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Short communication

# Cover cropping with oilseed radish (*Raphanus sativus*) alone does not enhance deep burrowing earthworm (*Lumbricus terrestris*) midden counts

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ABSTRACT

Deep burrowing earthworms are important ecosystem service providers but their populations are reduced by arable cultivations. We need to both better understand the impact of changes in crop management on earthworms and implement practices to enhance their in-field populations. Two current trends in arable cropping are the increased use of non-inversion tillage and over winter cover crops. *Lumbricus terrestris* abundances were estimated using midden counting on two field trials comparing tillage and cover cropping management practices. The long running (8-year) field trial showed that shallow non-inversion tillage had significantly ($p < 0.01$) greater *L. terrestris* abundances at 4.3 middens per $m^2$, in comparison to deep (ca. 20 cm) non-inversion tillage (3 middens per $m^2$) and conventional ploughing (ca. 25 cm) (1.9 middens per $m^2$), indicating that it is inversion rather than the depth of soil disturbance that is detrimental to their abundance. All trials showed that cover cropping with oilseed radish had no significant ($p > 0.05$) effect on *L. terrestris* midden counts, either during winter cover cropping or within the arable rotation. Field observations identified that 93% of middens were associated with crop leaves (still attached to the stem) incorporated into the burrow which may be a novel route of agrochemical exposure and needs further investigation.



## 1. Introduction

Deep burrowing earthworms such as *Lumbricus terrestris* are beneficial to agricultural soils, for example, their burrowing activity delivers an important ecosystem service improving water infiltration and crop growth (Andriuzzi et al., 2015). These anecic worms are highly sensitive to soil disturbance, with inversion tillage (ploughing) significantly reducing their populations (Chan, 2001; van Capelle et al., 2012) and local extinctions occurring on intensively cultivated soils (Kladivko et al., 1997). Ploughing has dominated UK tillage regimes for the past 30 years, and still account for 60% of wheat cultivations (Knight et al., 2012). The common anecic earthworm *L. terrestris* have slow reproduction rates (Lowe and Butt, 2014) and are slow colonisers of agricultural fields from margins (Nuutinen et al., 2011). Whilst field margins have high *L. terrestris* densities, it is thought that these do not act as a source of earthworms for field recolonization and that their recovery relies on the residual, surviving in-field worm populations (Roarty and Schmidt, 2013). There is a need to find management practices that enhance their in-field populations. Two current trends in UK arable cropping are the increased use of non-inversion tillage to improve soil structure and reduce operational costs and the use of over winter cover crops; this has increased in parallel with more spring cropping, often to improve soil management. While these changes in management would be expected to be beneficial to earthworm populations, empirical data are lacking.

Reduced intensity tillage (depth and/or inversion) is associated with higher *L. terrestris* abundances (Joschko et al., 2009). To the authors knowledge there have been no studies at species level to determine the effects of non-inversion tillage depth (shallow or deep) on *L. terrestris* earthworm populations. Potentially deep (ca. 20 cm depth) non-inversion tillage may cause as much disturbance to *L. terrestris* as moldboard ploughing (ca. 25 cm depth), and needs investigation to best inform on tillage practices.

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The second most important factor to anecic earthworm abundance after tillage is organic matter (Simonsen et al., 2010). A potential source of organic matter is cover cropping, with *L. terrestris* known to prefer relatively fresh litter (Piearce, 1978). In terms of cover crops, *L. terrestris* has a feeding and habitat preference in the order of grasses < brassicas < oats cover crops (Valckx et al., 2011). None the less, cover crops such as oilseed radish, exude glucosinolates, allelochemicals that supress crop pests and soil-borne diseases and may cause avoidance behaviours by earthworms. Further, cover cropping is often destroyed using agrochemicals such as glyphosate, which is known to reduce *L. terrestris* reproduction rates (Gaupp-Berghausen et al., 2015). A better knowledge of cover cropping effects on deep burrowing earthworm populations from field studies is needed to inform the choice of cover crop species and method of destruction and incorporation of residues.

