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RESEARCH

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Developing a systematic sampling method for earthworms in and around deadwood

Frank Ashwood^{1*} , Elena I. Vanguelova¹, Sue Benham¹ and Kevin R. Butt²

Abstract

Background: The ecological importance of deadwood is widely acknowledged, however popular forestry practices may reduce deadwood from a site, and most European forests now fall below recommended targets, putting deadwood-associated species at risk. There is increasing evidence that earthworm species which live in alternative habitats such as deadwood can be missed by traditional sampling methods, which can lead to false classifications regarding species distributions and conservation status and value. Resolving the current lack of a systematic and quantitative methodology for surveying earthworms in microhabitats such as deadwood may therefore lead to valuable insights into earthworm species ecologies in forest ecosystems. The main aim of this research was to develop and trial a systematic method for surveying deadwood-associated earthworms, with potential future application to other invertebrates. Sampling of earthworms within soil, deadwood and soil beneath deadwood was carried out across a chronosequence of unmanaged oak forest stands. The results were then used to investigate the influence of soil and deadwood environmental factors and woodland age on the earthworm populations of oak-dominated broadleaf woodlands.

Results: Results from our surveys successfully show that in oak woodland habitats with deadwood, omitting deadwood microhabitats from earthworm sampling can lead to underestimates of total earthworm species richness, abundance and biomass. We also found a significantly greater proportion of juveniles within the earthworm communities of broadleaf deadwood, where temperature and moisture conditions were more favourable than surrounding open soil habitats.

Conclusions: The systematic method presented should be considered as additional and complementary to traditional sampling protocols, to provide a realistic estimate of earthworm populations in woodland systems. Adopting this quantitative approach to surveying the biodiversity value of deadwood may enable forest management practices to more effectively balance wood production against ecological and conservation values. Opportunities for further development of the sampling methodology are proposed.

Keywords: Earthworms, Coarse woody debris, Deadwood, Microhabitat, Deciduous woodland, Oak, Soil, Sampling method

Background

As ecosystem engineers, earthworms are associated with a range of soil processes and functions linked with the development of sustainable forest ecosystems (Lavelle et al. 1997; Blouin et al. 2013). Earthworms are typically classified across three ecological groups based on their life strategies: epigeic (surface/litter dwelling), endogeic (shallow, mineral soil dwelling) or anecic (dwelling in deep vertical soil burrows) (Lee 1959; Bouché 1977),

however, not all species conform to such rigid classifications, and sub-divisions exist (Lavelle 1988). An additional group has been devised for 'corticolous' or 'arboreal' species, which are associated with trees; living in accumulations of organic matter in tree canopies, within rot holes and decaying wood, and under the bark of standing trees and rotting logs (Bouché 1972; Lee 1985; Mogi 2004; Römbke et al. 2017). Such decaying wood serves a key functional role in forests by acting as sites of plant nutrient exchange and seed germination, moisture retention and promoting soil structural improvements thorough its decay into humic substances

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(Harmon et al. 1986; McWinn and Crossley Jr 1996). Fallen large branches, logs, stumps and standing dead trees (snags) are also an important habitat in forest ecosystems; acting as a substrate for fungi and invertebrates and providing various other organisms shelter from predators (Bunnell and Houde 2010). Decaying wood habitats not only provide juvenile and adult earthworms with refuge from predators and a food resource, but may also enable overwintering of cocoons and extend periods of earthworm activity if favourable moisture and temperature conditions are provided (Hendrix 1996; Geraskina 2016). Earthworms are known to colonise deadwood following the initial stages of fungal decay and wood channelisation by invertebrates - further advancing deadwood decomposition through transport of soil, water, nutrients and microbes (Ausmus 1977; Caldwell 1993; Hendrix 1996). The diversity of earthworm populations of a forest may affect the rate of deadwood decay, with arboreal, epigeic and endogeic species potentially the most important at various stages (Hendrix 1996). Furthermore, earthworm communities and deadwood volume are both affected by tree species and woodland age (Muys et al. 1992; McWinn and Crossley Jr 1996).

Whilst deadwood colonisation processes have been described (e.g. Caldwell 1993), very few studies exist which have investigated earthworm: deadwood interactions and the effects of deadwood management on woodland earthworm populations (Hendrix 1996; Geraskina 2016; Zuo et al. 2018). This paucity of research is likely due to the current lack of a systematic and quantitative methodology for surveying earthworms and other invertebrates in microhabitats such as deadwood. Retaining undisturbed areas (and therefore deadwood) within managed forests may provide long-term benefits to the biodiversity of earthworms and other important forest soil organisms (Franklin and Waring 1980; Hendrix 1996). However, intensive forest management techniques results in decreases or the complete removal of deadwood from managed forest systems (Hodge and Peterken 1998). For example, short-rotation forestry periods may be too short for sufficient deadwood to develop, and early thinning operations in longer-term forestry may remove future deadwood trees from the forest (Van Lear 1996). Silvicultural techniques which utilise the entirety of the tree, such as Whole Tree Harvesting (WTH), can result in the complete removal of potential aboveground deadwood from a site, posing a threat to wildlife habitat and thus biodiversity (Hodge and Peterken 1998; Martikainen et al. 1999; Grove 2002; Dudley and Vallauri 2005; Davies et al. 2008). More information on the ecological importance and benefits of deadwood to forest health may help to promote a balanced approach between intensive and low-impact forest management practices.

