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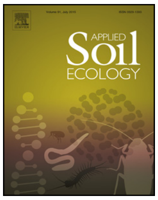

Lumbricus terrestris middens are biological and chemical hotspots in a minimum tillage arable ecosystem

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ABSTRACT

The biological (macrofauna and mesofauna), physical (size) and chemical (nutrient availability) properties of *Lumbricus terrestris* middens were studied on a minimum tillage field trial amended with farmyard manure, compost or unamended treatments. Results showed that herbivorous-type interactions with wheat crop leaves were common. Midden microhabitats containing these fresh leaves were significantly enhanced by up to 2.8-fold in mesofauna (springtails, enchytraeidae, mites, millipedes) abundance. Midden microhabitats on plots amended with farmyard manure had significantly ($p < 0.05$) more endogeic earthworms than compost amended or control plots. Further, middens found on farmyard manure amended plots were significantly ($p < 0.05$) larger, being twice the size (20.6 ± 1.7 g dry weight) of middens found on compost or control plots, demonstrating that farmyard manure improves midden microhabitat size and quality. Middens were enriched in extractable plant nutrients including P, K, S and Mn.

1. Introduction

Deep burrowing earthworms such as *Lumbricus terrestris* play an important role in arable ecosystems: for example their vertical tunnels alleviate waterlogging on soil and crops (Andriuzzi et al., 2015). Above their permanent vertical tunnel is a midden microhabitat, an accumulation of surface organic matter, mineral soil, stones and castings. Middens serve multiple functions, such as a shelter from predators, are a cache of food and modify the microclimate of the vertical tunnel. Few studies have investigated midden effects in arable ecosystems, but results from the USA have shown that middens are microbiological and chemical hotspots in no till cornfields (Bohlen et al., 1997; Subler and Kirsch, 1998). A study from Germany has shown that middens are hotspots of mesofauna activity throughout the growth season of beans, lupins and mustard crops (Schrader and Seibel, 2001). A study from no till arable fields in Finland has shown that middens are slug and earthworm hotspots (Nieminen et al., 2015).

Counting the abundance of middens is a useful tool to estimate deep burrowing earthworm populations in arable field trials (Singh et al., 2015; Stroud et al., 2016). Arable land management practices are critical to deep burrowing earthworm abundances, with midden based earthworm population estimates ranging between none (locally extinct) under conventional tillage (Kladivko et al., 1997) and up to 49 middens m^{-2} under minimum tillage (Bohlen et al., 1997). Conventional ploughing has accounted for 80–95% of wheat cultivations for the past 20-years in the UK, although minimum tillage is an increasingly adopted soil management practice (Knight et al., 2012).

A recent study (Stroud et al., 2016) showed that application of farmyard manure (FYM) amendments supported the conversion from conventional ploughing to minimum tillage regimes by enhancing the abundance of *L. terrestris* by 38% in comparison to compost amendments or control plots. In that study, detailed field descriptions identified a diversity of midden microhabitats. These included novel observations that green wheat leaves, still attached to the crop, were frequently incorporated into middens and that there was a midden size difference between the compost amended plots and the controls. This led to the hypothesis that management practices influence midden microhabitat quality and their effects in arable ecosystems. Further, that agro-chemical treatments on the crops could impact the microhabitat quality. The increasing occurrence of midden microhabitats in arable ecosystems is likely to have an effect on soil health, as middens can cover up to 25% of

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soil surfaces in minimum tillage cultivations (Subler and Kirsch, 1998). Hence, there is a need to study these microhabitats in arable ecosystems.

The aim of this study was to characterise the physical (size), biological (mesofauna and macrofauna abundance) and chemical properties of midden microhabitats in a minimum tillage wheat field trial.

