or reclaimed sites has led to either relatively rapid earthworm colonisation and community development, or maintenance and enhancement of an established earthworm community after five to six years. SRF species, tree age, land-use history and soil type exhibited an interactive effect on overall earthworm community development.

2. SRF litter quality showed a direct effect on earthworm food selection, growth and reproduction. The native *A. glutinosa, B. pendula* and *F. excelsior* litter supported earthworms and their activities over non-native *A. pseudoplatanus, C. sativa,* and *E. nitens*. Evidence suggests that earthworms preferred *E. nitens* litter over *A. pseudoplatanus* or *C. sativa.*

3. Earthworms showed a significant contribution to SRF litter decomposition and C nutrient release within SRF systems and the degree of contribution varied with SRF species, earthworm density and diversity. Field studies demonstrated that a mixed earthworm community accepted litter provision from some non-native species but favoured particular native trees.

4. Earthworm influence on nutrient uptake, growth and biomass production varied with tree species. A one year field experiment showed that rapidly growing *E. nitens* benefited more from earthworm activity than relatively slow growing *B. pendula.*

5. The current work supports the production of SRF as being beneficial to belowground biodiversity and carbon and nutrient cycling, as with only one exception (*C. sativa*), results tended to show that SRF-earthworm interactions were positive. It is perhaps most interesting that non-native *E. nitens* showed a positive interaction with native British earthworms.

## 8.4 Implication for sustainable SRF expansion

Besides demonstrating benefits from the presence of earthworms in SRF systems, this study revealed the direct and indirect effects of SRF species on earthworm community development. The earthworm density and diversity results at SRF study sites will be very useful for SRF scientists when modelling the effect of SRF growth on soils and soil biodiversity and their feedback effects on nutrient uptake and aboveground growth. Since earthworms have a direct link with above-ground plants and animals, earthworm data will be beneficial in assessing overall ecosystem sustainability. The baseline earthworm and soil survey data will be advantageous in the future when assessing and comparing the long-term effects of SRF on soil sustainability. The comparative laboratory and field findings related to SRF litter quality will support practitioners to decide the appropriate tree species for sustainable SRF expansion within the UK. The litter decomposition, carbon and nutrient release/loading data will be useful for modelling carbon sequestration and nutrient cycling within these systems. The earthworm-mediated nutrient release/loading data will be further beneficial for nutrient management and fertiliser application process. The tree growth experiment indicated that earthworm inoculation, especially with litter feeding *L. terrestris* in the short-term can, benefit the rapidly growing SRF systems such as eucalyptus in terms of litter decomposition, nutrient cycling, tree growth and biomass production. These data will be useful for reforestation of loamy soil sites with such trees. Overall, this work supports the production of SRF, as with only one exception (*C. sativa*), results tended to show that SRF species support, maintain or enhance soil biodiversity functions, hence soil sustainability. In respect of the use of eucalyptus (particularly *E. nitens*), during the time frame investigated, this research has found no reason to restrict expansion of planting in terms of earthworms and soil sustainability.

## 8.5 Limitations

This research showed clear interactions between SRF species and earthworms. However, a number of caveats need to be noted regarding the present study. The baseline earthworm survey did not permit comparison of all SRF species due to the limited availability of appropriate sites. Further, some of the sites used in this study were not actual SRF trial sites (e.g. Gisburn Forest, Rogate) but were general forest sites which consisted of monoculture plantations of potential SRF species. A seasonal or annual comparison of earthworm density or diversity could not be performed in selected SRF sites due to limited labour and resource availability. A tree ageing or harvesting effect could not be investigated due to time constraints during the current project. Laboratory earthworm growth and reproduction experiments were limited to a major litter-feeding earthworm (*L. terrestris*) and six SRF species due to usual scientific limitations. Choice-chamber experiments were expanded to four species of earthworm (in monoculture), but were still had to be limited to six SRF species. The webcam experiment could not be replicated as much as was desired due to the requirement for large amounts of storage capacity to save images and human workload associated with setting-up, viewing and reviewing of recorded materials. Field litter decomposition studies were limited to five SRF species because of the material costs, in addition to limitations of finding appropriate sites for such an investigation. The laboratory casting experiment was limited to three SRF species due to the associated analytical cost. The field-based SRF-earthworm experiment was limited to two tree species and two species of earthworm and only one year of duration due to maintenance, analytical costs and time constraints of this research.