There is increasing evidence that earthworm species which live in alternative habitats to soil (e.g. microhabitats such as decaying wood) can be missed by traditional quantitative sampling methods (Butt and Lowe 2004; Schmidt et al. 2015; Römbke et al. 2017). Such under-sampling can lead to false classifications regarding earthworm species distributions and conservation status, as demonstrated by the recent discovery of *Dendrobaena attemsi* in Ireland, and the reclassification of the same species from 'rare' to 'moderately common' in Germany, both following forest microhabitat surveys (Schmidt et al. 2015; Lehmitz et al. 2016; Römbke et al. 2017). Micro-habitat surveying may also reveal a wealth of new information on earthworm species ecologies. For example, through investigating a variety of micro-habitats on the Isle of Rum, Butt and Lowe (2004) found individuals of *Lumbricus rubellus* in soil-free loose scree, *Bimastos rubidus* and *Bimastos eiseni* under rocks in a crag, and *B. rubidus* below the bark of a dead tree. Resolving the current lack of a systematic and quantitative methodology for surveying earthworms and other invertebrates in microhabitats such as deadwood may therefore lead to valuable and fundamental insights into earthworm species ecologies, distributions and diversity in forest ecosystems (Hendrix 1996). As observed by Paoletti (1999), earthworm sampling methods must satisfy the objectives of the individual research project, and always represents a trade-off between available resources and sampling accuracy. Resolving the current lack of method for invertebrate sampling in coarse woody debris may prove to be particularly advantageous, as this ecological system is available in discrete units in a range of sizes, ages and species, and can be experimentally manipulated (Carroll 1996; Cornelissen et al. 2012; Zuo et al. 2018).

The primary objective of this research was to develop and trial a systematic method for surveying deadwood-associated earthworms in a woodland habitat. The success of the method was evaluated by comparing results against those gathered through standard soil earthworm sampling methods (Butt and Grigoropoulou 2010), in terms of species, abundance, biomass and ecotypes collected. The results from these surveys were then used to address the secondary objective of this research, which was to investigate the influence of soil and deadwood environmental factors and woodland age on the earthworm populations of oak-dominated broadleaf woodlands.

Methods

Study sites

Alice Holt Forest in Surrey, England (latitude 0°50'W; longitude 51°10'N), is one of 11 terrestrial Environmental Change Network (ECN) long-term monitoring sites

across the UK (Benham 2008). The forest is situated on surface-water gley soils on underlying gault clay, and largely consists of conifer species, with around 140 ha of common oak (*Quercus robur*) woodland. Classified as semi-natural ancient woodland, some oak stands are over 190 years of age. Further information regarding the soil, climate and woodland management history is given in Benham et al. (2012). Good management records have allowed a chronosequence of replicated plots to be established within the forest, representing a range of woodland stand ages with comparable soil and woodland management conditions (Benham et al. 2012). Three woodland age groups were available from the chronosequence plots: young (30 to 40 years), mid-rotation (70 to 90 years) and old (> 190 years). Four replicated stands from each age group were sampled over the course of one month, from mid-October to mid-November 2017 (twelve stands in total).

Sampling methodology

A small pilot study was initially conducted to inform the design of the main systematic sampling method. The full range of deadwood diameters (range in cm) and decay classes (grading from 1 - least decayed to 5 - most decayed) were investigated and only deadwood of decay class 3 onwards was found to contain earthworms. Furthermore, only decay class 5 was found to have earthworms within the decaying heartwood, rather than

exclusively beneath loose bark or within moss. In these cases, the number of earthworms recovered was comparatively small, and deadwood without a cover of loose bark or moss also yielded few earthworms (a few individuals of the species *B. eiseni* and *B. rubidus*). Further, deadwood of smaller diameter than 10 cm, such as thin fallen branches, yielded few earthworms. In total, five species of moss were identified growing on the deadwood in this study: *Kindbergia praelonga* (*Eurinchium praelonga*), *Isothecium myosuroides*, *Eurinchium striatum*, *Rhytidiadelphus triquetrus* and *Brachythecium rutabulum*. All were associated with supporting (predominantly epigeic) earthworm populations, with small body-sized species (e.g. *B. rubidus*) regularly found within or beneath damp moss.

The main systematic study utilised a square plot of 10 m × 10 m within each forest stand ($n = 12$), previously marked out following ECN protocols as detailed in Benham et al. (2012) (Fig. 1). Each plot was then surveyed for the total volume of coarse deadwood greater than 10 cm in diameter ('Coarse Woody Debris' or CWD) within the plot (following the methodology presented by the EU BioSoil project field manual - see Bastrup-Birk et al. 2007). From the total available deadwood per plot, five pieces of any size and decay class were randomly selected and sampled for earthworms. This involved relocating the deadwood onto a tarpaulin sheet, and immediately digging a standard square or modified

