

RESEARCH ARTICLE

Title: Woodland restoration on agricultural land: long-term impacts on soil quality**Running Head: Woodland restoration impacts on agricultural soils****Authors and addresses**

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Abstract

Woodland restoration is underway globally, to counter the negative soil quality and ecological impacts of agricultural expansion and woodland fragmentation, and restore or enhance biodiversity, ecosystem functions and services. However, we lack information about the long-term effects of woodland restoration on agricultural soils, particularly at temporal scales meaningful to woodland and soil development. This study utilised soil and earthworm sampling across a chronosequence of sites transitioning from 'agricultural land' to 'secondary woodland' (50-110 years) and 'ancient woodland' (>400 years), with the goal of quantifying the effects of woodland restoration on agricultural land, on key soil quality parameters (soil bulk density, pH, carbon and nitrogen stocks, and earthworm abundance, biomass, species richness and diversity). Broad-leaved woodland restoration led to significantly greater soil organic carbon (SOC) stocks compared to arable land, and young (50-60 years) secondary woodland increased earthworm species and functional diversity compared to both arable and pasture agricultural land. SOC stocks in secondary broad-leaved woodlands (50-110 years) were comparable to those found in long-term ancient woodlands (>400 years). Our findings show that broad-leaved woodland restoration of agricultural land can lead to meaningful soil ecological improvement and gains in SOC within 50 to 110 years, and provide intel on how restoration activities may be best targeted to maximise soil quality and functions.

Keywords: agricultural land, earthworm, ecosystem restoration, soil quality, soil organic carbon, woodland restoration

Implications for practice

- Restoring broad-leaved woodland onto agricultural land improves earthworm diversity and increases soil organic carbon (SOC) stocks, overcoming some negative aspects of agricultural intensification on soils.
- Targeting such woodland restoration onto arable land increases total SOC stocks, and restoring broad-leaved woodland onto either type of agricultural land increases earthworm functional diversity.
- Within the first 50-60 years following restoration, broad-leaved woodlands support greater earthworm species diversity than older (>100 years) woodlands or agricultural land.
- Within a short timeframe (50 to 110 years) restored broad-leaved woodland can increase SOC stocks to levels comparable with long-term (ancient) woodland.

Introduction

The restoration of degraded ecosystems and the services they provide is a global imperative for humanity in the face of increasing demand for resources (Foley et al. 2005). Globally, much environmental degradation is a result of agricultural expansion and intensification, particularly at the expense of woodland (FAO 2010; Foley et al. 2011; Rey Benayas & Bullock 2012). The restoration of lost woodland ecosystems represents an opportunity to counter the negative ecological impacts of agricultural expansion and woodland fragmentation, and restore or enhance biodiversity, ecosystem functions and services (Rey Benayas & Bullock 2012). Many countries have set targets for woodland expansion; for example in the UK, England alone could see the creation of 180,000 hectares of new woodland over the next 25 years (HM Government 2018). However, this must be undertaken strategically (Lee et al. 2002; Quine & Watts 2009), taking into account conflicting demands for other land uses (such as agriculture) and ensuring sustainable management of soil and biological resources (Rey Benayas & Bullock 2012).

Soil is a crucial component of forests, delivering fundamental ecosystem functions, including carbon (C) sequestration, biomass production, water and nutrient regulation (Quine et al. 2011). Forest soils contain the majority of the total forest C stock, and the stability of this C is key to its sequestration and climate change mitigation (Vanguelova et al. 2013, 2016). The accumulation of stable soil C is affected by a wide range of biotic and abiotic factors (Benham et al. 2012; Vanguelova et al. 2016), and understanding how soils respond to woodland creation is crucial to ensure long-term sustainable woodland habitat restoration (Benham et al. 2012). Likewise, it is important to understand the effects that woodland restoration has on key groups of organisms on agricultural land, in particular earthworm communities, since these ecosystem engineers are inextricably linked

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to soil chemical, physical and biological quality (Lavelle et al. 1997; Blouin et al. 2013; Vellend & Flinn 2015). Earthworms are typically classified into three ecological or functional groups based on their life strategies: epigeic (surface/litter dwelling), endogeic (shallow, mineral soil dwelling) or anecic (dwelling in deep, vertical soil burrows) (Bouché 1977; Lee 1959). Earthworms provide a range of ecosystem services depending on their ecological grouping, including improving soil structure, soil formation, nutrient cycling and soil organic carbon (SOC) sequestration (Lavelle et al. 1997; Blouin et al. 2013). For example, within earthworm casts, SOC may be protected for long periods in the form of stable aggregates (McInerney et al. 2001; Shipitalo & Le Bayon 2004). However, not all species produce such casts, and the composition of earthworm communities (and associated services) is affected by a range of factors including soil properties, habitat constraints, and land use and management (Decaëns et al. 2008). Since earthworm functional groups influence soil ecosystem processes differently, understanding the impacts of land management practices on earthworm functional and taxonomic diversity can enable better management of soil resources, in both agricultural landscapes and woodland ecosystems (Muys et al. 1992; Blouin et al. 2013; Pelosi et al. 2014; Cardinael et al. 2019).

Despite growing international pressure to restore agricultural land to woodland, very few studies have investigated the long-term effects of woodland restoration on soil ecology and soil physical and chemical quality in comparison to former agricultural land (Muys et al. 1992; Poulton 2006). The outcome of reforesting agricultural land is heavily influenced by factors such as tree species, management, soil type and the nature of the former land-use, and can be highly situation-specific (Bouché 1972; Muys et al. 1992). For example, Muys (1990) reported that the establishment of monoculture beech forests on highly productive agricultural soils led to soil degradation, and subsequently benefited from the experimental introduction of additional tree species, soil

management and earthworm inoculation. In a comparison of meadow with old and young broad-leaved forest stands, Muys et al. (1992) found significantly lower earthworm biomass in old forest stands compared to either young plantations or meadows. Wider forest surveys indicate that lower total soil C stocks in the forest floors, but greater organic C in the mineral soil (stable C) could be accumulated in broad-leaved than under coniferous woodlands (Morison et al. 2012; Vanguelova et al. 2013). In general, broad-leaved woodland establishment also results in greater earthworm abundance and diversity than coniferous woodland (Verstraeten et al. 2018). However, studies of soil and earthworm population dynamics on well-characterised sites over long timeframes meaningful to woodland and soil development are very rare (Muys et al. 2003; Curry 2004; Blouin et al. 2013; Sigurdsson & Gudleifsson 2013; Schwarz et al. 2015) and more research into long-term soil - woodland interactions is required, to inform new woodland restoration activities across a range of former land uses and soil properties. In the UK, long-term, large-scale woodland creation has been well-documented in historic maps (Watts et al. 2016); this provides a unique opportunity to investigate soil changes associated with woodland restoration over large spatial and temporal scales. A better understanding of soil changes following conversion from agriculture to woodland will enable more sustainable and strategic future woodland establishment.

