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# Earthworm selection of Short Rotation Forestry leaf litter assessed through preference testing and direct observation

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A B S T R A C T

Short Rotation Forestry (SRF) practice which includes use of rapidly growing native and non-native tree species has been introduced to the UK as a method to increase woody biomass production. A largely unknown aspect of SRF is the quality of leaf litter, and its palatability to the soil decomposer community of which earthworms are a major component. The aims of the present study were to investigate and compare the preference of selected native British earthworms for selected SRF species litter. These were addressed through a series of controlled laboratory experiments. Choice chambers were used to quantify litter removal by *Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea longa* and *Lumbricus terrestris* over a period up to five weeks. In addition, an infrared webcam recording technique was used to directly observe litter selection behaviour of *L. terrestris* under cover of darkness. Choice chamber experiments revealed that earthworms significantly preferred ($p < 0.05$) leaf litter of native alder (*Alnus glutinosa*), ash (*Fraxinus excelsior*) and birch (*Betula pendula*) over non-native eucalyptus (*Eucalyptus nitens*), sweet chestnut (*Castanea sativa*) and sycamore (*Acer pseudoplatanus*). Sweet chestnut litter was the least preferred by all selected earthworms. Webcam results revealed that *L. terrestris* demonstrates clear leaf litter selection behaviour and it was not a random activity. Direct webcam observation results corroborated the results of choice chamber experiments.



## 1. Introduction

Short Rotation Forestry (SRF) was introduced to the UK as a potentially efficient method to increase biomass feedstocks for the heat and power sectors (FC, 2010). Potential SRF practice includes use of rapidly growing native and non-native trees that reach their economically optimum size after 8–20 years; each plant producing a single stem to be harvested at around 0.15 m diameter (McKay, 2011). Expansion of SRF planting in the UK is set to increase.

Different tree species are known to have a major influence on soils and the associated soil fauna depending on leaf litter quality and quantity (Pigott, 1989; Muys et al., 1992; Zou, 1993; Neirynck et al., 2000; Reich et al., 2005). Tree litter palatability has a strong influence on aggregation of decomposer communities and overall establishment of soil faunal populations (Swift et al., 1979). A largely unknown aspect of SRF is the quality of leaf litter, and its palatability to the soil decomposer community, of which earthworms are a major component in the UK.

Earthworms, which have a pronounced effect on litter decomposition and nutrient cycling in many temperate woodlands (Curry, 1987; Scheu and Wolters, 1991; Benham et al., 2012; Rajapaksha et al., 2013), demonstrate a preference for certain types of leaf litter over others (Darwin, 1881; Satchell and Lowe, 1967; Edwards and Heath, 1963; Hendriksen, 1990). These litter preference studies have identified that chemical composition, particularly nitrogen content, carbon to nitrogen ratio, lignin content, phenolic compounds (tannins), calcium content and protein content, greatly influence earthworm litter selection in addition to hardness, hairiness, water content and shape of the litter material. Some investigators observed that earthworms preferred partially decomposed litter over fresh litter and concluded that bacterial and fungal activity on leaf litter enhances its palatability to earthworms (e.g. Satchell and Lowe, 1967; Wright, 1972; Hendriksen, 1990). However, most of the aforementioned tree litter preferential studies were limited to one large, deep burrowing species (*Lumbricus terrestris*) and hardly considered other earthworms. Further, these investigations focused on common temperate forest tree species such as alder (*Alnus glutinosa*), ash (*Fraxinus excelsior*), elder (*Sambucus nigra*), elm (*Ulmus glabra*), larch (*Larix deciduas*), oak (*Quercus petraea*) and beech (*Fagus sylvatica*). The preference of

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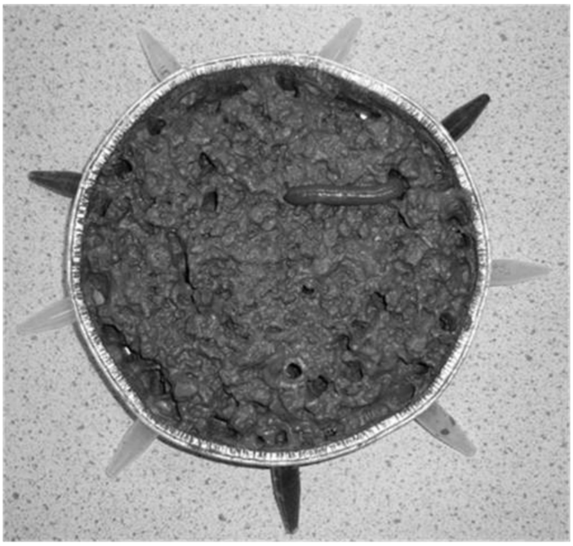

**Fig. 1.** A choice chamber with upper foil covering removed, seen from above (diameter and depth of tray respectively 0.16 and 0.03 m; food tube cap diameter and depth respectively 0.01 and 0.04 m). Leaf litter removal from food tubes by *L. terrestris* in Experiment 2 supplied with three types of litter materials; sycamore, sweet chestnut and eucalyptus; all three sweet chestnut food tubes remain full after 35 days (note: the tail of one earthworm is visible at the soil surface).