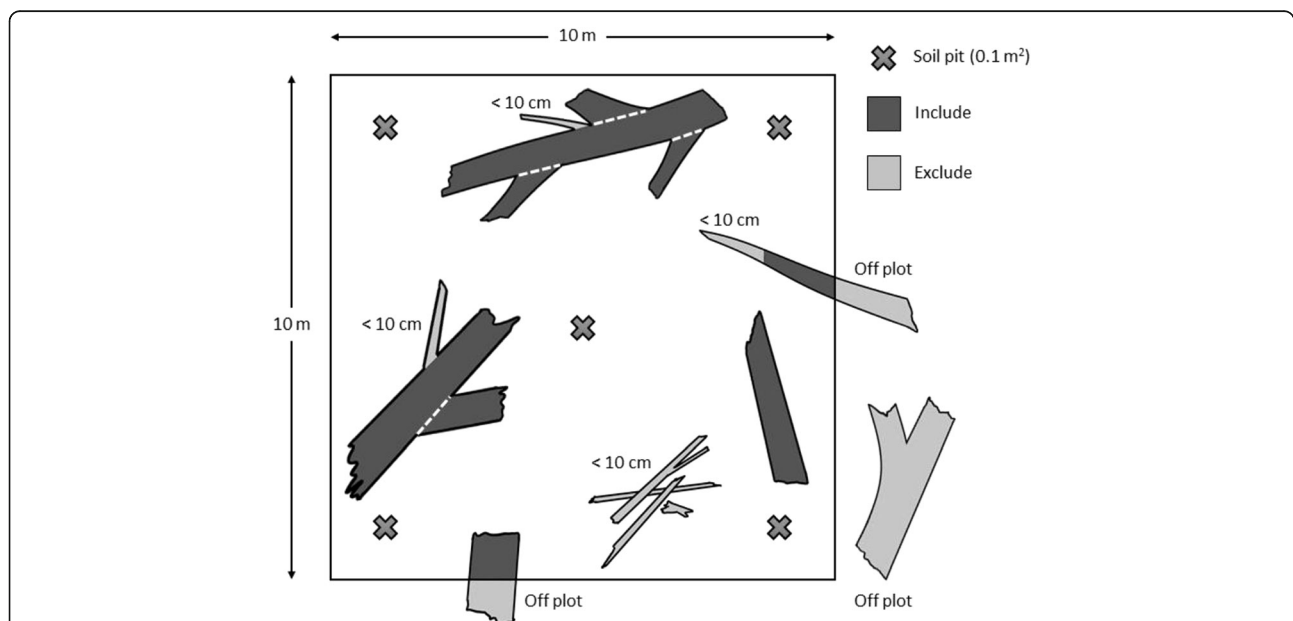


Fig. 1 Layout of the sampling method being undertaken in a 100-m² oak forest plot (adapted from Spetich 2007). Dashed white lines within deadwood indicate sections divided into separate pieces. All deadwood ≥ 10 cm in diameter and within the plot were measured for total length and midpoint diameter (dark grey), and five randomly selected pieces also sampled for earthworms within the deadwood and in the soil beneath (using 0.1 m² soil pits). All deadwood < 10 cm in diameter or outside the plot were excluded from the survey (light grey). Five standalone 0.1 m² soil pits (indicated by crosses) were sampled for soil-dwelling earthworms

rectangular soil pit (both 0.1 m² area and 10 cm depth) where the deadwood had been lying, then excavating this soil onto a separate sheet to be hand-sorted for earthworms. Whilst processing soil, the deadwood was routinely observed to catch any escaping earthworms. To each pit, 5 l of mustard suspension vermifuge (at concentration of 50 g mustard powder in 10 l water) was applied to the pits to extract deep-burrowing earthworms, and the pit observed for 15 min for emerging earthworms (Eisenhauer et al. 2008; Butt and Grigoropoulou 2010). Where possible, moisture and temperature measurements were taken (using a delta-T theta probe calibrated to clay soils and a thermometer) on an immediately adjacent area of soil that was beneath the deadwood, or otherwise this was taken before digging the soil pit. Soil samples were collected from the top 10 cm of each soil pit for chemical analysis. Following hand-sorting, soil was replaced, and attention turned to sampling the deadwood. Deadwood diameter and length was measured, and the likely tree species and decay class were estimated based on the criteria of Maser et al. (1979) and Hunter Jr (1990). This consisted of a 1 to 5 ranking system, whereby 1 is least decayed (freshly fallen) and 5 the most advanced stage of decay (complete incorporation of the deadwood into the soil profile). All deadwood tree species sampled in this study were deciduous: mainly common oak (*Quercus robur*) and silver birch (*Betula pendula*). Deadwood temperature was measured by placing the thermometer beneath any bark present. Any moss and loose bark were removed and inspected for earthworms, and if the deadwood was decay class 5, the remaining wood was also dismantled and inspected. Organo-mineral accumulations (decaying organic matter mixed with mineral soil) beneath loose bark were collected for chemical analysis. Any earthworms were collected into separate and clearly labelled tubes of 80% ethanol. All pieces of deadwood were returned to their original location once sampled, with moss and loose bark replaced as best possible.

Additionally, five standard soil pits (0.1 m² area and 10 cm depth) were dug in each plot (Fig. 1), and the soil excavated onto a sheet and hand-sorted for earthworms. Mustard vermifuge was applied to all pits as above, and earthworms collected and preserved as described above. Soil moisture and temperature measurements were taken in the top 10 cm soil immediately adjacent to each pit. Soil samples were also collected from this depth in each soil pit for chemical analysis by Forest Research laboratory services at Alice Holt Lodge, Farnham, UK. Soil bulk density and soil moisture content were analysed by oven drying at 105 °C for 24 h, and soil pH was measured in water suspension. All collected earthworms had 80% ethanol replaced within 24 h, had preserved mass recorded and were identified using the key of Sims and

Gerard (1999) and named using the key of Sherlock (2018).