In this study we utilised historical woodland creation to investigate changes in soil biological, physical and chemical quality due to woodland restoration, and compared this with surrounding agricultural land. Specific research objectives were to identify and quantify the effects of woodland restoration on agricultural land, and woodland age, on: (i) important soil physical (bulk density and structure), chemical (acidity, carbon (C) and nitrogen (N)), and (ii) biological parameters (earthworm abundance, biomass, species richness and diversity).

Methods

Study sites

This study utilised the research site network established by the Woodland Creation and Ecological Networks project (WrEN; www.wren-project.com), a natural experiment designed to study the effects of 160 years of woodland creation on biodiversity and inform landscape-scale conservation (Watts et al. 2016). The subset of sites used in this study centred around the National Forest area of the midlands of England (Figure 1), an intensely farmed agricultural landscape with fragmented woodland cover which has seen significant increases in woodland creation over the past 25 years (Harmer et al. 2015, Watts et al. 2016). As the woodlands selected for this study were created on former agricultural land (between 50 and 120 years ago) they lack any remnant woodland biodiversity or soils, thereby providing a novel system to study biodiversity and soil development in restored woodlands over long time scales. By focussing on a relatively homogeneous lowland agricultural landscape, we minimised the influence of other covariates on the study, such as topography and climate (Watts et al. 2016). The greater midlands area receives approx. 1460 h sunshine and 741 mm rainfall per annum, and has a mean daily maximum temperature of 14.1 °C (Met Office 2018). In total, 21 sites were sampled to form a chronosequence, consisting of seven ancient semi-natural woodlands of >400 years age, seven planted secondary woodland sites split into two age groups: young (50-60 years, four sites), and mature (110-116 years, three sites), and seven agricultural sites (three arable and four pasture) adjacent to the selected woodlands – representing the previous land-use prior to woodland restoration (Figure 1).

[Insert Figure 1]

We used a systematic process to select comparable woodland and agricultural sites within the study landscape. Initial site screening utilised historical maps (EDINA 2013, Natural England 2013) to confirm agricultural land-use prior to woodland planting and determine woodland age as detailed in Watts et al. (2016). Additionally, the selected woodland sites were spatially independent; at least 3 km distance apart (Watts et al. 2016). Soil variability can be high on a local scale, especially under woodland (Vanguelova et al. 2016). Therefore, to ensure comparability between sites, we selected woodland sites on directly comparable soil types. These were surface-water gleys of the Worcester, Denchworth and Ragdale series (Cranfield University 2016), known to have very little spatial variability in soil C (Vanguelova et al. 2013, 2016). The size of the woodlands was also controlled as much as possible (between 2 and 5 ha) to account for patch size and edge-effects (Vanguelova & Pitman 2019). All woodland sites were unmanaged broad-leaved woodlands, with a canopy dominated by the tree species *Quercus robur*, *Fraxinus excelsior*, *Betula pendula*, *Prunus avium*, *Acer pseudoplatanus*, *Salix sp.* and *Populus sp.* Pastoral agriculture sites were managed by livestock grazing at the time of sampling, whilst arable agricultural sites were used for cereal production and routinely ploughed. For more detailed information on the WrEN woodland site selection, see Watts et al. (2016).

Sampling methodology

Sampling was undertaken across all sites in October 2016, following established European soil protocols appropriate for the scale of sampling and spatial soil variability (Cools & De Vos 2010; Vanguelova et al. 2016). A sampling area (plot size of 20 x 20 m = 400 m²) was marked out in the centre of each site, where possible allowing a buffer of at least 50 m to the woodland edge to account for likely edge effects (Vanguelova & Pitman 2019). A systematic grid sampling approach

was applied in this central sampling area, providing ten sampling points. The distance between the sampling points was at least 5 m and with 1 m distance from tree stems (Vanguelova et al. 2016). At each sampling point, both the organic layer and two mineral soil depths (0-20 cm and 20-40 cm) were collected. Soil sampling followed the ICP Forests Soil Manual methods (Cools & De Vos 2010) using Dutch augers to collect mineral soil. An additional three quadrats (25 x 25cm) were sampled from the organic layer and three cylindrical core rings (100 cm³) were taken from the mineral soil horizons, to determine organic layer and soil bulk densities and allow estimation of organic layer and soil C and nutrient stocks.

Soil samples were prepared and analysed for chemical and physical properties by Forest Research laboratory services at Alice Holt Lodge, Farnham, UK. Soil samples had total C and N concentrations measured using a C:N Elemental Analyser (Carlo Erba (THERMO), FLASH EA 1112 Series). Soil moisture content was analysed by oven drying at 105 °C for 24 h, and soil pH was measured in water suspension (soil:water ratio 1:2.5). Extraction by 1M KCL was used on fresh soil for determining levels of available soil nitrate (NO₃⁻) and ammonium (NH₄⁺), by colorimeter analysis (MAFF 1993). Organic soil samples (litter and fermentation layers) were analysed for total major elements (P, K, Ca and Mg) through inductively coupled plasma-optical emission spectrophotometry (ICP-OES) after sulphuric acid digestion.

Earthworm surveys were carried out at each site by digging eleven 0.1 m² soil pits per plot (five within the central 20 x 20 m sampling area, and six outside this to account for edge-effects), and hand sorting for earthworms (Butt & Grigoropoulou 2010). Collected earthworms were placed directly into pre-labelled plastic bottles containing 80% ethanol solution, then transported to the laboratory and placed into fresh 80% ethanol for preservation. All adults were identified to species

level following the keys of Sims & Gerard (1999) and Sherlock (2018), assigned an ecological group (epigeic, endogeic or anecic, *sensu* Bouché 1972), and had their preserved biomass (wet weight) determined.

Statistical analysis

We tested the effects of land use type (four groups: pasture and arable agriculture, and secondary or ancient woodland), woodland age (three groups: young secondary (50-60 years), mature secondary (110-116 years) or ancient (>400 years)) and soil depth (0-20, 20-40 cm) (all categorical variables) on soil chemical and physical parameters and on earthworm population density, biomass, community composition, species richness and diversity. Where there was no difference in response variables within woodland age groups or agricultural types, these groups were pooled for analysis. Statistical analysis was carried out using Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey-Kramer post-hoc multiple comparison test. Where the normality and homogeneity of variance assumptions of ANOVA were not met, the non-parametric Kruskal-Wallis ANOVA test was applied. Additionally, mixed effects models were run to account for site-specific random effects. Goodness of fit of mixed effects models was compared to the linear regression models using Akaike's Information Criterion (AIC); since model fit was not improved by including site as a random effect ($AIC_{LMM} > AIC_{LM}$ with random site effects often explaining less than 1% of the variation), the linear regression approach was deemed an appropriate model for this study. Practical significance of results was interpreted using the effect size thresholds for Eta-squared (η^2) and Cohen's *d* (as appropriate to the statistical test employed) described by Cohen (1988) and Sawilowsky (2009). Statistical analysis was performed using the statistical software JASP (Release 0.9.0.1) and R Studio version 1.1.447 using R version 3.5.0.