2. Materials and methods

2.1. Field experiment

The field experiment was conducted at the Rothamsted Experimental Farm, Harpenden, (51.80°N, −0.36°, 128 m altitude), which has a temperate climate in the South of England. The soil was characterised as a flinty clay loam of the Batcombe soil series, with a pH 6.9 and organic matter content of 2.2%. All sampling for this study was performed in 2015, when the field experiment was in the third year of its conversion from conventional tillage (ploughing) to minimum tillage, the latter using a Lemken Karat stubble cultivator consisting of tines, discs and a crumbler roll to a depth of ca. 10 cm. The experiment is an arable rotation, with previous crops of Spring Barley (*Hordeum vulgare* cv Tipple, 2013) and Winter Oil Seed Rape (*Brassica napus* cv Quartz, 2014); the straw is baled and removed each year. At the time of sampling, the crop was Winter Wheat (*Triticum aestivum* cv. Crusoe). The experiment is a complete randomized block design with three replicate plots per treatment, each plot with an area of 9 m × 4 m. The trial is conventionally managed (agrochemicals including the split application of inorganic N, applied by hand). The treatments studied were on plots amended with 180 kg N ha^{-1} and (a) control (no organic amendment), (b) green waste compost at 3.5 t C per ha^{-1} yr^{-1} and (c) farmyard manure 3.5 t C per ha^{-1} yr^{-1}, which were applied by hand in September 2014, before tillage and wheat drilling.

2.2. Midden microhabitats and formation

Middens were identified as 5–10 cm diameter piles up to 5 cm in height of casts, flints and organic debris (twigs, leaves, straw) underlain by a ca. 5–7 mm diameter hole. Detailed observations of midden microhabitats were recorded throughout the cropping season. Midden building was filmed using an iPhone5s and headtorch between 10 pm and 2 am on warm summer (July, August) nights, confirming that wheat crop leaves are often used for midden building activities. Selected middens (n = 10) were removed and mustard solutions (10 g mustard powder per 750 ml water) were syringed into the hole to recover *L. terrestris* for speciation confirmation and biomass measurements (field weight, earthworms were rinsed with water and returned to the soil immediately after measurement).

2.3. Effect of midden composition on mesofauna community

During midden population estimates, different types of middens were marked with different coloured flags. Two types of middens occurring within a 1 m^2 quadrat in the control plots were selected for analysis. In total, six middens where wheat leaves had been incorporated into the burrow and six middens without wheat leaves were studied (n = 2 per type per plot, n = 3 control plots) to represent the two main types of midden microhabitat in June. Intact cores (4.5 cm diameter) of middens and soil immediately below (to 15 cm depth) were collected using a coring ring.

Fig. 1. Photographs showing the diversity of *Lumbricus terrestris* midden microhabitats across the minimum tillage wheat field trial dominantly composed of (a) fresh, green wheat leaves (Spring), (b) brown wheat leaves (Summer), (c) incorporated leaves into the tunnel (Summer) (d) surface debris (Summer), and (e) after harvest, straw (Autumn). (For interpretation of the references to colour in this figure legend and the text, the reader is referred to the web version of this article.)

Soil invertebrates were extracted from the cores on a Berlese-Tullgren funnel system (mesh 5 mm) (Burkard Manufacturing Co., Ltd., Rickmansworth, UK) over 10 days (Crotty et al., 2014). Extracted invertebrate specimens were stored in 70% ethanol. The arthropod groups were identified and separated into the Collembola superfamilies (*Entomobryomorpha, Poduromorpha, Neelipleona and Symphypleona*); the Acari were separated into *Oribatida, Mesostigmata* and *Astigmata*; and other invertebrates were identified at different taxonomic levels (Crotty et al., 2014).

2.4. Effect of organic amendments on earthworms associated with L. terrestris middens

After harvest, in the autumn (October 2015) when earthworms are most active, three middens were randomly selected from control plots, and plots amended with FYM or compost (n = 27). Middens and the soils directly below the midden were collected by pressing a coring ring (8 cm diameter) to 4 cm depth directly over each selected midden. A reference sample, 15 cm away from a midden was also collected using the coring ring. The samples were hand-sorted, adult earthworms identified using the (OPAL) key and total earthworm biomass determined (field weight, earthworms returned to the soil after assessment). The simple OPAL key was used as cryptic endogeic earthworm species have been recorded at this site (King et al., 2008) and analyses of cryptic diversity was beyond the scope of this investigation.

2.5. Physical and chemical properties of middens

Midden sizes (g, dry weight) were characterised by sieving (stainless steel sieve) midden samples to 4 mm to remove flints and plant debris, then oven drying at 105 °C. These were further sieved to 2 mm for chemical assessment. Soil samples (5 g) were weighed into polypropylene bottles and 50 ml 0.01$CaCl_2$ added. The bottles were shaken on a reciprocal shaker at 120 rpm for 2 h. The solutions were filtered and acidified prior to ICP-OES analysis for major and minor elements. For quality assurance, blanks, sample repeats and an internal soil standard reference (Ter Munch) were used. The analytical procedures gave satisfactory values of within 10% of the reference materials.