Statistical analysis

Statistical models were applied to data on earthworm population density and biomass (expressed for deadwood data as value per m² deadwood surface area), species richness and diversity, and soil chemical and physical parameters; with decay class, woodland age, soil pH and moisture content as explanatory variables. Data were first tested for normality using the Shapiro-Wilk test, which is suited to small sample sizes. Where data had a normal distribution, they were analysed using Student's t-test or one and two-way analysis of variance (ANOVA) with the Tukey-Kramer post-hoc multiple comparison test applied to significant treatment interactions. Where the assumptions of ANOVA were not met, we applied non-parametric Kruskal-Wallis ANOVA test followed by Dunn's Post-Hoc test as appropriate. Proportion data (adult vs juvenile) were analysed using Generalized Linear Mixed Model (GLMM) with logit link and binomial errors, followed by Chi-squared test. Sample-based species rarefaction curves were calculated using the *specpool* function in R Studio, utilising a range of popular analytical formulas (Chao 1987; Colwell and Coddington 1994; Chiu et al. 2014). Practical significance of results was interpreted using the effect size thresholds for Eta-squared (η^2) and Cohen's *d* 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 "Joy in Playing".

Results

Earthworm populations of deadwood and soil habitats

In total 1,012 earthworms were collected, representing 13 species, seven genera and all three main ecological groups (Bouché 1977): epigeic: *Bimastos eiseni* (formerly *Allolobophoridaella eiseni*), *Bimastos rubidus*, *Dendrobaena attemsi*, *Dendrobaena octaedra*, *Dendrobaena pygmaea*, *Eisenia fetida*, *Lumbricus castaneus* and *Lumbricus rubellus*; endogeic: *Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea rosea* and *Octolasion lacteum*; anecic: *Aporrectodea longa*. Total earthworm species richness varied by habitat type, with seven species found in deadwood, eleven species within the soil beneath deadwood, and twelve species found in open (uncovered) soil (Table 1). One species, *E. fetida*, was found exclusively within deadwood. Five additional species were found in both soil habitat types, and one species, *A. rosea*, was found only within open soil. Earthworm species diversity (Shannon-Wiener H) did not differ significantly between the three habitat types

Table 1 Abundance (individuals·m⁻², ± SD) of earthworm species found in the three habitats surveyed, arranged alphabetically by scientific name following the nomenclature of Sherlock (2018)

Earthworm species	Habitat		
	Soil	Deadwood soil	Deadwood
<i>Allolobophora chlorotica</i>	19.5 ± 23.8 a	9.8 ± 13.9 a	0.6 ± 1.3 b*
<i>Aporrectodea caliginosa</i>	2.2 ± 3.9	1.0 ± 2.9	–
<i>Aporrectodea longa</i>	0.8 ± 2.9	0.2 ± 0.6	–
<i>Aporrectodea rosea</i>	0.5 ± 1.2 †	–	–
<i>Bimastos eiseni</i>	0.2 ± 0.6 a	0.3 ± 0.8 a	1.9 ± 2.3 b**
<i>Bimastos rubidus</i>	4.5 ± 7.5	6.5 ± 7.8	2.8 ± 3.1
<i>Dendrobaena attemsi</i>	8.8 ± 25.3	6.7 ± 17.8	0.9 ± 2.4
<i>Dendrobaena octaedra</i>	16.8 ± 23.8	12.5 ± 17.2	3.2 ± 4.6
<i>Dendrobaena pygmaea</i>	0.3 ± 1.8	0.2 ± 0.6	–
<i>Eisenia fetida</i>	–	–	0.2 ± 0.5 †
<i>Lumbricus castaneus</i>	0.3 ± 1.2	0.3 ± 1.2	–
<i>Lumbricus rubellus</i>	19.2 ± 10.5 a	13.8 ± 9.9 a	1.6 ± 1.4 b***
<i>Octolasion lacteum</i>	0.2 ± 0.6	0.5 ± 1.7	–
Total abundance (Individuals·m ⁻²)	102.0 ± 63.8 a***	21.33 ± 15.0 b	21.18 ± 10.1 b
Total biomass (g·m ⁻²)	23.8 ± 9.1 a***	5.0 ± 2.8 b	2.6 ± 1.3 b

Different letters indicate significant differences, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Kruskal-Wallis non-parametric ANOVA, $n = 12$. † Unique species to this habitat, – species absent from this habitat

investigated. Stand age had no effect on any of the earthworm variables measured.

Total earthworm abundance (individuals·m⁻²) was significantly greater in open soil than in soil beneath deadwood and within deadwood (Kruskal-Wallis $H(2) = 21.16$, $p < 0.001$) (Table 1 & Fig. 2). Similarly, total earthworm biomass (g·m⁻²) was significantly greater in open soil than in soil beneath deadwood and within deadwood ($H(2) = 25.74$, $p < 0.001$). There was an effect of habitat type on the abundance (individuals·m⁻²) of three earthworm species: *B. eiseni* abundance was significantly greater in deadwood than either soil habitat type ($H(2) = 10.49$, $p = 0.005$). Conversely, the abundance of *A. chlorotica* and *L. rubellus* was significantly greater in both soil habitat types than in deadwood ($H(2) = 5.943$, $p = 0.05$ and $H(2) = 16.06$, $p < 0.001$, respectively) (Table 1). Deadwood decay class had no significant effect on total earthworm abundance or biomass, or on species richness and diversity. However, decay class did affect the abundance (individuals·m⁻²) of the earthworm species *B. eiseni*; with significantly greater abundance in deadwood of decay class 3 ($N = 50$, $M = 2.73$, $SD = 5.14$) than decay class 4 ($N = 15$, $M = 0.17$, $SD = 0.44$) (Kruskal-Wallis $H(1) = 6.07$, $p = 0.014$). The proportion of epigeic earthworms (% total population) in deadwood was higher in decay class 3 (86.2%) than in decay class 4 (72.7%); conversely, the proportion of endogeic earthworms increased from 13.8% in decay class 3, to 27.3% in decay class 4.