A sensitive technique to determine active anecic earthworm populations in field studies is midden counting. Middens are the surface accumulations of castings, leaves and other organic materials embedded into the burrow and surrounding area (Brown et al., 2000). The low numbers of middens in tilled organic farming systems precludes sampling (Moos et al., 2016), however, surveying 20% of the plot area on conventionally farmed fields was sufficient to determine treatment effects (Stroud et al., 2016a). Middens are formed by sub-adults and adult *L. terrestris*, and are surface features that are counted to provide a reliable estimate of their populations (Rossi and Nuutinen, 2004; Simonsen et al., 2010; Singh et al., 2015; Stroud et al., 2016a).

We hypothesised that a radish cover cropping would be associated with higher abundances of *L. terrestris* middens in comparison to non-cover cropping management practices, and that the differences would be greater as tillage intensity is reduced because it provides more surface residues and less soil disturbance. Further, that long-term arable management practices that include cover cropping would significantly enhance *L. terrestris* midden counts in-field during the arable rotation series.

## 2. Materials and methods

### 2.1. Field experiments

The long-term field experiments conducted at NIAB as part of the New Farming Systems (NFS) programme, Morley, Norfolk, UK, which has a sandy loam soil (Ashley series) with sand 69%, silt 18%, clay 13% and organic matter content 2%. The experiments are arable rotation trials that have been running since 2008 and have been described elsewhere (Stobart and Morris, 2014). The cultivations trial is a factorial randomised block experiment, with the earthworm survey sub plot unit size 12 m × 12 m. The trial compares conventional moldboard ploughing (ca. 25 cm), deep non-inversion (20 cm using a combination of surface cultivation and deeper legs), and shallow non-inversion (just working the soil surface to no greater than 10 cm) and is managed with or without oilseed radish cover cropping at a seed rate of 10 kg $ha^{-1}$. Wider research examining soil physical conditions and other production characteristics are being assessed under contrasting tillage regimes (Stobart et al., 2014) has identified some changes in soil structure and stability; however, yield differences due to tillage regime in winter wheat were generally small. The fertility building trial is a randomized block design experiment, with earthworm survey sub plot size of 12 m × 12 m. The trial is managed using shallow non-inversion tillage and the treatments studied were those managed at the 100% N-fertilisation dose (RB209) with or without oilseed radish cover cropping.

A conventionally managed cover cropping field trial was conducted at the Rothamsted Experimental Farm, Harpenden, (51.80°N, −0.36°W, 128 m altitude), which has a flinty silty clay loam soil (Batcombe series) with sand 28%, silt 52% and clay 20%, and organic matter content of 2%. The experiment was started in Autumn 2015, and is a complete randomized block design with four replicate plots per treatment, each plot with an area of 9 m × 6 $m^2$. The plot treatments studied included (a) control (ploughed), (b) shallow non-inversion tillage, (c) shallow non-inversion tillage and cover cropping, (d) direct drilling, and (e) direct drilling and cover cropping following a winter wheat crop. In terms of tillage, a plough was used to ca. 25 cm depth for conventional stubble management, shallow non-inversion tillage was performed using a Lemken Karat stubble cultivator consisting of tines, discs and a crumbler roll to a depth of ca. 10 cm. Direct drilling was performed using a Moore uni-drill. The winter cover crop, oilseed radish (150 seeds $m^2$) was established in September 2015.

### 2.2. L. Terrestris *population estimates using midden counting*

Field descriptions of midden characteristics were recorded (size, organic debris). Midden counting and verification was performed as previously described (Stroud et al., 2016a). Briefly, a 1 $m^2$ quadrat was used to survey 6 $m^2$ per plot; 20 plots were surveyed at Rothamsted (120 $m^2$) and 24 plots were surveyed at the cultivations trial at Morley (144 $m^2$) and 8 plots were surveyed on the fertility building trial at Morley (48 $m^2$). Following Singh et al. (2015), these counts were verified by using a vermifuge (mustard solution, 10 g Colmans® mustard powder added to 1 litre of water) on 10 × 0.25 $m^2$ areas representing midden densities across the field. Earthworms were collected, rinsed, identified and biomass (field weight, returned to the field after measurement) was recorded. Spearman's rank correlation co-efficient was used to assess the correlation to midden counts (n = 15, correlation = 0.6, $p < 0.01$).