## 8.6 Future Research

Based on current findings, innumerable investigations could be developed for further research in this area. However, a number of important studies using similar experimental designs are suggested.

1. Expanded field survey work to new SRF sites to included monitoring of other types of soil fauna. This would help to establish the effects of SRF species on overall soil biodiversity.
2. Longer-term surveys, throughout the SRF cycle, to monitor effects of tree growth and harvesting on the earthworm community.
3. Further laboratory growth and reproduction experiments with a greater number of earthworm species to investigate combinations of species (interactions) to present more realistic results comparable with field data.
4. Longitudinal litterbag studies in SRF systems to establish litter decomposition and nutrient cycling as SRF trees grow with associated soil faunal development.
5. Longer-term (e.g. two-four years) tree growth experiments with selected species of SRF and earthworms to reveal the direct effects of earthworms on SRF nutrient uptake and tree growth.
6. Further laboratory and field experiments to confirm direct effect of eucalyptus root exudates on earthworms and other soil fauna.

With a growing requirement for biomass production to satisfy energy demand, SRF could be one part of the solution. Nevertheless, this could lead to vast areas of the UK diverted into SRF, e.g. eucalyptus, but perhaps food production will be more important. Either way, earthworms will have a vital role to play.

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**Appendix I. Project Milestones (Oct 2009 – Dec 2012)**

| **Activity** | Months | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | **3** | **6** | **9** | **12** | **15** | **18** | **21** | **24** | **27** | **30** | **33** | **36** | **39** |
| 1. Comprehensive literature survey | | | | | | | | | | | | | | |
| 2. Development of an experimental design | | | | | | | | | | | | | | |
| 3. Field earthworm/soil survey | | | | | | | | | | | | | | |
| 4. Laboratory earthworm growth and reproduction experiments | | | | | | | | | | | | | | |
| 5. Laboratory choice-chamber experiments | | | | | | | | | | | | | | |
| 6. Web-cam recording experiments | | | | | | | | | | | | | | |
| 7. Field litterbag studies | | | | | | | | | | | | | | |
| 8. Laboratory earthworm cast analysing experiment | | | | | | | | | | | | | | |
| 9. Field-based SRF-earthworm interaction experiment | | | | | | | | | | | | | | |
| 10. Data analysis & interpretation | | | | | | | | | | | | | | |
| 11. Thesis writing | | | | | | | | | | | | | | |
| 12. Conferences/Seminars | | | | | | | | | | | | | | |

## Appendix II. Application for safety and ethical approval for all projects

**Faculty of Science and Technology**

All undergraduate, postgraduate, commercial and research projects need ethical approval. No field work, experimentation or work with participants can start until approval is granted. The questions below should be completed by the Principal Investigator or supervisor of the proposed project. Where projects involve students, the Principal Investigator is always the supervisor and never the student.

For **undergraduate** and **postgraduate taught** projects: use the questions to identify whether the project should be referred to the relevant Ethics Committee.

- If you answer "No" to questions, then do not apply for approval.
- If you answer "Yes" to **any** of the questions, please discuss them with your supervisor. If your supervisor is confident that you can follow standard forms, protocols or approaches, then your supervisor can approve your application. If your supervisor is not, then the application should be sent for approval.

For **research, commercial and other** projects: use the questions to help compile suitable evidence to support your application.

- If you answer "No" to questions, then your application is likely to be approved quickly.
- If you answer "Yes" to **any** of the questions, please provide evidence relating to the management of the activity. If your approach seems appropriate, then your application is likely to be approved quickly.

Submit the application form and any supporting evidence to an appropriate Ethics Committee. Different committees might have different approval processes.

Principal Investigators, or project supervisors, are responsible for ensuring that all activities fall within the principles set down in the University Code of Conduct for Research and the University Ethical Principles for Teaching, Research, Knowledge Transfer, Consultancy and Related Activities. They are also responsible for exercising appropriate professional judgment in undertaking this review and evaluating the activity according to the criteria laid down in this application. If you are uncertain about any sections of this document, or need further information and guidance, please consult a member of the relevant Faculty/School Ethics Committee.

The Faculty and School Ethics and Safety Committees are to ensure that you comply with the University's ethical principles in the conduct of the activity. Committees can ask for clarification or set conditions for you to meet before approval is granted.