The proportion of adult earthworms (% total population) was significantly lower within deadwood ($M = 53.10$,

$SD = 18.40$) than in soil beneath deadwood ($M = 72.94$, $SD = 11.56$) and in open soil ($M = 70.47$, $SD = 8.31$) (Chi-squared, $\chi^2(2, N = 36) = 135.95$, $p < 0.001$), and vice versa for juveniles (Fig. 3). Within deadwood, there was no difference in the proportion of adult and juvenile earthworms ($p > 0.05$), but within soil beneath deadwood, the proportion (%) of adults ($M = 72.94$, $SD = 11.56$) was significantly greater than juveniles ($M = 27.06$, $SD = 11.56$) ($\chi^2(1, N = 12) = 47.0$, $p < 0.001$). This was also the case within open soil ($\chi^2(1, N = 12) = 20.9$, $p < 0.001$).

On average, the deadwood surveys contributed an additional 81 earthworms and 209 g earthworm biomass per 100 m² plot (or 8,100 earthworms and 20.9 kg per hectare). Compared with the estimated plot average of 10,200 earthworms and 2,378 g earthworm biomass yielded per 100 m² plot (equivalent to 1,020,000 earthworms and 237.8 kg per hectare) by traditional open soil sampling, deadwood surveying contributed an additional 0.8% to the total earthworm abundance and 8.8% to the total earthworm biomass data. Sample-based species rarefaction curves calculated the expected number of earthworm species against the cumulative number of deadwood samples, and indicated that a maximum sampling effort of 8 deadwood samples (using the Chao equation) is required to capture the total earthworm species richness within a plot.

Environmental factors

Habitat type influenced soil moisture content (%), with significantly greater moisture content in the organo-

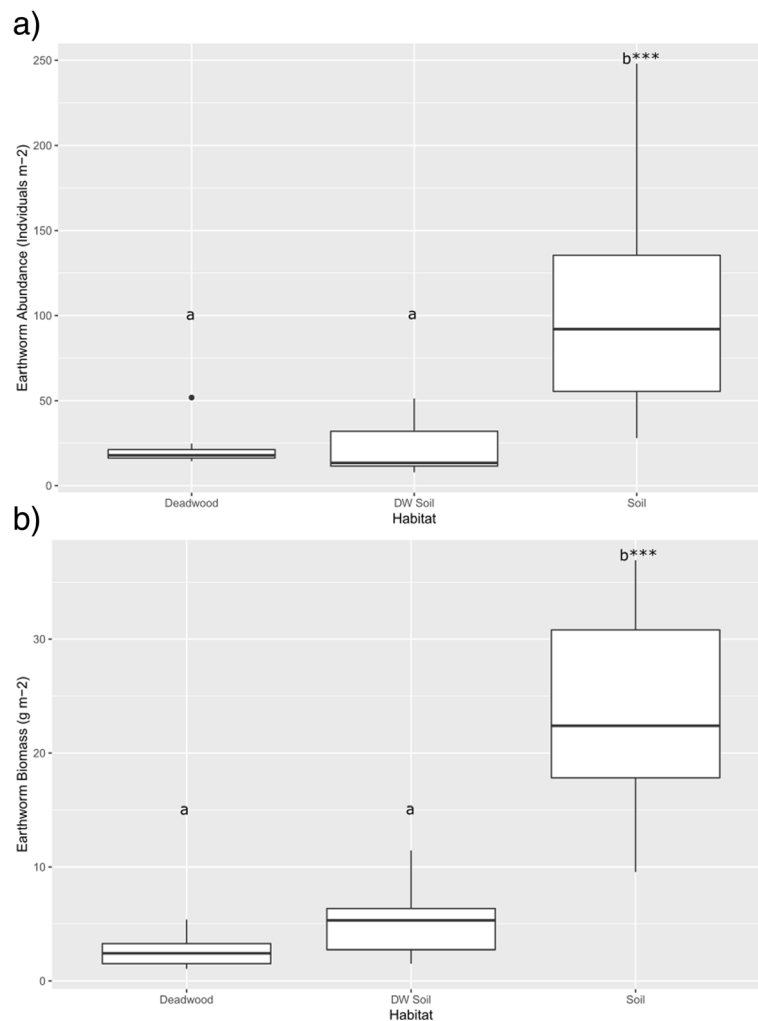


Fig. 2 Earthworm (a) abundance (individuals·m⁻²) and (b) biomass (g·m⁻²) in the three habitats surveyed. DW soil = Deadwood soil. Kruskal-Wallis non-parametric ANOVA, *** $p < 0.001$, $n = 12$

mineral accumulations beneath deadwood bark ($N = 12$, $M = 78.43$, $SD = 2.36$) than in soil beneath deadwood ($M = 24.31$, $SD = 9.34$) and open soil ($M = 23.82$, $SD = 8.30$) ($H(2) = 17.46$, $p < 0.001$). On average, deadwood was notably around 1 °C warmer ($M = 12.18$, $SD = 2.13$) than the soil beneath ($M = 10.98$, $SD = 1.74$) or open soil ($M = 10.88$, $SD = 1.84$), however this difference was not statistically significant. There was a significant effect of decay class on deadwood temperature, with decay class 4 ($N = 28$, $M = 12.43$, $SD = 2.10$) greater than decay class 3 ($N = 92$, $M = 11.33$, $SD = 1.88$), ANOVA (1,118) $F = 6.903$, $P = 0.01$, $\eta^2 = 0.055$. Soil pH was unaffected by habitat type, with the organo-mineral accumulations beneath loose bark of deadwood similar in pH ($M = 4.57$, $SD = 0.38$) to the 0–10 cm depth of soil beneath deadwood ($M = 4.44$, $SD = 0.47$) and open soil ($M = 4.39$, $SD = 0.28$). Likewise, soil bulk density was not significantly different between the two main soil habitat types

(soil beneath deadwood: $N = 12$, $M = 0.915$, $SD = 0.13$; open soil: $M = 1.00$, $SD = 0.15$). Forest stand age had no effect on deadwood volume; however, 190+ year old stands had a greater mean deadwood volume of 1.18 m³ ($SD \pm 2.05$) per 100 m² plot compared with 70–90 years (0.09 m³ \pm 0.02) and 30–40 years (0.18 m³ \pm 0.15). There was no effect of stand age on any of the measured environmental variables.