#### 2.2.1. Midden counting within the arable rotation series with and without cover cropping

Midden counting was performed on both Morley field trials in Spring (May) 2015, when earthworms are most active and when the trials were under winter wheat (cv. Relay). This was to study the effects of long term rotation management that includes cover cropping.

#### 2.2.2. Middens associated with and without the cover crop

Midden counting was performed on the Morley cultivations field trial and Rothamsted cover cropping trial in Winter (January) 2016, when the trial was sown with an oilseed radish to determine *L. terrestris* midden counts associated with this winter cover crop.

### 2.3. Statistical analyses

The midden abundance data were tested for normality (and if appropriate, log transformed) then analysis of variance was carried out to assess the statistical significance of midden abundance between the tillage and cover cropping treatments (Genstat, 2012, 14th addition, VSN International Ltd., UK).

## 3. Results

### 3.1. Midden description

Middens found on both these arable field trials were identified as a pile (minimum 2 cm high and 5 cm in diameter), composed of casts, small (<3 cm) stones and straw and/or wheat leaves underlain by a burrow (diameter ca. >5 mm) lined (up to 6 cm depth) by leaves. The species recovered from the midden

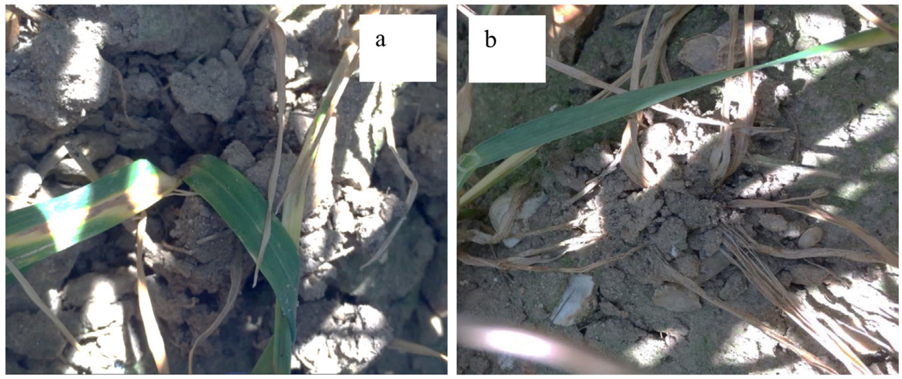


**Fig. 1.** Photographs showing (a) fresh green wheat leaves and (b) dessicated, brown wheat leaves embedded into *L. terrestris* middens. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

validations were adult and sub-adult *L. terrestris* with an average mass of $4.96 \pm 1.1$ g. In Spring, 93% middens (n = 206) were associated with wheat leaves, still attached to the plant and within the burrow entrance of the midden. These leaves were obtained from plants within 15 cm of the midden, and often several plants (either side of the row) were used, with between 1 and 14 leaves found in each midden. Green leaves were also found in 4% of middens and the green leaves were still attached to the plant, identifying active foraging of living plant tissues (Fig. 1a and b).