Results

Soil physical and chemical properties

[Insert table 1]

Mineral soil organic carbon (SOC) stock (t C ha^{-1}) and SOC concentration (%) at 0-20 cm depth was significantly greater under ancient woodland (>400 years old) than arable land ($F(4,16) = 3.244$, $p = 0.040$, $\eta^2 = 0.45$, and $F(4,16) = 3.244$, $p = 0.023$, $\eta^2 = 0.49$, respectively) (Table 1). Total SOC stock (t C ha^{-1}) down to depth of 40 cm (including organic horizon) was also significantly greater on ancient woodland ($N = 7$, $M = 204.40$, $SD = 75.59$) than arable land ($N = 4$, $M = 89.57$, $SD = 13.78$) ($F(4,16) = 3.153$, $p = 0.043$, $\eta^2 = 0.44$) (Figure 2). Woodland SOC stocks generally increased with woodland age but mean secondary woodland SOC stocks were not significantly different from those found in ancient woodlands. There was a significant effect of soil depth on SOC stocks under secondary woodland (young and mature pooled), with 0-20 cm depth mineral soil containing significantly greater SOC than 20-40 cm depth ($t(12) = 2.316$, $p = 0.039$, $d = 1.24$) (Table 1 and Figure 2). Besides this, there were no woodland age effects on SOC stocks and concentrations at different soil depths or overall down to 40 cm depth. No woodland age effects were found on N concentrations or N stocks at each soil depth, however the total soil N concentration in mature (110-116 years), secondary woodland at 0-20 cm mineral soil was significantly greater than at 20-40 cm depth ($t(4) = 3.474$, $p = 0.025$, $d = 2.84$) (Table 1).

[Insert Figure 2]

Mineral soil C:N ratio at both 0-20 cm and 20-40 cm depths was significantly greater under both young and mature secondary woodland and ancient woodland than agricultural land (arable and

pasture pooled) ($F(2, 18) = 10.83, p < .001, \eta^2 = 0.55$, and $F(2, 18) = 8.603, p = 0.002, \eta^2 = 0.49$, respectfully) (Table 1 and Figure 3). There was no effect of woodland age on soil C:N; however, at 0-20 and 20-40 cm, the soil C:N ratio of mature secondary woodland (110-116 years) was significantly greater than that of both arable and pasture ($F(4, 16) = 6.589, p = 0.002, \eta^2 = 0.62$ and $F(4, 16) = 7.252, p = 0.002, \eta^2 = 0.65$, respectively) (Table 1).

[Insert Figure 3]

Soil bulk density (g m^{-2}) at 0-20 cm depth was significantly greater on arable sites than young (50-60 years) secondary woodland ($F(4, 16) = 3.269, p = 0.039$). This relationship had a very large effect size ($\eta^2 = 0.45$), indicating that land use type accounted for almost half of the variance in soil bulk density. The lowest mean soil pH was recorded under young secondary and ancient woodland, and the highest was on arable land; however, there were no statistically significant effects of woodland land use, woodland age or soil depth on soil pH (Table 1).

Earthworm populations

In total 3,541 earthworms were collected, belonging to thirteen species and six genera (Table 2). Only two species were found exclusively on agricultural land (*Dendrobaena pygmaea* and *Lumbricus festivus*), and three species found only in woodlands (*Octolasion cyaneum*, *Eisenia fetida*, and *Dendrobaena octaedra*). Total earthworm abundance was significantly greater on agricultural land (arable and pasture pooled) ($N = 7, M = 242.7 \text{ m}^{-2}, SD = 161.98$) than both young and mature secondary woodlands ($N = 7, M = 120.52 \text{ m}^{-2}, SD = 47.83$) and ancient woodlands ($N = 7, M = 120.73 \text{ m}^{-2}, SD = 161.98$) ($F(2,18) = 4.22, p = 0.031, \eta^2 = 0.32$). There was however, no difference in abundance between the two main woodland types (secondary and ancient). The greater earthworm

abundance on agricultural land is explained by the pasture sites, which was significantly greater than all other land uses ($F(4,16) = 4.738, p = 0.010, \eta^2 = 0.54$) (Table 2 and Figure 4). Earthworm total biomass (g m^{-2}) was also significantly greater on pasture sites than all other sites ($F(4,16) = 7.810, p = 0.001, \eta^2 = 0.66$) (Table 2 and Figure 4).

[Insert Table 2]

[Insert Figure 4]

Earthworm species richness and diversity

Earthworm species diversity, expressed as Shannon's H diversity index, was significantly greater in secondary woodland ($N = 7, M = 1.399, SD = 0.255$) than agricultural land (both types) ($N = 7, M = 1.106, SD = 0.196$) ($t(12) = -2.411, p = 0.033, \text{Cohen's } d = -1.29$). Earthworm diversity was also greater in ancient woodland ($N = 7, M = 1.322, SD = 0.190$) than agricultural land (both types) ($t(12) = 2.085, p = 0.059$), and whilst this was not statistically significant, the effect size was large ($d = 1.12$). Earthworm species diversity was highest in young secondary woodland, and significantly higher than on arable land ($F(4,16) = 3.569, p = 0.029, \eta^2 = 0.47$) (Table 2). No significant effects of land-use or woodland age were observed on earthworm species richness.

Earthworm functional group dynamics

Compared with agricultural land (arable and pasture pooled), woodland (all age groups pooled) showed a 4% increase in epigeic (surface-living) species, an 18% decrease in endogeic (shallow-burrowing) species and an 11% increase in anecic (deep-burrowing) species. The abundance (individuals m^{-2}) of endogeic earthworms was similar across all woodland ages, but significantly greater on agricultural land (arable and pasture pooled) ($N = 7, M = 85.58, SD = 66.94$) than in

woodland soils (all woodland age groups pooled) (N = 14, M = 34.48, SD = 18.85) (H(1) = 4.699, $p = 0.030$, $d = 1.23$). Notably, the endogeic earthworm species *A. chlorotica* was significantly more abundant (individuals m⁻²) on pasture sites than on all other land uses (H(4) = 13.26, $p = 0.010$) (Table 2). In terms of total abundance, the proportion (%) of endogeic earthworms was significantly greater on agricultural sites (both types pooled) (N = 7, M = 87.42, SD = 8.439) than secondary woodland (N = 7, M = 63.14, SD = 18.071 (F(2, 18) = 6.232, $p = 0.009$, $\eta^2 = 0.41$).

The anecic species *L. terrestris* was significantly less abundant on agricultural land (arable and pasture pooled) (N = 7, M = 1.04, SD = 1.33) than woodland sites (all ages pooled) (N = 14, M = 3.38, SD = 2.46) (H(1) = 4.796, $p = 0.029$). However, another anecic species, *A. longa*, was significantly more abundant on pasture sites than all other sites (H(4) = 10.01, $p = 0.040$) (Table 2). The proportion (%) of anecic species was lower on agricultural land (arable and pasture pooled) (N = 7, M = 9.985, SD = 7.560) than woodland (all ages pooled) (N = 14, M = 22.588, SD = 14.935) (F(1, 19) = 4.343, $p = 0.051$). Whilst not statistically significant, this had a medium-large practical significance ($\eta^2 = 0.19$).