Further observations

Alongside the systematic sampling, additional forest microhabitats were casually investigated for earthworm presence. Within a young (30–40 years) plot, *D. octaedra* and *D. attemsi* were found within an organic matter accumulation (decaying foliar material and other organic debris) in the saddle of a mature silver birch (*B. pendula*), at approximately one metre above ground height. Within the same plot, a specimen of *B. eiseni* was

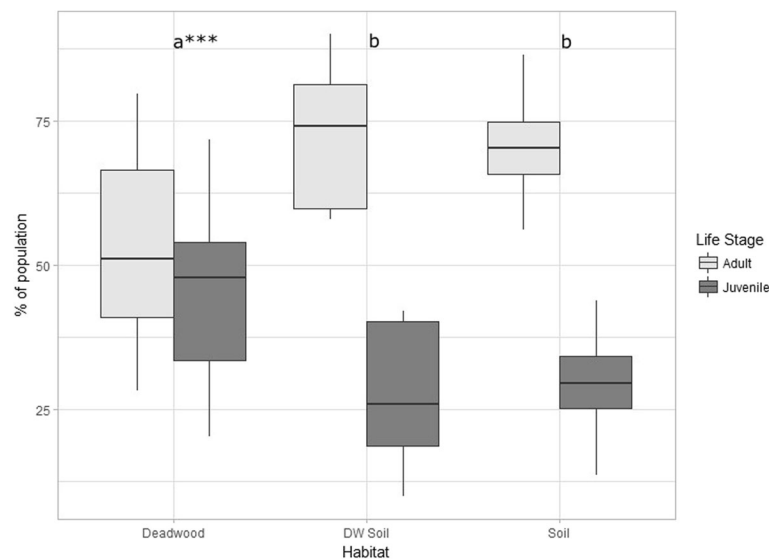


Fig. 3 The proportion (% of total abundance) of adult and juvenile earthworms in the three habitats surveyed. DW soil = Deadwood soil. Generalized Linear Mixed Model (GLMM) with logit link and binomial errors, followed by Chi-squared test, *** $p < 0.001$

collected from beneath the bark of a standing dead tree. On another young plot, *B. eiseni* were collected from within a bark fissure/bleed on a diseased common oak (*Q. robur*) at approximately 2 m height. Additionally, on numerous occasions where adult *L. rubellus* were collected from within organo-mineral accumulations under the bark of lying deadwood, their cocoons were also recovered.

Discussion

Deadwood earthworm populations

Earthworms are considered secondary colonisers of deadwood, exploiting previous microbial conditioning of the wood and channels created by invertebrates and fungal hyphae (Hendrix 1996). With limited capacity for digesting plant residues, the available fungal hyphae, microbial biomass and particulate organic matter are considered to support the resident earthworm populations, and their feeding on these further regulates deadwood decomposition rates (Ausmus 1977; Lee 1985; Hendrix 1996). Our study confirmed that earthworms were only located within deadwood which was mid-stage decay and onwards; and, besides a difference in the abundance of *B. eiseni*, we found little change in earthworm diversity and abundance between the two main mid-stage decay classes investigated (classes three and four) (Hunter Jr 1990; Bastrup-Birk et al. 2007). However, the proportion of endogeic earthworms within the deadwood earthworm community increased as decay advanced, as first hypothesised by Hendrix (1996) and observed for mixed broadleaf deadwood by Kooch (2012). Only within deadwood of the most advanced decay class were earthworms recovered from the

heartwood and sapwood (the majority of the deadwood biomass), and in such cases the number of both individual earthworms and the number of species recovered was minimal. Degree of loose bark affected earthworm abundance, with few earthworms recovered from deadwood lacking a loose bark cover, likely due to unsuitable conditions and insufficient availability of food resources. Such wood was case-hardened and showed arrested decay (Van Lear 1996), and our findings support those of Zuo et al. (2016, 2018), who found that deadwood earthworm assemblages are influenced by deadwood decomposition stage and degree of bark fissures. Deadwood earthworm populations are also affected by tree species identity (Zuo et al. 2016), however since the tree species sampled in this study were limited to *Q. robur* and *B. pedula*, this could not be tested further.

Hendrix (1996) proposed that maximum earthworm diversity in deadwood is reached during the secondary colonisation and maceration phases, eventually reflecting that of surrounding soil following complete incorporation into the soil. Our data supports the former proposition; however we found no evidence that earthworm diversity in deadwood approaches that of surrounding open soil during the most advanced stage of decay – with such highly decomposed material observed as hostile and dry in the field. This may be related to increased humification of soils beneath deadwood compared to open soil, which was found by Shannon et al. (unpublished data) for the same forest stands as investigated in our study, which would provide less palatable organic matter food source for earthworms beneath the deadwood. Open soil earthworm abundance and biomass showed comparable levels to similar mixed deciduous

and oak forests (Zajonc 1971; Satchell 1983; Muys et al. 1992; Butt 2011).