### 3.2. Midden counts within the arable rotation series managed with and without cover cropping

There was a significantly ($p < 0.01$) different abundance in *L. terrestris* middens between the different tillage regimes in Spring (Fig. 2a). Ploughing had the least number of middens with a mean of 1.9 middens per m$^2$. Middens were 38% more abundant on the deep non-inversion tillage and 126% more abundant on the shallow non-inversion tillage plots in comparison to ploughing. The greatest number of middens (18 per m$^2$) was recorded on one shallow non-inversion tillage plot, although the mean was 4.3 middens per m$^2$ for this regime. There were fewer middens in the order of 0.5 per m$^2$ on deep and 0.6 per m$^2$ on shallow non-inversion tillage regimes associated with cover cropping management practices although this was not statistically significant ($p = 0.07$). There was also no significant difference ($p > 0.05$) in midden abundance on the fertility building trial, with the cover cropping regime associated with 6.4 middens per m$^2$ in comparison to the non cover cropping regime associated with 5.7 middens per m$^2$ (Fig. 2b).

### 3.3. Midden counts associated with and without the cover crop

There was a significantly ($p < 0.01$) different abundance in *L. terrestris* middens between the different tillage regimes in Winter on both field trials (Fig. 3a and b). Ploughing had the least number of middens, with zero found on the Rothamsted trial and 1–1.4 middens per m$^2$ found on the Morley trial. Cover cropping had no significant ($p > 0.05$) difference on midden abundance for either trial.

## 4. Discussion

Tillage regimes had a significant effect on *L. terrestris* midden counts, with ploughing on both the Rothamsted and Morley field trials associated with the lowest midden abundances (Figs. 2 and 3). Ploughing is known to significantly reduce deep burrowing earthworm populations (Chan, 2001; van Capelle et al., 2012). Investigating midden numbers in different long term rotations identified that the numbers of *L. terrestris* middens found on the

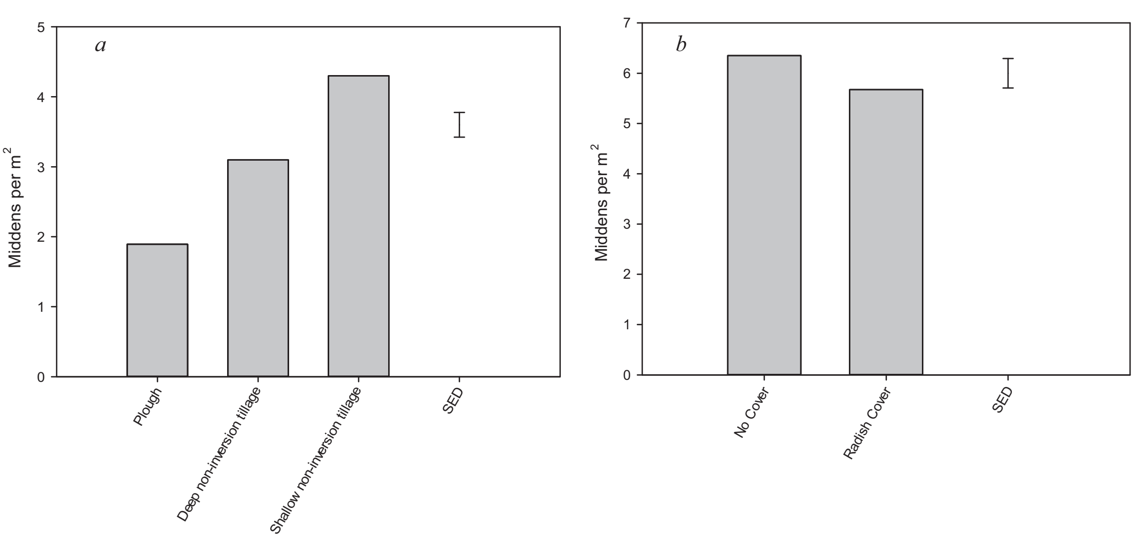


**Fig. 2.** Mean number of *L. terrestris* middens per m$^2$ from Spring sampling (whilst trials were under winter wheat) showing (a) from the cultivations trial, effects of tillage and (b) from the fertility building trial (managed using shallow non-inversion tillage), effects of prior cover cropping. The standard errors of the differences of the means are presented as the SED. Tillage effects were significantly different ($p < 0.01$), cover cropping was not significantly different ($p > 0.05$).