Discussion

Woodland restoration effects on soil properties

Long-term woodland significantly increased the mineral soil organic carbon (SOC) stock compared with arable land, with ancient woodlands having a relatively high average SOC stock of 180 t C ha⁻¹ to 40 cm depth compared with an average UK woodland soil C stock of 118 t C ha⁻¹ on Gleysols reported by Vanguelova et al. (2013). Importantly, mean SOC stocks (149 t C ha⁻¹) in our restored (i.e. planted) secondary woodlands were not significantly different to those found in our ancient

woodlands (which represent the historical land use prior to agricultural expansion). This indicates that mineral soil SOC accumulation is initially rapid, but slows over time between secondary and ancient woodland; This trend was previously recorded by Benham et al. (2012), with most of the SOC accumulation at shallow soil depths under oak forest being achieved within the first 100 years of afforestation. A comparable study exists at Geescroft Wilderness in Rothamsted where arable land has reverted to acid woodland after being abandoned in 1886. Soil organic C (to 69 cm depth) increased from 29 t C ha⁻¹ in the 1880s to 62 t C ha⁻¹ in the 1980s (Poulton 1996). Where arable farmland was the previous land-use for our secondary woodland sites, with an average starting topsoil C stock of 48 t C ha⁻¹ as recorded in our study, the topsoil of our secondary woodlands shows an average SOC accumulation rate of 0.49 t C ha⁻¹ Y⁻¹ (across 75 years - the mean age of our secondary woodlands). This estimate falls between the 0.1-0.3 recorded by Benham et al. (2012) and the 0.38 t C ha⁻¹ y⁻¹ at Geesecroft Wilderness, and the 0.56 t C ha⁻¹ Y⁻¹ at Broadbalk; and fits with a range of surveys of UK and European forest soils (Chamberlain et al. 2010; Jenkinson 1990). Overall, the rate of C accumulation depends strongly on previous land use, soil type, forest type and management. Lack of accurate information regarding prior land use makes it difficult to reliably calculate soil carbon accumulation rates, since different agricultural practices are known to affect the soil C stock baseline and subsequent changes (Jenkinson 1990). Limitations on historical maps meant we were unable to tell in every case whether former agricultural sites were arable or pasture; nevertheless, we detect clear changes in SOC according to land use and forest restoration. Forest management practices are also known to affect SOC accumulation, with activities such as thinning, whole tree harvesting and brash removal, leading generally to decreases in C stock (Morison et al. 2012). At the time of sampling, the woodlands investigated in our study were unmanaged, which may explain the high estimated SOC accumulation rates and measured topsoil C stocks in

comparison to other studies (Jenkinson 1990; Chamberlain et al. 2010; Benham et al. 2012; Vanguelova et al. 2013).

Pasture land in this study held a topsoil total SOC stock comparable to ancient woodland – potentially making woodland restoration on pasture a less rewarding option for soil C sequestration than on the SOC-poor arable land. However, it should be noted that woodlands additionally store substantial C aboveground as tree biomass (Morison et al. 2012). As part of the national soil survey of England and Wales, surveys of afforested mineral soil sites over the past 30 years showed that permanent grassland conversion to forestry was the only land use change which significantly reduced the C and N stocks from the mineral soil and overall soil profile (Hannam et al. 2016). However, a deep soil investigation comparing grassland and >200 years broadleaved woodland chronosequence on clay soils suggested that woodland creation has contributed to higher C accumulation within soil depth of more than 20-40 cm and down to 1m, likely due to C inputs from tree roots (Razauskaite 2018). This comparison between managed grassland and broadleaved woodland development on clay soil types showed similar soil C stocks in the topsoil under both land uses; however, a higher proportion of the C in the grassland topsoil was in the labile C pool in comparison to greater stable C pool in the woodland soils (Razauskaite 2018). For future restoration practices, establishing woodland on arable land is likely to be more directly beneficial to soil quality and biological value than on pasture land, which is already a biologically and organic C rich habitat. However, woodland restoration on pasture land may still lead to the development of more stable SOC stocks and resilient soil ecosystem than the previous land use.

SOC dynamics are linked with soil N dynamics (Kirby et al. 2005; Benham et al. 2012). In our study, total soil N stocks followed a similar pattern to total SOC, with higher total N stocks under young and

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mature secondary and ancient woodland in comparison to agricultural land (both types) when the organic layer was taken into account. However, woodland mineral soil N stocks showed only a small increase in total N stock (an extra 2.6 t N ha⁻¹ at 0-40 cm depth) over arable land, which had a constant input of N by fertilisers, and there was no effect of woodland age likely due to increased demand of N uptake with age. Such processes are very likely in N cycling of broad-leaved woodland ecosystems, as litter decomposition is faster than conifers, and broad-leaves are more demanding and have higher uptake of N than conifers (Vanguelova & Pitman 2019). In an oak and Corsican pine woodland chronosequence study in the south of England, an increase of topsoil N concentrations and stocks was found with woodlands aged from young (20-40 years) to mature secondary (50-110 years) and to old (150-200 years) on surface water gley soils (Pitman et al. 2014). Since the maximum depth sampled here was 40 cm, there is limited information on likely N translocation with soil depth.

Soil C:N ratio reflected the above trends in C and N stock and showed a decrease at lower mineral soil depths reflecting the breakdown of organic plant materials by the soil microbial community, which possesses a lower C:N ratio than the plant residues (Cools et al. 2014). Both types of agricultural land in our study had significantly lower C:N ratio in mineral soil compared with the woodland sites (particularly ancient woodland), indicating lower mineralisation in woodland soils leading to greater capture of N due to C accumulation. N leaching at similar lowland broadleaved sites in England on surface water gley soils is minimal and sometimes nitrate can be under detection limit (Vanguelova et al. 2010), thus the lower C:N ratio in deeper mineral layer suggests lower C, but higher N accumulation in the clay lattice. Clay soils are well known for their capacity for N and stable C accumulation, with >70% of total C in the stable pool (Villada et al. 2013, 2016). Soil C and N dynamics are heavily influenced by biotic and abiotic factors, including soil texture and biological

activity (Benham et al. 2012). Soil organic matter content and C concentration have been negatively related with soil bulk density, with mineral soils generally having higher bulk densities than organic soil types (Chamberlain et al. 2010, Vangelova et al. 2013). In our study, woodland restoration led to significantly lower soil bulk density than on arable agricultural land. Whilst there was no significant effect of woodland age on soil bulk density, a small decrease in bulk density over time corresponded with an increase in mineral SOC stock. This may also relate to increased root activity, earthworm diversity, and abundance of anecic earthworms, leading to increased creation of macropores within the soil and a reduction in soil compaction (Muys 1990; Capowiez et al. 2003; Colloff et al. 2010; Lavelle 2012).

