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Original article

Earthworms from soils developed after 80 years under tree monocultures at Holt Down, Hampshire, UK

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ABSTRACT

Experimental research from the 1980s showed that tree species influenced soil development where stands of beech (*Fagus sylvatica*) and small-leaved lime (*Tilia cordata*) respectivey started to develop a podzolic soil and a brown forest soil after 50 years from near identical origins. Evidence of earthworms was reported but no detail provided. Current work re-examined these soils and a further adjacent spruce (*Picea abies*) plantation and specifically sampled for earthworms. Standard soil and litter measurements were made, and earthworms were collected by a combined digging and hand-sorting, plus vermifuge technique. The soil surface below lime was covered with *Mercurialis perennis*, but deep leaf litter was present below beech, with needle cover below spruce. Significantly more earthworms were present below lime, at a density of 29 m^{-2}, when compared with beech (<2 m^{-2}) with spruce intermediate (11 m^{-2}), with a significantly greater earthworm biomass below lime. Of 8 earthworm species collected, more than 70% were from below lime, including *Aporrectodea longa*, *Lumbricus terrestris*, *A. caliginosa*, *Octolasion cyaneum* and *L. rubellus*. Those below spruce were mainly *Dendrobaena octaedra* and only *A. longa* was found below beech. These observations, after 80 years of differential soil development below tree stands, clearly show continued interactive influences on soils of monoculture tree species with associated ecosystem engineering earthworms.

Quality of leaf litter, particularly in afforested habitats, is known to dramatically affect soil development [1], as examined from natural and experimental investigations [e.g. 2–5]. Such organic inputs directly impact soil organisms, such as earthworms [6] and it has been suggested that earthworms are a vital part, potentially a driver, of the soil developmental process [7,8]. Research by Pigott [9] at Holt Down (in southern England) reported changes to soils and vegetation development below pure stands of small-leaved lime (*Tilia cordata* – hereafter referred to as lime) and beech (*Fagus sylvatica*), following more than 50 years of growth on former arable land. A green understory of *Mercurialis perennis* (dog's mercury) was present below lime. Below beech, a 0.04 m layer of fallen leaves was present and this above 0.07 m of stone-free loam, with a pH of 3.8. Deeper than this, greater stone content and more compacted clay was encountered with little structure until chalk and flint rubble was reached at 0.55–0.70 m. By comparison, beneath lime, a brown loam, with a pH of 5.1, was present with no leaf litter to 0.22 m, with semi-structured loam and clay to 0.48 m below which chalk and rubble was again reached [9].

The current work was undertaken after reading intriguing information [9] on earthworms from the plots at Holt Down e.g., with reference to the structure of the soil: "… aggregates are mostly the product of the activity of large earthworms, *Lumbricus terrestris* and *Allelobophora* spp., which are abundant in the soil under lime and apparently absent under beech, where only small pigmented worms are present in the bottom of the litter." Obviously, there is no genus "*Allelobophora*", so likely a typographical error for *Allolobophora*, now generally considered as *Aporrectodea* [10]. In addition, the identification of *L. terrestris* as the deep burrowing, soil aggregate-forming species was worthy of verification as was clarification of the other earthworms. A pilot investigation was undertaken in 2005 [11], followed by this systematic sampling in May 2013.

Using published literature [9], advice from site managers [12] and acquired knowledge [11], the sites sampled by Pigott in the 1980s, within present day Queen Elizabeth Country Park (QECP), were located (50.957636, −0.964813). In addition to beech and lime sites, separated only by a ride, an adjacent, similar-aged stand of spruce (*Picea abies*) was also investigated, providing 3 contiguous stands. The site, with an altitude of 165–170 m sits on Holt Down and has mean annual rainfall of