Deadwood earthworm abundances in our study appear to be high, however this is because the few available comparable studies focus on systems which are inherently low in earthworms, for example coniferous woodlands (Hendrix 1996; Kooch 2012; Geraskina 2016). Deadwood has been found to support proportionately high numbers of juvenile Lumbricid earthworms in such forests (Geraskina 2016), and we similarly found a significantly greater proportion of juveniles within the Lumbricid earthworm communities of broadleaf deadwood, compared with surrounding soil-based habitats. The collection of large earthworm cocoons alongside adult specimens of *L. rubellus* from within deadwood, in addition to smaller cocoons typical to epigeic species, indicates that both epigeic and endogeic species (Bouché 1977) may exploit deadwood for their complete lifecycle. Given that the organo-mineral accumulations beneath the bark of deadwood were significantly moister and on average a degree warmer than open soil habitats, deadwood habitats seemingly provide favourable conditions for juveniles and cocoons. Earthworms, like most soil fauna, are highly temperature and moisture dependant, and soil-based earthworm sampling efficiency is accordingly sensitive to seasonal weather and soil conditions (Callahan Jr and Hendrix 1997; Eggleton et al. 2009). The buffering of moisture and temperature fluctuations by bark and within decaying deadwood are likely to extend woodland earthworm activity throughout the year, particularly during summer drought and winter freezing conditions (Hendrix 1996; Geraskina 2016; Zuo et al. 2016). The average pH of organo-mineral material beneath bark in decayed wood was similar to surrounding open soil (around 4.4), falling within the tolerance range of the species found (Sims and Gerard 1999), but below the lower limits of most endogeic and anecic species – explaining the low number of anecic earthworms found overall (Graefe and Beylich 2003).

Investigating earthworms in traditionally under-sampled habitats (e.g. woodland) and microhabitats (e.g. deadwood) has been shown to yield valuable information on individual species distributions and ecologies (Butt and Lowe 2004; Schmidt et al. 2015; Römbke et al. 2017). Through a novel methodological approach utilising elector traps on live trees, Römbke et al. (2017) gathered compelling evidence for assigning the epigeic species *Bimastos eiseni* as an arboreal earthworm species, associated more with trees than litter habitats. We found significantly greater abundance of *B. eiseni* in deadwood than in soil habitats, as well as individuals beneath the bark of a standing dead tree and within a bark fissure/bleed on a diseased common oak (*Q. robur*), further supporting the classification of *B. eiseni* as an

arboricolous earthworm species. Another epigeic species of interest is *Dendrobaena attemsi*, which has been increasingly located across the British Isles in recent years, following increased sampling in woodland and deadwood habitats (Butt and Lowe 2004; Eggleton et al. 2009; Schmidt et al. 2015). Our collection of *D. attemsi* showed very localised (restricted to the plot level) but abundant populations, in keeping with the species' highly stenotopic habitat requirements for acidic and organic matter rich woodland habitat (Bouché 1972). The value of systematic woodland and microhabitat surveying was further demonstrated by the collection of the UK nationally very rare (Eggleton et al. 2009; Sherlock 2018) earthworm species *Dendrobaena pygmaea* from the mineral clay nutrient rich soil beneath deadwood within a young oak plot. This expands upon current descriptions of this species' ecology, which currently limit its distribution to high organic matter habitats in broadleaf woodlands and mossy banks of streams (Sims and Gerard 1999; Sheppard et al. 2014; Sherlock 2018).

Evaluating the presented methodology

Our research demonstrates that in woodland habitats with deadwood, omitting these microhabitats from earthworm sampling can lead to underestimates of total earthworm populations and richness. However, we also found that the soil beneath deadwood supports much reduced earthworm populations compared to open soil; thus, woodland-based research proposing estimates of soil-dwelling earthworm populations which do not account for microhabitat effects could be over-estimating such populations. The importance of woodland microhabitat surveying is increasingly being recognised by earthworm researchers, however most research thus far has utilised casual sampling methods with limited systematic applicability (Butt and Lowe 2004; Kooch 2012; Schmidt et al. 2015; Römbke et al. 2017). Based on our results, the systematic earthworm surveying methodology as presented cannot replace traditional soil pit sampling alone, but should be considered as additional and complementary to provide a realistic estimate of earthworm populations in woodland systems. As well as improving quantitative earthworm data, such holistic sampling may enable the gathering of fundamental knowledge on different earthworm species life histories and ecological roles (Butt and Grigoropoulou 2010; Römbke et al. 2017), but also other faunal, fungal and microbial communities. The most conservative estimate from our species accumulation curve indicated that future surveys should include up to 8 deadwood samples (randomly selected across deadwood size and decay class) in order to reliably capture the total species richness of a forest stand – a reasonable sampling effort compared to estimates of ten or more soil pits necessary

for soil-dwelling earthworm sampling (Valckx et al. 2011). When reporting earthworm population density and biomass in deadwood, it was considered that unlike other studies which use raw density data or present density as earthworms per m^3 volume of deadwood (e.g. Kooch 2012; Geraskina 2016), it is more appropriate to provide data in per m^2 surface area. Ecologically this is sensible, since in most cases there are few earthworms located within the heartwood and sapwood – instead being closely associated with the degree of loose bark cover on the deadwood surface (Zuo et al. 2018). Presenting deadwood earthworm data as individuals per surface area has the additional benefit of making these data directly comparable to those from standard earthworm soil sampling (Butt and Grigoropoulou 2010; Valckx et al. 2011), overcoming such incompatibility issues as encountered by Römbke et al. (2017), however this does require the measurement of total deadwood volume/area within the whole plot for upscaling the results.