Expiry and review: The principal investigator is responsible for ensuring activities are reviewed. Normally:

- each year: review risk assessments: check for changes to hazards and training refreshers
- after 5 years: review ethics: check for new laws, practices
- closure: dispose of materials and sensitive data properly

Refer to the relevant documents from the following links:

1. Ethical Principles for Research, Consultancy, Practical Work and Related Activities
2. Research Governance (Multiple documents)
3. Health, Safety & Environment (Multiple documents)

1 Project synopsis | Approver: | Cmte number:

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.1 Title | Soil fauna diversity under short rotation forestry – impacts and responses | | | | | | | | | | |
| 1.2 Project type | Original research | | Research degree | x | PG taught | | UG taught | | Commercial | | |
| 1.3 Short description in layman's terms [no acronyms or jargon] | The overall aim of this project is to investigate effects of interacting factors of SRF species and soil types on the below-ground diversity of earthworms. In addition, the effects of varying earthworm population on SRF tree growth will be assessed. Results on earthworm diversity and functions will be linked with litter quality and quantities, soil carbon and nutrient cycling of these systems in addition to above-ground biodiversity. A range of field and laboratory experiments will be used to investigate interactive effects between plants, soils and earthworms within SRF ecosystem. | | | | | | | | | | |
| 1.4 Dates | Start 01/10/2009 | | | | End 30/12/2012 | | | | | | |
| 1.5 School of ….. | Built and Natural Environment | | | | | | | | | | |

2 Participants

| | |
|---|---|
| 2.1 Project supervisor /principal investigator: name, position and original signature | Dr. Kevin Butt (DoS)<br>Dr. Elena Vanguelova (2nd Supervisor)<br>Prof. Andy Moffat (3rd Supervisor) |
| 2.2 Co-workers: names and positions [eg student] | Nalika Rajapaksha (Research student) |

3 External collaborators

3.1 List external collaborating bodies

Forestry Commission

3.2 Provide evidence of any ethical approvals obtained [or needed] by external collaborators

N/A

3.3 Indicate whether confidentiality agreements have been or will be completed

N/A

Read any associated procedures and guidance or follow any associated checklist, and delete, Yes or No, for each characteristic in A) to F) below.

If you respond **No**, then in your judgment you believe that the characteristic is irrelevant to the activity.

If you respond **Yes**, then you should **provide relevant documentation** [including risk assessments] with the application, and cross-reference to it, eg A2 or B9. **Use reference numbers of standard** forms, protocols and approaches and risk assessments where they exist.

| | |
|---|---|
| A) Does the activity involve field work or travel to unfamiliar places? If Yes: | A) Yes/~~No~~ |
| 1. Does the activity involve field work or leaving the campus [eg overseas]? | 1. Yes/~~No~~ |
| 2. Does the field work involve a 'party' of participants or lone working ? | 2. Yes/~~No~~ |
| 3. Does the activity involve children visiting from schools? | 3. ~~Yes~~/No |
| B) Does the activity involve humans other than the investigators? If Yes: | B) ~~Yes~~/No |
| 1. Will the activity involve any external organisation for which separate and specific ethics clearance is required (e.g. NHS; school; any criminal justice agencies including the Police, CPS, Prison Service)? – start this now [CRB clearance process at Loughborough; Uclan contact Carole Knight] | 1. ~~Yes~~/No |
| 2. Does the activity involve participants who are unable to give their informed consent (e.g. children, people with severe learning disabilities, unconscious patients etc.) or who may not be able to give valid consent (e.g. people experiencing mental health difficulties)? | 2. ~~Yes~~/No |
| 3. Does the activity require participants to give informed consent? [consent guidance at City U] | 3. ~~Yes~~/No |
| 4. Does the activity raise issues involving the potential abuse or misuse of power and authority which might compromise the validity of participants' consent (e.g. relationships of line management or training)? | 4. ~~Yes~~/No |
| 5. Is there a potential risk arising from the project of physical, social, emotional or psychological harm to the researchers or participants? | 5. ~~Yes~~/No |