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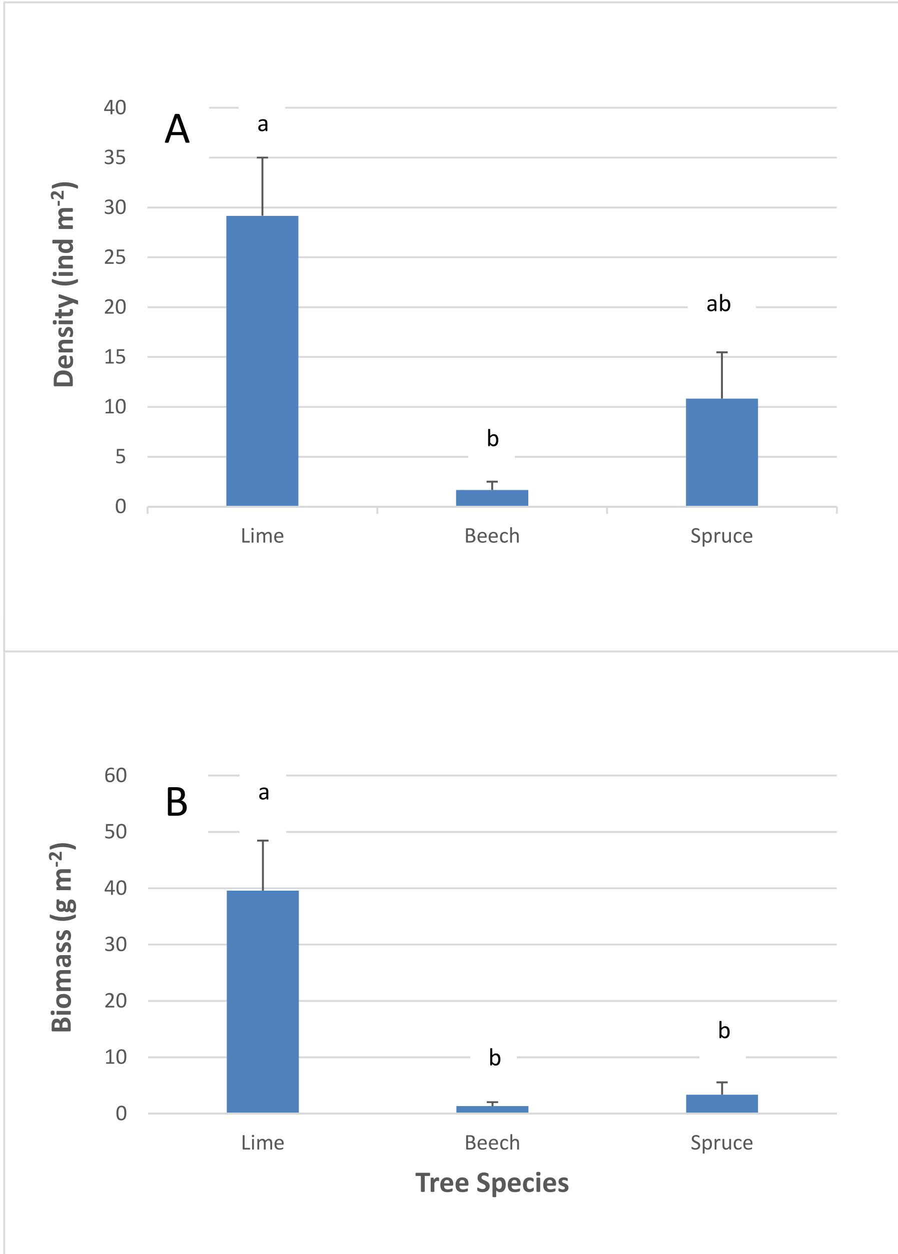

Fig. 1. Earthworm density (A) and biomass (B) at Holt Down from soils collected in May 2013 under plantations of three tree species. (From n = 12, bars represent mean values and standard errors; different letters indicate $p < 0.05$.)

1030 mm with mean monthly rainfall of >55 mm in the driest months (April- June) [12]. QECP is wholely on chalk and the soils are mainly rendzinas except for the well marked clay with flints, 1.0–1.2 m deep over chalk on Holt Down. Soils here are clay-loams, often with numerous flints and a lower pH of 5.5–6.5 [13]. The tree stands of interest are growing on an approximately 3° slope. Prior to planting in 1930–1935 [9], Holt Down had been cultivated and a mixed grass-herb array of species was found [13].

At a good time of year for earthworm collection (late spring), a standard method of earthworm sampling was employed which involved digging a soil pit of 0.1 m^2 to a depth of 0.2 m and hand-sorting the leaf litter and soil, with a mustard vermifuge (5 g L^{-1}) added to the pit [14]. Within each of the three tree species (*T. cordata*, *F. sylvatica*, and *P. abies*), replicated pits (n = 4), no closer than 5 m, were sampled in three blocks at 0, 25, and 50 m down a north-south slope (3 × 4 x 3 = 36 samples). Earthworms were preserved in formalin and identified to species in the laboratory [10,15]. Litter and soil were collected for mass determination and standard chemical analyses [16]. Non-woody litter was determined by removal of twigs and cones. In addition to standard sampling, earthworm middens were investigated for earthworms and a mustard vermifuge injected directly into the burrow below. Any juvenile earthworms located were kept alive and returned to the laboratory for growth to maturity and identification. Analyses of variance were performed using Minitab version 21.4.1.0.