Woodland restoration effects on earthworm communities

Overall, there were clear differences in earthworm community structure under woodland (regardless of age) compared to both agricultural land types, with an increase in the proportion of anecic (deep-burrowing) and epigeic (surface-living) species, and a decrease in endogeic (shallow-burrowing) species. Forest soils are particularly suitable for epigeic species due to the development of a litter layer, but may support few endogeic species if the organo-mineral layer is small; whilst anecic species abundance in forests is influenced by the quality and quantity of litter, as well as soil physical and chemical status (Decaëns et al. 2008). The observed increase in proportion of anecic earthworms in woodland compared with agricultural land is likely a direct response to the availability of surface organic matter, and the observed increases in SOC under woodland may reflect the incorporation and accumulation of this organic matter into stable soil aggregates through earthworm activity (Six et al. 2004; Pulleman et al. 2005). A positive relationship has been observed between organic matter content and stability of earthworm casts, and in absence of soil disturbance

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such organic matter can be protected within casts for long periods (McInerney et al. 2001; Shipitalo & Le Bayon 2004). Earthworm casts from soils with a high clay content such as those in our study may possess higher aggregate stability than in other soil types (Schrader & Zhang 1997). However, the stabilisation of aggregates within earthworm casts is influenced by many other factors, including organic matter quality and quantity, environmental and climatic conditions, and earthworm species (Shipitalo & Le Bayon 2004).

Compared with arable agricultural land specifically, woodland restoration increased earthworm diversity and species richness, with young secondary woodland (50-60 years) having highest species richness and significantly higher diversity. Young woodland on former arable land removes habitat constraints and partitions trophic and spatial resources, leading to increases in earthworm diversity (Lavelle & Spain 2001; Decaëns et al. 2008; Palo et al. 2013). Similar findings have been made within rows of individual trees on silvoarable agroforestry sites in France (Cardinael et al. 2019). Arable land supports lower species richness due to decreased availability of organic matter, changes in soil water content and temperature, and physical disturbance from agricultural management activities (Edwards & Bohlen 1996; Pelosi et al. 2014; Cardinael et al. 2019). In our study, arable sites were used for cereal crops and are known to have received routine soil disturbance due to ploughing activity. Such sites tend to be dominated by smaller endogeic species which can survive disturbance, or by anecic species with high dispersal and colonisation behaviour, as found in our study (Edwards 1983; Lee 1985; Decaëns et al. 2008). When compared with pasture land, however, woodland restoration resulted in a significant reduction in total earthworm abundance and biomass. Such differences in earthworm abundance between woodland and pasture/grassland have been previously documented, and are attributed to changes in the soil microclimate, organic matter inputs and vegetation effects (Lee 1985; Decaëns et al. 2008). Pasture land may support large

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populations of anecic species due to high quality organic matter resources, and of endogeic species through the development of a rich organo-mineral horizon, but is generally unsuitable for most epigeic species which require surface litter habitat (Bouché 1972,1977; Decaëns et al. 2008). This was indeed the case in our study, in which pasture soils contained significantly higher abundance of the endogeic species *A. chlorotica*, and the anecic species *A. longa* than all other land-use types. *A. longa* has flexible feeding abilities for an anecic earthworm, being able to forage within the soil profile (Lowe & Butt 2002). Given the poor earthworm populations we found on arable land, restoration of woodland onto such land is a means of achieving significant soil ecological improvement. Despite the greater abundance of earthworms on pasture land, restoring broad-leaved woodland may lead to the development of a more diverse earthworm population, with the associated benefits to soil quality supported by the full range of earthworm ecological groups.

We found that earthworm population density and biomass under young and mature secondary woodland was generally higher than under ancient semi-natural woodlands. This reflects similar findings by Muys et al. (1992), who compared meadow, old and young forest stands and found that young plantations had significantly higher earthworm biomasses than old forest stands, with levels comparable to the previous meadow land use. Such trends have been linked to lower pH values under ancient woodland, however, within our study, soil pH tended to remain within favourable levels for most earthworm species following long-term woodland restoration. As observed in our study, the surface water gley soils tend to fall within a pH range of between 5 to 6 due to a high clay content, nutrient content and calcium carbonate secondary formations (Benham et al. 2012). Soil pH, as well as earthworm population responses to woodland restoration on agricultural land, are highly dependent on the tree species planted and the associated litter quality and quantity (Muys 1990; Muys et al. 1992; Curry 2004; Rajapaksha et al. 2013, Schelfhout et al. 2017). Tree species

identity has been shown to be more important than tree diversity in driving earthworm communities in European forests (De Wandeler et al. 2018). Muys et al. (1992) demonstrated that the relatively unpalatable and poor-quality litter of *Quercus palustris* led to reduction in earthworm biomass in old forest stands, leading to litter accumulation and the formation of moder humus. Whilst relatively unpalatable, *Quercus sp.* litter is still capable of supporting populations of detritivorous earthworms in broad-leaved woodlands where oak leaf litter is typically mixed with that of trees with litter of a higher quality such as *Corylus*, *Tilia* and *Acer* species (Perel & Sokolov 1964; Satchell 1983; Hendricksen 1990; Hobbie et al. 2006). Where tree litter is of poorer quality, such as that of *Betula*, *Larix* and *Picea*, earthworm populations may in fact benefit from having oak as a co-occurring tree species (Schwarz et al. 2015; Schelfhout et al. 2017). In our study, all woodlands were broad-leaved with a canopy mostly dominated by *Quercus robur*, *Fraxinus excelsior*, *Prunus avium* and *Acer pseudoplatanus*, species which support high earthworm biomass under secondary woodland (Neiryneck et al. 2000; Curry 2004; Rajapaksha et al. 2013, 2014). Thus, a mixture of appropriate broadleaved tree species planting during reforestation of agricultural land will be important for supporting a diverse earthworm community, and therefore resilient forest soil systems (Muys 1990).

Globally, the restoration of lost woodland ecosystems represents an opportunity to counter the negative impacts of agricultural expansion and woodland fragmentation on biodiversity and soil quality, and restore or enhance biodiversity, ecosystem functions and services (Rey Benayas & Bullock 2012). However, to ensure the sustainable management of soil and biological resources, any new woodland planting must be undertaken strategically taking into account conflicting demands for other land uses (Lee et al. 2002; Quine & Watts 2009). Our research demonstrates that restoring broad-leaved woodland onto agricultural land increases earthworm species and functional diversity and the accumulation of soil C stocks, overcoming some negative soil aspects of agricultural

intensification. Much of these gains are made within a short timeframe; within 50 to 110 years restored broad-leaved can increase SOC stocks to levels comparable with ancient woodland. To further inform international future woodland restoration, we suggest that the chronosequence approach adopted in this study be expanded to investigate alternative forestry practices, tree species, soil types and additional soil quality indicators.

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Table 1. Mean (and standard deviation) soil properties across all land types investigated. OH= Organic horizon. Significant interactions reported in main text.