| | |
|---|---|
| 6. Does the activity involve the researchers and/or participants in the potential disclosure of any information relating to illegal activities; the observation of illegal activities; or the possession, viewing or storage (whether in hard copy of electronic format) which may be illegal? | 6. ~~Yes~~/No |
| 7. Will deception of the participant be necessary during the activity? | 7. ~~Yes~~/No |
| 8. Does the activity (e.g. art) aim to shock or offend? | 8. ~~Yes~~/No |
| 9. Will the activity involve invasion of privacy or access to confidential information about people without their permission? | 9. ~~Yes~~/No |
| 10. Does the activity involve medical research with humans, clinical trials or use human tissue samples or body fluids? | 10. ~~Yes~~/No |
| 11. Does the activity involve excavation and study of human remains? | 11. ~~Yes~~/No |
| C) Does the activity involve animals and other forms of life? If Yes: | C) Yes/~~No~~ |
| 1. Does the activity involve scientific procedures being applied to a vertebrate animal (other than humans) or an octopus? | 1. ~~Yes~~/No |
| 2. Does the activity involve work with micro-organisms? | 2. ~~Yes~~/No |
| 3. Does the activity involve genetic modification? | 3. ~~Yes~~/No |
| 4. Does the activity involve collection of rare plants? | 4. ~~Yes~~/No |
| D) Does the activity involve data about human subjects? If Yes: | D) ~~Yes~~/No |
| 1. After using the data protection compliance checklist, have you any data protection requirements? | 1. ~~Yes~~/No |
| 2. After answering the data protection security processing questions, have you any security requirements? [Data storage] [keep raw data for 5 years] | 2. ~~Yes~~/No |
| E) Does the activity involve hazardous substances? If Yes: | E) Yes/~~No~~ |
| 1. Does the activity involve substances injurious to human or animal health or to the environment? Substances must be disposed properly. | 1. Yes/~~No~~ |
| 2. Does the activity involve igniting, exploding, heating or freezing substances? | 2. ~~Yes~~/No |
| F) Other activities: | F) |
| 1. Does the activity relate to military equipment, weapons or the Defence Industry? | 1. ~~Yes~~/No |
| 2. Are you aware of any ethical concerns about the company/ organisation, e.g. its product has a harmful effect on humans, animals or the environment; it has a record of supporting repressive regimes; does it have ethical practices for its workers and for the safe disposal of products? | 2. ~~Yes~~/No |
| Note: in all cases funding should not be accepted from tobacco-related industries | |

If you respond **Yes**, then you should **provide relevant documentation** [including risk assessments] with the application, and cross-reference to it, eg A2 or B9. **Use reference numbers of standard** forms, protocols and approaches and risk assessments where they exist.

These standard forms are being followed [cross reference to the characteristic, eg A2]:

**A1** – This research includes travelling and field work within UK. Complete risk assessments for travelling and all the field works are attached.
**A2** – The particular project include lone field work. A complete risk assessment for lone field work is attached.
**C2** - This research involve with soil invertebrate fauna mainly earthworms.
**E1** - Some project activities are engaged with hazardous substances (Formaldehyde). CoSHH risk assessment for Formaldehyde is attached

## Appendix III. Risk Assessments

**Safety, Health and Environment Section**

**RISK ASSESSMENT FORM**

| **Risk Assessment For** |
|---|
| **Service / Faculty / Dept**: School of Built and Natural Environment |
| **Location of Activity**: Forestry sites in England and Scotland |
| **Activity:** Travelling by Hired vehicle/train<br><br>**REF:** |

| **Assessment Undertaken By** |
|---|
| **Name:** Nalika Rajapaksha |
| **Date: 01/01/10** |
| **Signed by Head of Dept / equivalent**<br>  |
| **Date : 10/01/10** |

| **Assessment Reviewed** |
|---|
| **Name:** |
| **Date:** |
| |

| **List significant hazards here:** | **List groups of people who are at risk:** | **List existing controls, or refer to safety procedures etc.** | **For risks, which are not adequately controlled, list the action needed.** | **Remaining level of risk: high, med or low** |
|---|---|---|---|---|
| Vehicle | Nalika | Driver to complete pre-trip check of vehicle, Car Use Self Declaration Statement has been completed, Drivers to familiarise themselves with controls of hire cars before setting off, Seat belts must be worn | | **Low** |
| Slips trips and falls | Nalika | Particular care should be used when walk | | **Low** |
| Manualhandling (luggage) | Nalika | Manual handling assessments, trolley available, information provision, training | | **Low** |
| Hotel | Nalika | Approved hotel list, Should read evacuation procedures at hotel | | **Low** |
| Personal Security | Nalika | Route will be planned in advance and the Line Manager Should be notified of the estimated time of arrival. Mobile phone | | **Low** |
| Environmental Conditions (weather) | Nalika | Check weather forecast, Personal protective equipment as appropriate | | **Low** |