Six earthworm species were collected from the pits. These were *Aporrectodea caliginosa*, *Aporrectodea longa*, *Dendrobaena octaedra*, *Octolasion cyaneum*, *Lumbricus rubellus*, and *L. terrestris*. Overall, community densities (±s.e.) of 29.17 ± 8.57, 1.67 ± 1.12 and 10.83 ± 4.99 ind. m^{-2} were recorded for lime, beech and spruce, with respective biomasses of 39.59 ± 13.0, 1.35 ± 0.92 and 3.38 ± 2.54 g m^{-2}. A significant difference was present for density between lime and beech stands ($p = 0.006$, $F_{(2, 33)} = 5.90$) (Fig. 1A). Earthworm biomass, below

Table 1
Soil and litter properties under plantation monocultures after 80 years of differential soil development, Holt Down, Hampshire, England, May 2013 (Mean values ± s.e.; n = number of replicates for each measurement; different letters in a column indicate $p < 0.05$).

Tree species	Soil pH	Soil Moisture content (%)	Soil Loss on ignition (%)	Non-woody litter dry mass (g 0.1 m^{-2})	Litter description
	(n = 6)	(n = 12)	(n = 12)	(n = 12)	
Lime (*Tilia*)	6.42 ± 0.07^a	31.10 ± 0.50^a	10.66 ± 0.34^a	14.99 ± 2.15^a	Sparse, vegetated
Beech (*Fagus*)	4.02 ± 0.10^b	33.53 ± 0.96^a	12.33 ± 0.61^a	98.84 ± 4.61^b	Litter to 0.04 m
Spruce (*Picea*)	3.57 ± 0.16^c	33.60 ± 1.08^a	17.36 ± 0.98^b	140.58 ± 10.90^b	Needles and cones

lime was significanlty greater than both spruce and beech ($p = 0.002$, $F_{(2, 33)} = 7.87$) (Fig. 1B.). Greatest species richness (n = 5) was observed under lime, where anecic *A. longa* (23%) and *L. terrestris* (3%); endogeic *A. caliginosa* (37%) and *O. cyaneum* (34%); and epigeic *L. rubellus* (3%) were present. A single, mature *L. terrestris* located was the largest (5.03 g) and only earthworm extracted with a mustard vermifuge during standard sampling. The two *A. longa* found below beech were both immature with mean mass 0.81 g. Eighty-five percent of earthworms found below spruce were epigeic *D. octaedra*, with this acidophilic species absent under either lime or beech. Middens of *L. terrestris* were only located below lime trees. Qualitative searching revealed juvenile *Lumbricus* spp. (masses of 0.12–0.62 g) which grew to be identified as *L. terrestris*. *A. caliginosa* and *L. rubellus* were also present in middens, as were *Aporrectodea rosea* and *Satchellius mammalis*, bringing the species count to eight. Each large burrow below a midden revealed an adult *L. terrestris*, the largest with mass of 5.63 g.

Observations of the ground layer were very similar to those seen thirty years earlier, plus needle cover below spruce (Table 1; see Supplementary Fig. 1). At sampling (May 2013), soil moisture content (%) did not differ significantly across the 3 tree species ($p = 0.089$, $F_{(2, 33)} = 2.61$). All soils sampled were acidic, with those below lime significantly less than those below either beech or spruce ($p = 0.001$, $F_{(2, 15)} = 180.90$) (Table 1). Soil below lime was determined a brown forest soil whilst that below beech and spruce more closely aligned to a brown podzol. Loss on ignition showed differences between spruce when compared with lime and beech ($p = 0.001$, $F_{(2, 33)} = 24.99$). Comparisons of non-woody litter showed major differences between the 3 tree species ($p = 0.001$, $F_{(2, 33)} = 85.49$) (Table 1).

Tree species, planted on near identical soils more than eighty years earlier (1930s), have profoundly influenced soil development and resulted in different chemical, physical, and biological characteristics [8]. The role of earthworms in this process seems intimately linked, as these are the major ecosystem engineering animals present [17], and are themselves affected by tree litter-influenced soil properties such as pH and organic matter content [15]. The earthworms, particularly *L. terrestris*, have removed all lime leaves into the soil whilst beech leaves and spruce needles are largely left untouched. Primarily this is because no litter-burying earthworm species are present below beech or spruce, as the litter is not palatable [1]. Numbers and biomasses below given species were equivalent or less than earthworm records from similar habitats [18]. *Lumbricus castaneus* and *Bimastos rubidus*, found during the pilot study [12], were not located this time, but the latter had been found below rotting bark of lime, so earthworm sampling techniques may need to be adapted to achieve full assessment of communities in forest areas [19]. Earthworm communities that have developed under different tree species have dramatically affected soil function, e.g., through decomposition and incorporation rate of leaf litter plus soil physical attributes such as aggregation [20]. Current results demonstrate the importance of interactions between above and below ground biological components in forest pedogenesis. Long term monitoring of this nature is vital to better understand the effects of specific trees and earthworms on ecosystem engineering.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2023.103560.

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