2.6. Statistical analyses

Statistical analysis was performed by using Genstat® (2012, 14th edition, VSN International Ltd., UK). Earthworm populations from the three middens per plot were pooled to compare between treatments. All element concentrations are reported on a dry weight basis. The Shannon Diversity index (H') and Taxon richness (S) were calculated for mesofauna data. Each variable was analysed using the general analysis of variance (ANOVA) and differences obtained at levels $p < 0.05$ were reported as significant. ANOVA assumptions of normality were checked by plotting the distributions of the residuals.

3. Results

Middens were built by *L. terrestris* adults with an average biomass of 6.65 ± 1.2 g (standard deviation, n = 14). Middens were 5–10 cm in diameter and up to 5 cm in height. In spring, middens were often composed of green wheat leaves (Fig. 1a); by summer, brown wheat leaves (Fig. 1b), still attached to the crop plant, were newly incorporated into the existing burrow (Fig. 1c, Video 1). There were also middens composed of only straw, twigs and/or tree leaves (Fig. 1d). In the autumn, straw debris after harvest dominated midden compositions (Fig. 1e). Midden building activities were recorded on video in July, when wheat leaves closest to the soil surface are brown. They show an *L. terrestris* earthworm attempting to pull wheat leaves (Video 2), and shredding wheat leaves (Video 3).

The middens with wheat straw had almost three times the total invertebrate population of those middens with straw, twigs and tree leaves (29964 ± 5179 vs 10824 ± 2336 respectively). The mites and Collembola were the most abundant groups present in both midden types. The abundance of the Entomobryomorpha and Oribatida was greatest in those middens with wheat leaves than those middens with straw, twigs and tree leaves (Table 1). Overall,

Table 1
Mesofauna abundance and diversity indices in *Lumbricus terrestris* midden microhabitats.

Microhabitat:	Midden microhabitat type 1	Midden microhabitat type 2
Details	Straw, twigs, tree leaves Mean ind m^{-2}	With wheat leaves Mean ind m^{-2}
Entomobryomorpha	3729 ± 734.5	10395 ± 2239.4
Poduromorpha	33 ± 33.0	198 ± 161.7
Symphypleona	33 ± 33.0	330 ± 97.9
Neelipleona	0	33 ± 33.0
Oribatida	2244 ± 997.5	11946 ± 3069.8
Mesostigmata	1023 ± 200.7	2574 ± 701.0
Astigmatina	3003 ± 832.9	1287 ± 654.2
Haplotaxida	165 ± 94.5	1914 ± 394.9
Diptera	165 ± 94.5	297 ± 151.2
Julida	132 ± 41.7	726 ± 208.7
Lithobiomorpha	66 ± 41.7	0
Diptera Larvae	66 ± 66.0	33 ± 33.0
Hemiptera	33 ± 33.0	0
Staphylinidae	33 ± 33.0	0
Tipulidae	33 ± 33.0	0
Megadrilacea	0	66 ± 41.7
Coccinellidae	0	33 ± 33.0
Carabidae Larvae	0	33 ± 33.0
Araneae	0	33 ± 33.0
Hemiptera	0	33 ± 33.0
Geophilomorpha	0	33 ± 33.0
Lepidoptera	66 ± 66.0	0
S (Taxon richness)	7.0 ± 0.5	8.7 ± 0.3
H' (Shannon diversity)	1.5 ± 0.1	1.5 ± 0.1

the invertebrate faunal taxon richness (S) was significantly enhanced ($p < 0.05$), but the diversity (H') was not. Middens and the soil directly beneath them were significantly ($p < 0.05$) enhanced in other earthworm species in comparison to the control soils (Fig. 2). Adult *Allolobophora chlorotica* and juvenile earthworms were associated within the middens. Middens found on FYM plots had significantly ($p < 0.05$) higher earthworm numbers and biomass associated (per 150 cm^3) with them in comparison to middens found on the other plots.

Farmyard manure amended plots were associated with significantly ($p < 0.05$) larger middens, twice the size of those on compost or unamended plots (Fig. 3). No differences in extractable nutrients were found between middens collected from soils amended with FYM, compost or no amendments (Table 2). However, middens were associated with at least 2 times higher extractable P, K and S concentrations in comparison to reference soils.