Site Type	Soil layer / depth (cm)	SOC (%)	SOC stock (t C ha ⁻¹)	Total N (%)	N stock (t N ha ⁻¹)	C:N ratio	Bulk Density (g cm ⁻³)	pH
Arable (N = 4)	0-20	2.06 (0.33)	48.42 (34.91)	0.25 (0.11)	5.64 (1.73)	9.03 (2.16)	1.18 (0.15)	6.59 (0.71)
	20-40	3.66 (1.50)	41.15 (6.80)	0.22 (0.09)	4.99 (1.37)	8.79 (2.76)	1.18 (0.18)	6.77 (0.58)
Pasture (N =3)	0-20	4.77 (0.88)	96.58 (15.89)	0.40 (0.07)	8.08 (1.20)	12.31 (3.89)	1.02 (0.06)	6.20 (0.42)
	20-40	3.39 (0.83)	85.19 (18.98)	0.29 (0.05)	7.15 (0.82)	12.18 (4.04)	1.27 (0.10)	6.42 (0.19)
Agricultural pooled (N =7)	0-20	3.51 (1.55)	69.06 (27.93)	0.31 (0.17)	6.30 (3.15)	11.60 (0.71)	1.01 (0.12)	6.40 (0.92)
	20-40	2.75 (1.03)	63.89 (17.98)	0.25 (0.14)	5.92 (1.58)	11.13 (0.60)	1.07 (0.14)	6.62 (0.89)
Young secondary woodland (50-60 y, N = 4)	OH	19.41 (9.67)	9.14 (2.15)	1.18 (0.36)	0.59 (0.18)	15.83 (3.33)	0.46 (0.14)	5.88 (1.26)
	0-20	5.06 (1.85)	85.63 (18.54)	0.48 (0.14)	8.16 (0.69)	10.42 (1.60)	0.88 (0.19)	5.69 (1.34)
	20-40	3.38 (1.17)	67.45 (23.56)	0.35 (0.10)	6.88 (1.70)	9.70 (1.71)	1.00 (0.14)	6.08 (1.38)
Mature secondary woodland (110- 116 y, N = 3)	OH	13.77 (3.50)	13.58 (3.24)	0.82 (0.22)	0.81 (0.19)	16.76 (0.65)	0.77 (0.13)	5.90 (0.71)
	0-20	3.67 (0.64)	86.21 (21.32)	0.29 (0.03)	6.80 (1.28)	12.62 (1.10)	1.17 (0.10)	6.02 (0.52)
	20-40	2.56 (0.22)	59.07 (5.93)	0.21 (0.02)	4.96 (0.47)	11.92 (0.31)	1.16 (0.04)	6.47 (0.35)
Secondary woodland pooled (N = 7)	0-20	4.46 (1.55)	85.88 (17.98)	0.40 (0.12)	6.82 (1.56)	10.26 (0.35) b	1.11 (0.14)	6.42 (0.60)
	20-40	3.03 (0.94)	63.75 (17.58)	0.29 (0.08)	6.06 (1.60)	10.12 (0.30) b	1.22 (0.15)	6.62 (0.46)
Ancient woodland (>400 y, N = 7)	OH	16.34 (5.45)	23.92 (12.94)	1.02 (0.30)	1.54 (0.84)	15.86 (2.13)	0.79 (0.58)	6.30 (0.70)
	0-20	5.16 (1.73)	103.28 (34.91)	0.45 (0.14)	7.95 (3.15)	11.99 (0.99)	1.00 (0.21)	5.83 (1.01)
	20-40	3.66 (1.50)	77.20 (34.91)	0.33 (0.10)	6.96 (2.74)	11.27 (0.72)	1.07 (0.13)	6.25 (1.02)

Table 2. Mean (and standard deviation) earthworm abundance, biomass and diversity across all site types investigated. Ecological groups assigned *sensu* Bouché (1972). Species rarity assigned according to Sherlock (2018). Different letters indicate significant differences between soil depths. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Species	Rarity (UK)	Land use				
		Arable	Pasture	Young woodland	Mature woodland	Ancient woodland
Epigeic species						
<i>Dendrobaena octaedra</i>	Uncommon	-	-	-	-	0.3 (0.7)
<i>Eisenia fetida</i>	Common	-	-	-	-	0.1 (0.3)
<i>Lumbricus castaneus</i>	Common	1.6 (3.2)	0.9 (1.6)	2.0 (4.1)	1.8 (2.4)	0.4 (1.0)
<i>Lumbricus festivus</i>	Rare	-	0.3 (0.5)	-	-	-
<i>Lumbricus rubellus</i>	V. common	-	2.7 (3.3)	0.7 (1.4)	2.4 (2.8)	1.0 (1.1)
Endogeic species						
<i>Allolobophora chlorotica</i>	V. common	22.3 (16.3) a	51.8 (18.9) b**	10.5 (18.5) a	0.9 (1.6) a	1.3 (1.7) a
<i>Aporrectodea caliginosa</i>	V. common	17.1 (16)	79.4 (55.1)	17.5 (13.5)	25.5 (3.6)	15.5 (7.5)
<i>Aporrectodea rosea</i>	Common	2.0 (3.0)	13 (5.6)	9.8 (10.9)	9.1 (8.1)	11.2 (8.6)
<i>Dendrobaena pygmaea</i>	V. rare	0.5 (0.9)	-	-	-	-
<i>Murchieona muldali</i>	Rare	-	0.3 (0.5)	0.7 (1.4)	0.3 (0.5)	0.7 (1.4)
<i>Octolasion lacteum</i>	Common	-	2.7 (3.3)	1.6 (3.2)	2.1 (3.7)	0.3 (0.7)
<i>Octolasion cyaneum</i>	Common	-	-	1.8 (3.6)	-	-
Anecic species						
<i>Aporrectodea longa</i>	Common	2.0 (1.6) a	28.5 (16.4) b*	9.8 (9.7) a	4.5 (5.7) a	2.7 (3.0) a
<i>Lumbricus terrestris</i>	Common	0.5 (0.5)	1.8 (1.8)	5.2 (2.0)	1.5 (1.4)	3.1 (2.5)
Juveniles (<i>L. sp</i>)		116.6 (93.4)	170.9 (101.4)	70.9 (32.3)	59.1 (1.8)	60.1 (32.4)
Abundance (Ind. m ⁻²)	-	162.5 (115.6) a	349.7 (169.3) b**	121.1 (63.2) a	119.7 (29.6) a	96.6 (45.2) a

Total biomass (g m ⁻²)	-	25 (12.4) a	111.7 (57.1) b***	30.7 (13.9) a	42.2 (24.5) a	22.1 (12.4) a
Shannon H diversity	-	0.97 (0.12) a	1.29 (0.05) ab	1.47 (0.31) b*	1.31 (0.18) ab	1.32 (0.19) ab