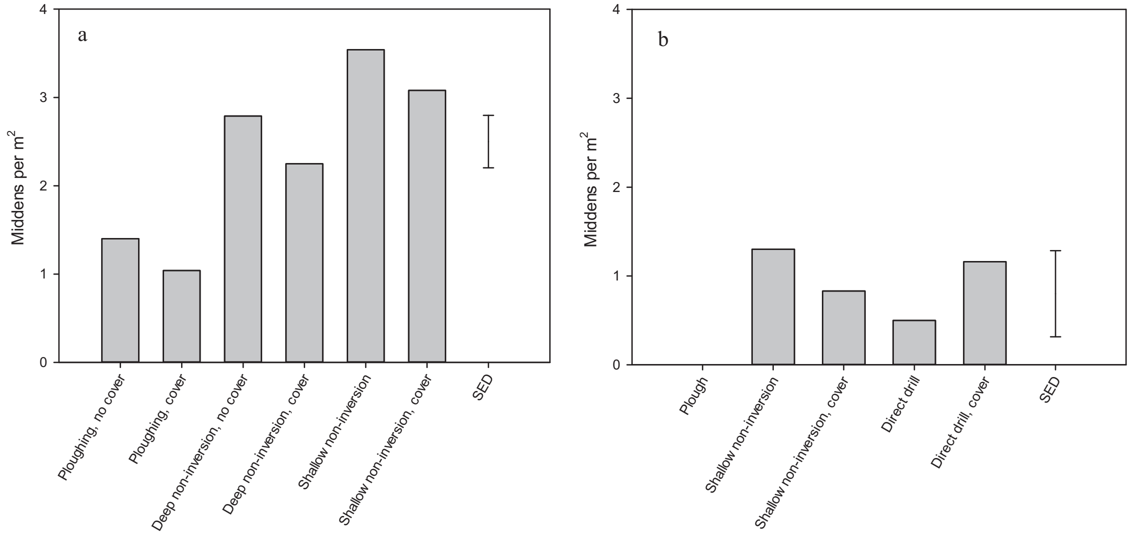


**Fig. 3.** Mean number of *L. terrestris* middens per $m^2$ in Winter on plots planted with the oilseed radish cover crop at (a) long-term cultivations trial at Morley and (b) recently established cover cropping trial at Rothamsted. The standard errors of the differences of the means are presented as the SED. Tillage effects were significantly different ($p < 0.01$), cover cropping was not significantly different ($p > 0.05$).

conventional ploughing plots (Fig. 2) are consistent with other studies that have found 1–3 middens per $m^2$ for conventional tillage regimes (Simonsen et al., 2010). Deep non-inversion tillage (ca. 20 cm depth) was associated with 60% higher *L. terrestris* midden abundances than conventional ploughing (ca. 25 cm depth). This shows that it is inversion which is highly detrimental, rather than depth per se. None the less, shallow non-inversion tillage (ca. 10 cm depth) had significantly higher *L. terrestris* midden abundances than deep non-inversion tillage regimes. The maximum number of middens detected under the shallow tillage regime was 18 middens per $m^2$ in one single plot, suggesting that this agro-ecosystem has a significant capacity for supporting much higher *L. terrestris* abundances. In agreement with another study of middens in arable ecosystems (Stroud et al., 2016b), middens were predominantly composed by crop leaves (still attached to the plant) (Figs. 1), which may present a novel exposure route to agrochemicals and needs further investigation. Midden abundances have been reported to be as high as 48.8 per $m^2$ (Bohlen et al., 1997) and can cover up to 25% of the soil surface (Subler and Kirsch, 1998), suggesting that midden abundances were low on these UK field trials.