4. Discussion

The results show that the deep burrowing earthworm, *L. terrestris* regularly interacts with above-ground crop leaves under these standard arable management practices (Fig. 1a–e, Videos 1–3). Herbivorous-type behaviours have been recorded on plant seedlings in a gravel driveway and in a laboratory study, but are thought to be rare (Griffith et al., 2013; Kirchberger et al., 2015). Our direct observations in the field trial contradict this interpretation, both green and senescent crop leaves being shredded, consumed and incorporated into middens by adult *L. terrestris* earthworms, demonstrating that this behaviour is common in this wheat cultivation. From an arable management perspective, this discovery suggests a need to increase plant diversity (e.g. companion cropping (Schmidt et al., 2003)) to better support this behaviour by *L. terrestris*. This would reduce the trade-off between herbivorous-type interactions with the crop and their beneficial tunnelling activities to soil water management that enhance crop production as shown by Andriuzzi et al. (2015).

Midden microhabitats are known mesofauna hotspots in comparison to reference soils throughout the cropping season for a range of crop types (Schrader and Seibel, 2001). Our results identified a diversity of midden microhabitats within an arable field trial, with many middens associated with wheat leaves (Fig. 1a–c). It was hypothesised that, as crop plants are treated with a range of conventional agro-chemical regime (herbicides, fungicides etc.), the presence of leaves from treated plants may negatively impact midden microhabitat quality. However, midden microhabitats containing wheat leaves had significantly higher species richness and abundance of mesofauna (Table 1) than midden microhabitats without wheat leaves. A crucial difference in midden composition was that wheat leaves were found up to 10 cm depth in the burrow in various stages of decomposition (Figure d), indicating that it is the amount and type of organic matter incorporated into the soil that is supporting greater mesofauna

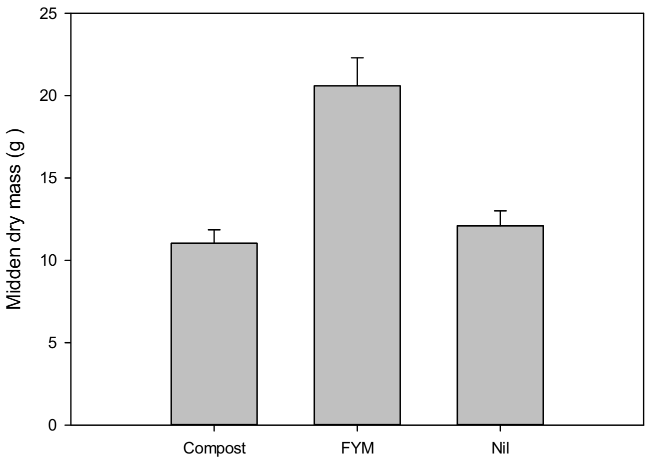

Fig. 3. Size comparison of *Lumbricus terrestris* middens in relation to farmyard manure or compost (3.5 t C ha^{-1} yr^{-1}) amendments, in comparison to the control (no amendment). The error bars are standard errors.

Table 2
The $CaCl_2$ extractable pool of plant nutrients from *Lumbricus terrestris* middens in comparison to reference soils presented as mean ± s.e. Values in a column followed by a different superscript letter are significantly different ($P < 0.05$).

Treatment		Extractable (mg kg^{-1})			
		P	K	S	Mn
FYM	Midden	4.8 ± 0.5^a	26.1 ± 0.2^a	$21 \pm 0.6\ ^a$	0.7 ± 0^a
	Reference soil	1.2 ± 0.2^b	16.3 ± 4^b	3.4 ± 0.4^b	0.1 ± 0.1^b
Compost	Midden	4.9 ± 0^a	23.8 ± 1.1^a	23.4 ± 1^a	0.4 ± 0^a
	Reference soil	1.1 ± 0.2^b	16.1 ± 5.4^b	3.7 ± 2.4^b	0.1 ± 0^b
Nil	Midden	3.9 ± 0.2^a	23.9 ± 0.4^a	28.7 ± 3.3^a	0.8 ± 0^a
	Reference soil	1.0 ± 0.2^b	10.4 ± 1.2^b	3.1 ± 0.1^b	0.1 ± 0.1^b