**Safety, Health and Environment Section**

**RISK ASSESSMENT FORM**

| **Risk Assessment For** |
|---|
| **Service / Faculty / Dept**: School of Built and Natural Environment |
| **Location of Activity**: Field/Le206/Forest research centre |
| **Activity:** Soil Analysis<br><br>**REF:** |

| **Assessment Undertaken By** |
|---|
| **Name:**<br><br>Nalika Rajapaksha |
| **Date: 01/01/10** |
| **Signed by Head of Dept / equivalent**<br>  |
| **Date : 10/01/10** |

| **Assessment Reviewed** |
|---|
| **Name:** |
| **Date:** |
| |

| **List significant hazards here:** | **List groups of people who are at risk:** | **List existing controls, or refer to safety procedures etc.** | **For risks, which are not adequately controlled, list the action needed.** | **Remaining level of risk: high, med or low** |
|---|---|---|---|---|
| Trips, slip and falls | Nalika | Avoid lone working, Mobile phone, 1st aid training | | Low |
| Microbial infection from soil | Nalika | Tetanus vaccination (current) | | Low |
| Mechanical injury to hands | Nalika | Use equipment as directed, Wear gloves | | Low |
| Burns (Oven and muffle furnace) | Nalika | Follow manufacture guideline | | Low |
| Inhalation of dust | Nalika | Ware protective mask | | Low |
| Broken glass | Nalika | Use glassware as instructed | | Low |
| Electrocution from mains | Nalika | Follow manufacture guidelines | | Low |

**Safety, Health and Environment Section**

**RISK ASSESSMENT FORM**

| **Risk Assessment For** |
|---|
| **Service / Faculty / Dept**:<br>School of Built and Natural Environment |
| **Location of Activity**: Field/Le206 |
| **Activity:** Field earthworm collection (Standard)<br><br>**REF:** |

| **Assessment Undertaken By** |
|---|
| **Name:**<br><br>Nalika Rajapaksha |
| **Date: 01/01/10** |
| **Signed by Head of Dept / equivalent**<br>  |
| **Date : 10/01/10** |

| **Assessment Reviewed** |
|---|
| **Name:** |
| **Date:** |
| |

| **List significant hazards here:** | **List groups of people who are at risk:** | **List existing controls, or refer to safety procedures etc.** | **For risks, which are not adequately controlled, list the action needed.** | **Remaining level of risk: high, med or low** |
|---|---|---|---|---|
| Contact formaldehyde with Skin | Nalika | Use of disposable gloves and lab coat to protect skin | | Low |
| Contact formaldehyde with eye | Nalika | Safety specs/goggles | | Low |
| Ingestion/inhalation | Nalika | Work with concentrated formaldehyde in fume cupboard | | Low |
| Slips, trips and falls | Nalika | Avoid long working, Mobile phones, 1st aid training | | Low |
| Microbial infection from soil (Tetanus) | Nalika | Ensure inoculation is current | | Low |
| Mechanical damage to body from spade | Nalika | Wear protective footwear/gloves | | Low |
| Electrocution from mains | Nalika | Follow manufacturers guidelines | | Low |
| Working alongside heavy plants | Nalika | Follow on site safety guidelines (specific training) | | Low |

**Safety, Health and Environment Section**

**RISK ASSESSMENT FORM**

| **Risk Assessment For** |
|---|
| **Service / Faculty / Dept**:<br>School of Built and Natural Environment |
| **Location of Activity**: Field/Le206 |
| **Activity:** Feed preparation for earthworms (Horse manure drying and granulation)<br><br>**REF:** |

| **Assessment Undertaken By** |
|---|
| **Name:**<br><br>Nalika Rajapaksha |
| **Date: 01/01/10** |
| **Signed by Head of Dept / equivalent**<br>  |
| **Date : 10/01/10** |

| **Assessment Reviewed** |
|---|
| **Name:** |
| **Date:** |
| |

| **List significant hazards here:** | **List groups of people who are at risk:** | **List existing controls, or refer to safety procedures etc.** | **For risks, which are not adequately controlled, list the action needed.** | **Remaining level of risk: high, med or low** |
|---|---|---|---|---|
| Microbial infection from manure | Nalika | Wear gloves, wash hands | | Low |
| Burns skin from 105$^{o}$C | Nalika | Follow manufacture guidelines | | Low |
| Electrocution | Nalika | Follow manufacture guidelines | | Low |
| Inhalation of small particles | Nalika | Wear protective mask | | Low |