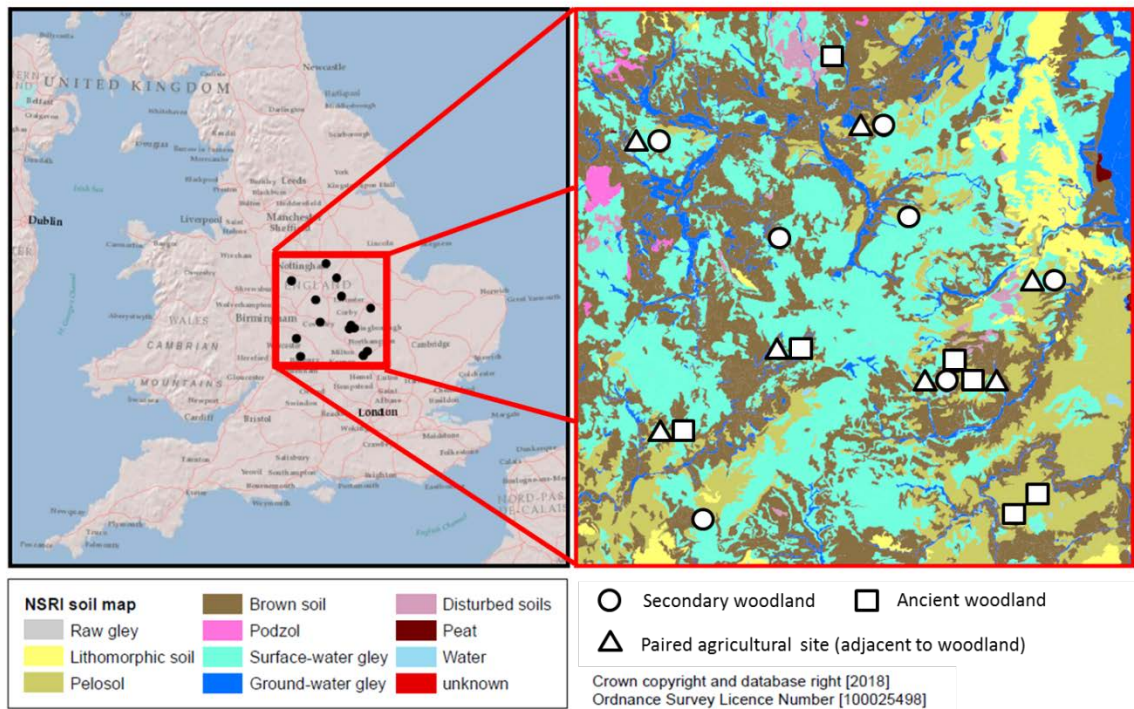


Figure 1. Maps showing the locations and the dominant soil types of the study sites in England (n = 21).

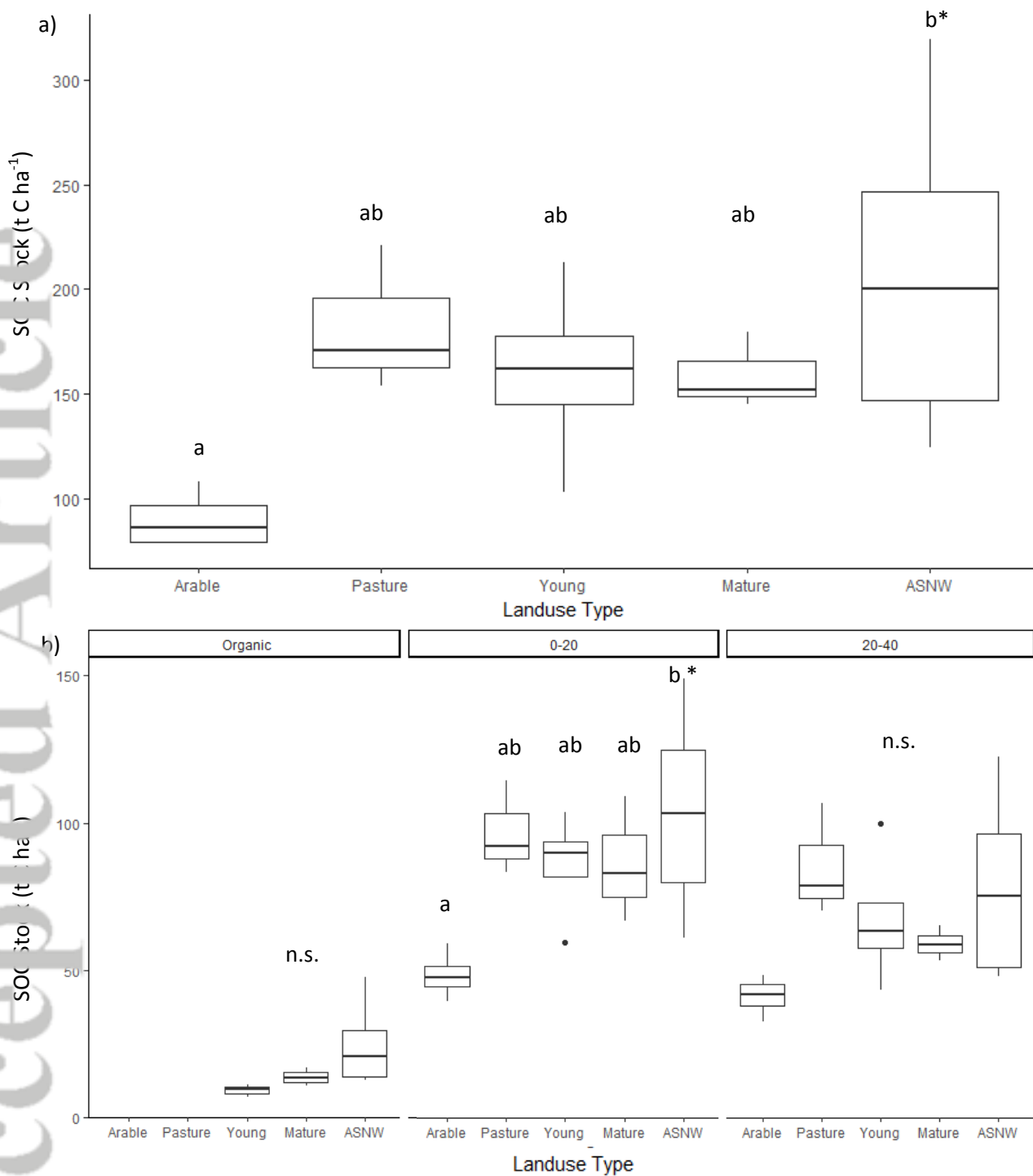


Figure 2. Boxplot showing a) the total SOC stock (t C ha⁻¹) (organic horizon plus 0-40 cm depth mineral soil), on arable and pasture agricultural land, young and mature secondary woodland and ancient woodland (ASNW), and b) soil organic carbon (SOC) stock (t C ha⁻¹) in the OH organic horizon 'Organic' and at 0-20 cm and 20-40 cm depth individually. Different letters in a row indicate significant differences, n.s. = no significant differences, one-way ANOVA followed by Tukey-Kramer post-hoc test, * p < 0.05.