European earthworms for non-native tree species such as eucalyptus is unrecorded.

The aim of the present study was therefore to investigate and compare the preference of native British earthworms for selected native and non-native SRF species litter. This was addressed through a series of controlled laboratory experiments; use of choice chambers to quantify litter removal by earthworms over time and direct observation by webcam recording. Objectives were:

1. To measure and compare SRF litter preference by four selected species of earthworm using a choice chamber technique;
2. To record direct evidence of SRF litter selection by *L. terrestris* using a webcam technique;
3. To compare direct observation results with indirect choice chamber results for *L. terrestris*.

## 2. Materials and methods

### 2.1. Choice chamber experiments

#### 2.1.1. General

To assess SRF leaf litter preference of earthworms, a modified choice chamber, similar to that described by Doube et al. (1997) was set up under controlled environmental conditions. This novel soil-mediated system provided more natural 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. 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 choice chambers. Eppendorf tube caps were separated and centres were drilled-out (approx. diameter 0.01 m), to permit passage of earthworms. Holes were made in the foil tray wall, so that drilled caps could be affixed to the inner side, equally spaced, such that the Eppendorf tubes could be attached from the outside (see Fig. 1). This technique allowed for removal of the Eppendorf tubes and replacement, without disturbance to the experimental system. Empty Eppendorf tubes were initially used to hold the cap in position. Trays were then filled with Kettering loam (25% moisture) as a proven substrate for earthworms (Butt et al., 1994).

Earthworms were introduced to each tray as designated and sprayed with water. Trays were covered with a sheet of aluminium foil, held in place with a rubber band, to prevent moisture loss and earthworm escape. Two holes were made with a mounted needle in the sheet to ensure air circulation. Trays were kept in darkness for 24 h at 15 °C in temperature-controlled incubators, for earthworms to equilibrate to the system.

Freshly fallen, air-dried SRF leaf-litter (previously collected) 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 to prevent undue influence of particle size on earthworm food selection. Individual masses of a new set of cap-less Eppendorf tubes were recorded. Tubes were filled (0.20–0.25 g) with dry leaf litter particles and tube masses were re-recorded. Leaf litter-filled tubes were soaked with water for two hours, and then excess was drained by inversion (5 min) on absorbent tissue paper. Masses of food-filled tubes were re-recorded and these were used to replace the empty Eppendorf tubes previously attached to the earthworm-containing choice chambers and maintained in incubators, as before.

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

All experiments used field-collected, adult earthworms that had been acclimated to laboratory conditions for eight weeks prior to experimentation (Fründ et al., 2010). During this period, the earthworms were fed with a mixture of leaves from the range of SRF species to be tested to ensure that no pre-conditioning occurred with respect to litter. The air-dried SRF material used throughout had been previously collected from known forest sites.

#### 2.1.2. Experiment 1

This experiment investigated SRF litter preference by four species of earthworm; *Allolobophora chlorotica* and *Aporrectodea caliginosa* (endogeic); *Aporrectodea longa* and *L. terrestris* (anecic) with initial individual mean masses of 0.19 ± 0.03 and 0.43 ± 0.05; 1.56 ± 0.09 and 4.27 ± 0.17 g, respectively. Experimental earthworms were collected from locations which have been used for previous earthworm collection associated with published work (Dupont et al., 2011). The *A. chlorotica* were all of the green morph, which are similar in nature despite lineage, and inter-breed freely. Six SRF species used were; alder (*A. glutinosa*), ash (*F. excelsior*), birch (*Betula pendula*), eucalyptus (*Eucalyptus nitens*), sweet chestnut (*Castanea sativa*) and sycamore (*Acer pseudoplatanus*). Each earthworm species was assessed separately with 30 *A. chlorotica*, 15 *A. caliginosa*, 4 *A. longa* or 2 *L. terrestris* per tray. These numbers were selected so that each treatment received an approximately similar earthworm biomass and that results might be observed in a reasonable time frame. Food tubes containing different SRF species litter were randomly arranged around each tray (total = 6; one from each SRF species) with four replicate trays for each earthworm species. SRF litter preference was assessed by calculating mean mass loss of litter in individual food tubes, measured three times per week over four weeks.