**Safety, Health and Environment Section**

**RISK ASSESSMENT FORM**

| **Risk Assessment For** |
|---|
| **Service / Faculty / Dept**:<br>School of Built and Natural Environment |
| **Location of Activity**: Le206 |
| **Activity: Cocoon collection by wet sieving**<br><br>**REF:** |

| **Assessment Undertaken By** |
|---|
| **Name:**<br><br>Nalika Rajapaksha |
| **Date: 01/01/10** |
| **Signed by Head of Dept / equivalent**<br>  |
| **Date : 10/01/10** |

| **Assessment Reviewed** |
|---|
| **Name:** |
| **Date:** |
| |

| **List significant hazards here:** | **List groups of people who are at risk:** | **List existing controls, or refer to safety procedures etc.** | **For risks, which are not adequately controlled, list the action needed.** | **Remaining level of risk: high, med or low** |
|---|---|---|---|---|
| Trips, slips and falls | Nalika | Avoid lone working, Mobile phone 1st aid training | | Low |
| Microbial infection from soil | Nalika | Tetanus vaccination current | | Low |
| Mechanical injury to hands | Nalika | Follow manufactures guidelines for sieve use | | Low |

**Safety, Health and Environment Section**

**RISK ASSESSMENT FORM**

| **Risk Assessment For** |
|---|
| **Service / Faculty / Dept**:<br>School of Built and Natural Environment |
| **Location of Activity**: Le206 |
| **Activity:** Laboratory culture of earthworms<br><br>**REF:** |

| **Assessment Undertaken By** |
|---|
| **Name:**<br><br>Nalika Rajapaksha |
| **Date: 01/01/10** |
| **Signed by Head of Dept / equivalent**<br>  |
| **Date : 10/01/10** |

| **Assessment Reviewed** |
|---|
| **Name:** |
| **Date:** |
| |

| **List significant hazards here:** | **List groups of people who are at risk:** | **List existing controls, or refer to safety procedures etc.** | **For risks, which are not adequately controlled, list the action needed.** | **Remaining level of risk: high, med or low** |
|---|---|---|---|---|
| Slips, trips and falls | Nalika | Avoid long working, Mobile phones, 1st aid training | | Low |
| Microbial infection from soil | Nalika | Ensure inoculation is current | | Low |
| Mechanical damage to hands | Nalika | Follow manufacturers guidelines | | Low |
| Electrocution | Nalika | Follow manufacturers guidelines | | Low |
| Back injury from lifting | Nalika | Follow established manual lifting and handling protocol | | Low |

**Safety, Health and Environment Section**

**RISK ASSESSMENT FORM**

| **Risk Assessment For** |
|---|
| **Service / Faculty / Dept**: School of Built and Natural Environment |
| **Location of Activity**: Forest sites in England and Scotland |
| **Activity:** Lone field work (soil/earthworm sampling)<br><br>**REF:** |

| **Assessment Undertaken By** |
|---|
| **Name:**<br><br>Nalika Rajapaksha |
| **Date: 01/01/10** |
| **Signed by Head of Dept / equivalent** |
| **Date : 10/01/10** |

| **Assessment Reviewed** |
|---|
| **Name:** |
| **Date:** |
| |

| **List significant hazards here:** | **List groups of people who are at risk:** | **List existing controls, or refer to safety procedures etc.** | **For risks, which are not adequately controlled, list the action needed.** | **Remaining level of risk: high, med or low** |
|---|---|---|---|---|
| Slips, trips and falls | Nalika | Mobile phones, Regular contact with a responsible person, 1st aid training | | Low |
| Microbial infection from soil (Tetanus) | Nalika | Ensure inoculation is current | | Low |
| Mechanical damage to body from spade/spike | Nalika | Wear protective cloths/footwear/gloves | | Low |
| Weather condition | Nalika | Appropriate clothing | | Low |
| Working alongside heavy plants | Nalika | Follow on site safety guidelines (specific training) | | |
| Skin/eye contact of Formaldehyde | Nalika | Wash off immediately, if appropriate seek medical aid. | | Low |
| Loss the way | Nalika | Carry a local map | | Low |

k

## Appendix IV. COSHH Risk Assessment

COSHH RISK ASSESSMENT FORM. (*Page 1 of 2)*

| **Faculty/Department**<br><br>School of Build and Natural Environment | **Assessors Name(s)**<br>**Nalika Rajapaksha** | **Job Title/Position**<br>**Research student** | ☎<br>**4218** |
|---|---|---|---|