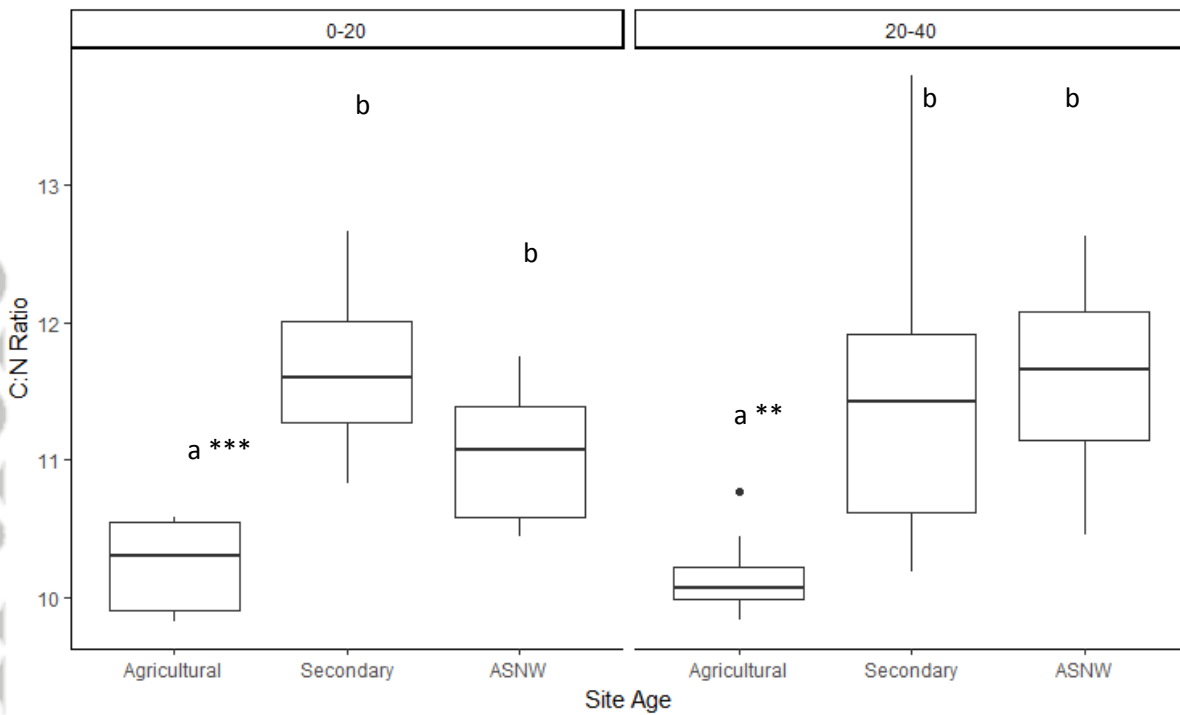


Figure 3. Boxplot showing the soil C:N ratio at two depths (0-20 cm and 20-40 cm) on pooled agricultural land, secondary woodland and ancient woodland (ASNW). Different letter in a row indicate significant differences, one-way ANOVA followed by Tukey-Kramer post-hoc test, ** $p < 0.01$, *** $p < 0.001$. (N = 7).

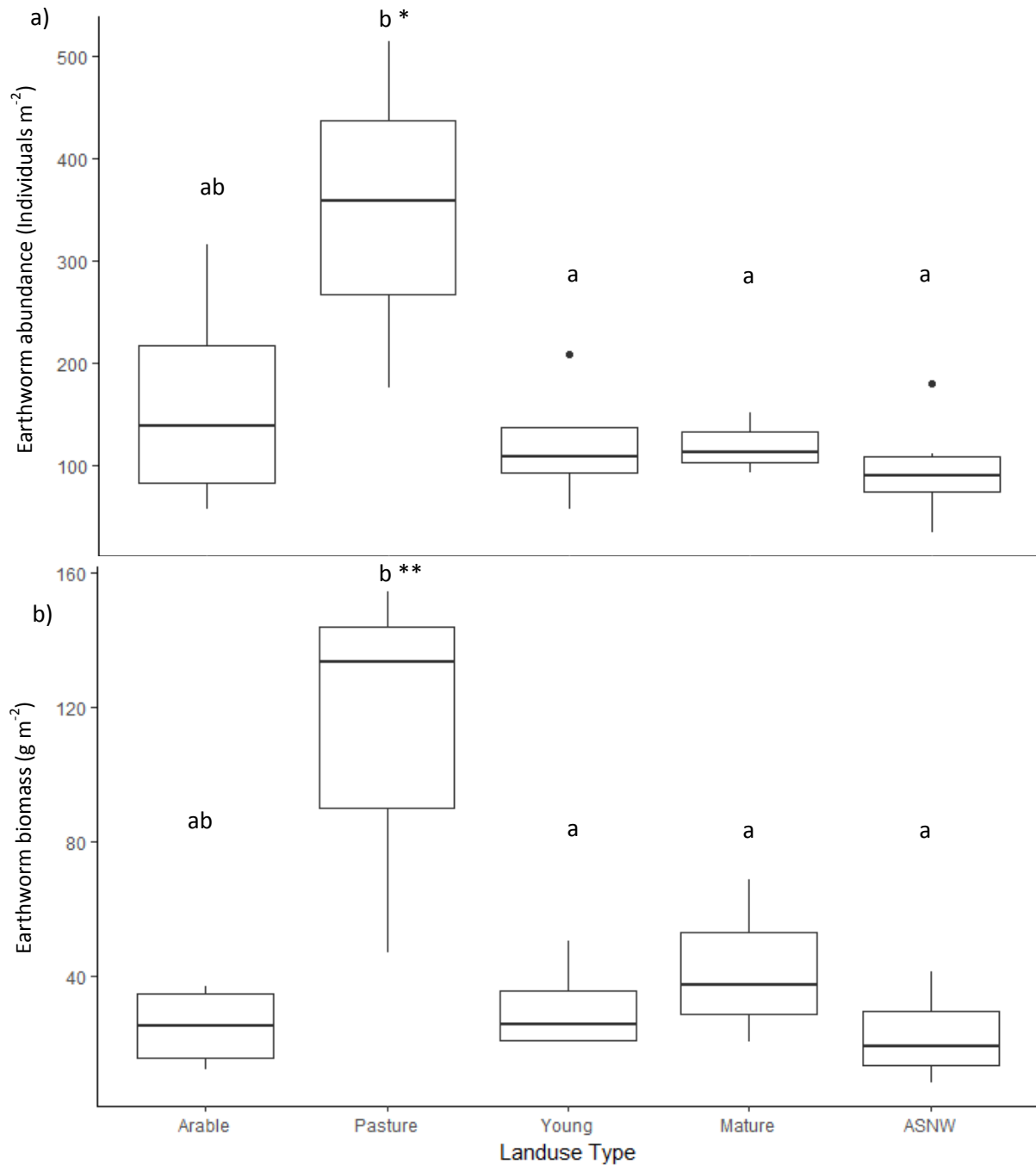


Figure 4. Boxplots showing earthworm abundance (individuals m⁻²) (a) and biomass (g m⁻²) (b) results from two agricultural classes and three woodland age classes (young secondary: 50-60 years old, mature secondary: 110-116 years old, ancient (ASNW): >400 years old) investigated in this study. Different letters in a row indicate significant differences, one-way ANOVA followed by Tukey-Kramer post-hoc test, * p < 0.05, ** p < 0.01.