Through the surveys carried out, we found no effect of stand age on any of the measured earthworm or soil variables. Previous research has indicated that young broad-leaf stands are capable of supporting higher earthworm species richness, biomass and population density than old woodland, through the removal of habitat constraints and partitioning of trophic and spatial resources (Muys et al. 1992; Lavelle and Spain 2001; Decaëns et al. 2008; Palo et al. 2013). Likewise, for soil chemistry, Pitman et al. (2013) found significant differences in soil moisture and pH between oak stands of different ages within Alice Holt forest. The discrepancies between our results and those above may be due to differences in sampling methodologies and seasonality of sampling; but it is most likely that the labour-intensive nature of this pilot study necessitated the use of a smaller overall sample size than is required to identify these trends. Future applications of this more labour-intensive methodology should seek to balance available resources and sampling accuracy for addressing the research objectives (Paoletti 1999).

The methodology employed in this study was experimental in nature, and as such a number of observations can now be made to improve future applications. Firstly, since few earthworms were present in less-decayed deadwood (classes 1 or 2, see Hunter Jr 1990), future surveys should consider focusing research effort on decay class 3 or higher (the most common decay class in our study, as with other studies such as Spetich 2007). Likewise, few earthworms were found in deadwood with little bark or moss cover, nor in deadwood much smaller than 10 cm in diameter (e.g. fallen small branches), so these could also be reasonably excluded from future surveys. Zuo et al. (2016) sampled macrofauna living within deadwood bark following fragmentation into smaller

pieces, a technique which could be further explored in the field. Future deadwood-earthworm surveys might also consider collecting and incubating/DNA sequencing cocoons found in deadwood, to elucidate which species utilise the habitat within their lifecycle. Additionally, future soil earthworm sampling might consider using mustard seed extract Allyl isothiocyanate (AITC) vermifuge, an effective chemical expellant to the mustard powder used in the present study (ISO (International Organization for Standardization) 2018). Further development of this surveying strategy could include alternative microhabitats depending on the availability of these in the ecosystem under investigation (e.g. under stones and other debris), which earthworms are known to inhabit (Butt and Lowe 2004). Finally, modified microhabitat surveys could be applied in the collection of other invertebrate fauna in deadwood habitats; to enable better quantitative assessment of its total biodiversity value (Johnston and Crossley Jr 1996; Snider 1996; Shaw 2013; Zuo et al. 2016).

Woodland management implications

The ecological importance of deadwood is widely acknowledged by forest managers and researchers, with management practices and harvesting targets having been set to achieve deadwood retention within forest stands (Warren and Key 1991; Hodge and Peterken 1998). We found little change in deadwood volume between stands of different ages, with large variability between plots - as previously noted by Taylor (2013) for the same forest stands. Deadwood accumulation is widely acknowledged to be highly variable at the stand level due to peculiarities in stand development and history, making statistical trends difficult to identify (Franklin and Waring 1980; Spies et al. 1988; Woldendorp et al. 2004). Forestry practices such as coppicing and whole tree harvesting (WTH) may reduce deadwood potential from a site, and research shows that most European forests now fall below the recommended target of $50 \text{ m}^3 \cdot \text{ha}^{-1}$ deadwood volume which mimics natural levels (Forestry Commission 1997; Hodge and Peterken 1998; Bunnell and Houde 2010; Puletti et al. 2017). Deadwood-associated species are at risk as a consequence of these reductions; for example, over 800 deadwood-dependent species were previously placed on the Swedish national Red List due to habitat loss (Dudley and Vallauri 2005), and over 350 saproxylic beetle species are considered threatened by harvesting activities within the EU (Cálix et al. 2018). International Forest inventories and monitoring such as the European ICP forests BioSoil survey record deadwood volume across forest stands (Neville and Bastrup-Birk 2006; Puletti et al. 2017). However, in most cases, only measurements of volume and quality of deadwood are used as an indicator or proxy measurement for biodiversity value, rather than

in-depth ecological surveys; sacrificing detailed information regarding specific species and groups of potential importance for more convenient data recording (Barsoum et al. 2016). The use of systematic deadwood sampling methodologies may enable the collection of fine-scale data on important ecological groups such as woodland invertebrates (Schmidt et al. 2015; Römbke et al. 2017; Fuller et al. 2018). Adopting this approach may enable forest management practices to more effectively balance commodity production against the ecological and conservation importance of deadwood retention (Van Lear 1996).

Conclusions

This trial of a systematic deadwood surveying method revealed that in mixed oak woodlands, omitting deadwood microhabitats from earthworm sampling can lead to underestimates of total earthworm abundance, biomass and species richness. Deadwood was found to support proportionately higher numbers of juvenile Lumbricid earthworms, compared with surrounding soil-based habitats, and this was linked to more favourable micro-environmental conditions. However, since the surrounding soil contained significantly higher total earthworm abundance and biomass than deadwood, the microhabitat surveying method should be considered as additional and complementary to traditional sampling protocols. Adopting this additional surveying method may provide a more realistic estimate of earthworm populations in woodland systems, and thus enable forest management practices to better balance the ecological and conservation value of deadwood retention against intensive wood production.

Abbreviations

ANOVA: Analysis of variance; CWD: Coarse Woody Debris; ECN: Environmental Change Network

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Authors' contributions

FA designed and implemented the study, with assistance from SB and EV. FA analysed the results and wrote the manuscript with contributions from all co-authors. All authors read and approved the final manuscript.

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Competing interests

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