Briefly describe the task/process. (description, use, users)
Preservation of collected earthworms in diluted formaldehyde (3.7%).
This dilution from concentrated (37%)
User: Nalika Rajapaksha

| **Substances** (used or produced as by-products or wastes) | **Quantity** | **Hazard Class** | **WEL** | **Exposure Route(s)** | **Frequency and Duration of Exposure** | **Known Health Effects:** |
|---|---|---|---|---|---|---|
| Water solution containing 37% formaldehyde, 1% methanol | 2 L | Toxic<br>Sensitisi ng | | Skin<br>Eye<br>Inhalation | | Toxic by inhalation, in contact with skin and if swallowed. Causes burns. Limited evidence of a carcinogenic effect. May cause sensitization by skin contact. |
| | | | | | | |

| Results of Relevant Health Surveillance | Results of Exposure Monitoring |
|---|---|
| | |

| **Control Measures** | | | | |
|---|---|---|---|---|
| ☐ Elimination | ☐ Substitution | ☐ Reduction | ☐ Isolation | X Eng. Control |
| *Details* | *Details* | *Details* | *Details (glovebox)* | *Details(LEV, fumehood)* |
| Further Details (if required)<br>Any work with concentrated (37%) to be undertaken in fume cupboard (dilution) | | | | |
| **Personal Protective Equipment** | | | | |
| X Gloves | X Eye protection | X Coverall/lab coat | ☐ Foot protection | X Respiratory protection |
| *Details* | *Details* | *Details* | *Details* | *Details:* |
| | | | | |
| ☐ Health Surveillance required | | ☐ Exposure monitoring required | | |

**Emergency Arrangements**

| **First Aid:** | |
|---|---|
| Eyes | Rinse immediately with plenty of water (15 min), seek medical advice |
| Skin | Irritant to skin, Wash off immediately, if appropriate seek medical aid. |
| Ingestion | Toxic if swallowed. Seek immediate medical attention. Do not induce vomiting |
| Inhalation | Irritant to respiratory track, move to fresh air. |

| **Fire: Extinguisher Type** | | | |
|---|---|---|---|
| X Water | X Foam | ☐ Powder | X $CO_2$ |

| **Spillage/release:** |
|---|

**Waste Disposal procedure**

| Disposal as special (hazardous) waste |
|---|

**Persons likely to be exposed**

| ☐Staff | X Student | ☐ Visitor | ☐ Contractor |
|---|---|---|---|
| ☐Public | ☐ Other (specify) | | |

| **Additional risks:** for example circumstances where work will involve exposure to more than one substance hazardous to health, consider the risk presented by exposure to such substances in combination. Also, non-routine maintenance may present additional risk of exposure. |
|---|
| Long Term Exposure Limit (LTEL) (8- hour TWA): 0.75 ppm<br>Short Term Exposure Limit (STEL) (15- minute TWA): 2 ppm |

| Authorised by (sign): | KRB JTP | Review date due: | |
|---|---|---|---|
| Date: 10/01/10 | | | |

Notes:

*Hierarchy of control*

| *Change the task or process so that the hazardous substance is not required or generated.* |
|---|
| *Replace the substances with a safer alternative.* |
| *Totally isolate or enclose the process.* |
| *Partially enclose the process and use local exhaust ventilation.* |
| *Ensure good general ventilation.* |
| *Use a system of work that minimises the chance and degree of exposure.* |
| *Provide personal protective equipment (PPE).* |
| *Train and inform staff in the safe system of work and risks.* |
| *Additional supervision.* |
| *Examination, testing and maintenance of engineering controls and/or PPE.* |
| *Monitoring of exposure.* |
| *Health Surveillance.* |
| *Other (specify).* |