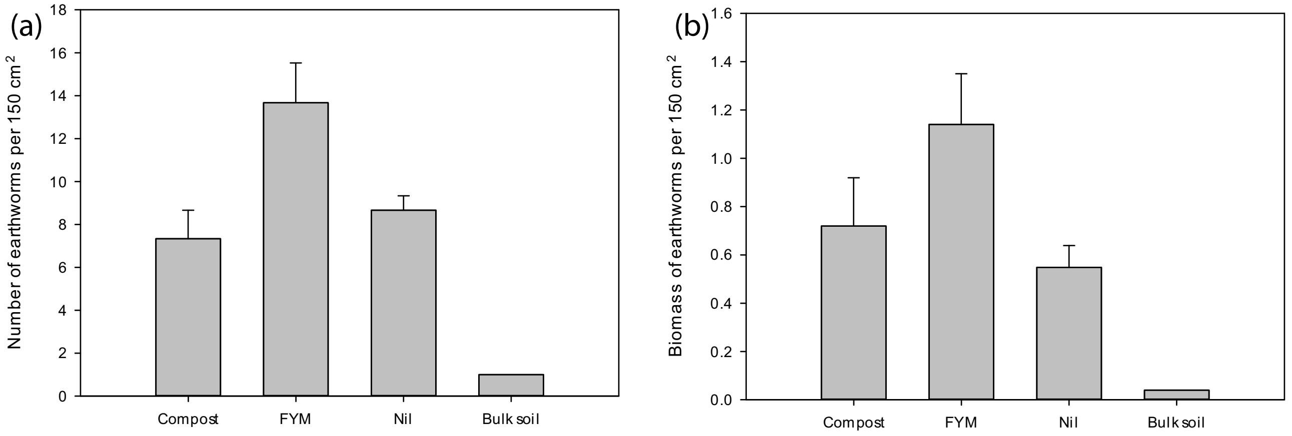

Fig. 2. Earthworm community associated within the three pooled *Lumbricus terrestris* middens (150 cm^2 per plot) in comparison to the three pooled reference soils, located 15 cm from a midden (150 cm^2 per plot) (a) earthworm numbers and (b) earthworm biomass in relation to farmyard manure or compost (3.5 t C ha^{-1} yr^{-1}) amendments, in comparison to the control (no amendment). The error bars are standard errors.

species richness and abundance. It is not clear why one type of midden appears to favour an enhanced faunal community, although it may be that the wheat leaf residues are less recalcitrant and therefore are more readily available to the decomposer community than the straw, twigs and tree leaves which are either more lignified or may contain secondary compounds. The greater numbers of soil fauna found in association with middens containing wheat leaf residues may lead to an enhancement of the multiple ecosystem functions, including decomposition, nutrient retention and cycling with knock-on effects on crop yields and health.

This is the first study to show that midden microhabitats, made by deep burrowing anecic earthworms, are also endogeic earthworm hotspots in comparison to reference soils in min till arable ecosystems (Fig. 2). This is in agreement with research in no till arable ecosystems that also have elevated endogeic earthworm populations (Nieminen et al., 2015) and natural systems (Butt and Lowe, 2007). Further, earthworms were twice as abundant in middens (and soil directly beneath the middens) formed on FYM-amended plots per 150 cm^3, in comparison to compost amended or control plots (Fig. 2) showing that the management practice of amending soils with FYM enhances the midden hotspot effect for macrofauna. As worms feed from their middens, it is likely that middens provide a superior food source than the bulk (reference) soil, supporting a multi-species microhabitat. Further, FYM is a more favourable amendment than compost (as both applied at the same rate, 3.5 $t\,C\,ha^{-1}$) for earthworm activity, given the size of middens (Fig. 3). This coupled to previously reported findings that *L. terrestris* middens are significantly more abundant on FYM plots (Stroud et al., 2016), indicates that FYM is useful for enhancing *L. terrestris* activities in arable ecosystems under minimum tillage conversions. General improvements in earthworm populations are known to occur under FYM amendments (Leroy et al., 2008). In terms of chemical properties, it is known that middens are C and N hotspots (Bohlen et al., 1997; Subler and Kirsch, 1998; Wilcox et al., 2002). The wider nutrient composition of middens, specifically the extractable pool of essential plant nutrients and transfer to crops, was unknown. These results show that the chemical composition of middens significantly differed from the reference soil, with enhancements in plant nutrients such as P, K, and S (Table 2). However, middens can cover up to 25% of the soil surface in some fields (Subler and Kirsch, 1998), which might have an influence on crop plant chemistry and should be investigated further.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2016.03.019.

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