The main hypothesis was that cover cropping with radish enhances *L. terrestris* midden counts, and this would have a long lasting effect (during the rotation series) and greater differences would be detected under reduced tillage regimes. However, we found no evidence to support these hypotheses. Cover cropping regimes with oilseed radish did not enhance the abundance of *L. terrestris middens*, either within a long-term rotation that includes cover cropping (Fig. 2) or at the time of cover cropping (Fig. 3). This could be due to a multitude of factors including the long lifecycle of *L. terrestris* in comparison to the short winter cover cropping period, feeding preference of this crop species, allelochemicals from the oilseed radish or agro-chemical management of cover cropping that might mitigate any benefits from increased organic matter supply. Further research is needed to investigate cover crop *L. terrestris* responses and their associated management practices in order to best support infield deep burrowing earthworm populations. In particular, *L. terrestris* was interacting directly with the above ground living vegetation which could be a novel route of agrochemical exposure which needs further investigation.

## 5. Conclusion

*L. terrestris* midden counts are highly sensitive to tillage regimes, with inversion tillage (ca. 25 cm) associated with the lowest numbers of middens, and shallow non-inversion tillage (ca. 10 cm) associated with approximately double the number of middens. Cover cropping with oilseed radish alone does not enhance the abundance of *L. terrestris* middens during, or in the subsequent arable rotation, regardless of tillage intensity.

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## References

Andriuzzi, W.S., Pulleman, M.M., Schmidt, O., Faber, J.H., Brussaard, L., 2015. Anecic earthworms (*Lumbricus terrestris*) alleviate negative effects of extreme rainfall events on soil and plants in field mesocosms. Plant Soil 397, 103–113.
Bohlen, P.J., Parmelee, R.W., McCartney, D.A., Edwards, C.A., 1997. Earthworm effects on carbon and nitrogen dynamics of surface litter in corn agroecosystems. Ecol. Appl. 7, 1341–1349.
Brown, G.G., Barois, I., Lavelle, P., 2000. Regulation of soil organic matter dynamics and microbial activityin the drilosphere and the role of interactionswith other edaphic functional domains. Eur. J. Soil Biol. 36, 177–198.
Chan, K.Y., 2001. An overview of some tillage impacts on earthworm population abundance and diversity—implications for functioning in soils. Soil Tillage Res. 57, 179–191.
Gaupp-Berghausen, M., Hofer, M., Rewald, B., Zaller, J.G., 2015. Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations. Sci. Rep. 5, 12886.
Joschko, M., Gebbers, R., Barkusky, D., Rogasik, J., Höhn, W., Hierold, W., Fox, C.A., Timmer, J., 2009. Location-dependency of earthworm response to reduced tillage on sandy soil. Soil Tillage Res. 102, 55–66.
Kladivko, E.J., Akhouri, N.M., Weesies, G., 1997. Earthworm populations and species distributions under no-till and conventional tillage in Indiana and Illinois. Soil Biol. Biochem. 29, 613–615.
Knight, S., Knightley, S., Bingham, I., Hoad, S., Lang, B., Philpott, H., Stobart, R., Thomas, J., Barnes, A., Ball, B., 2012. Desk study to evaluate contributory causes

of the current yield plateau in wheat and oilseed rape. HGCA Report No 502, Home Grown Cereals Authority, Stoneleigh, Warwickshire.
Lowe, C.N., Butt, K.R., 2014. Cocoon viability and evidence for delayed hatching by the earthworm *Lumbricus terrestris* in a laboratory based study. Zeszyty Naukowe 17, 61–67.
Moos, J.H., Schrader, S., Paulsen, H.M., Rahmann, G., 2016. Occasional reduced tillage in organic farming can promote earthworm performance and resource efficiency. Appl. Soil Ecol. 103, 22–30.
Nuutinen, V., Butt, K.R., Jauhiainen, L., 2011. Field margins and management affect settlement and spread of an introduced dew-worm (*Lumbricus terrestris* L.) population. Pedobiologia 54 (Supplement), S167–S172.
Piearce, T.G., 1978. Gut contents of some lumbricid earthworms. Pedobiologia 18, 153–157.
Roarty, S., Schmidt, O., 2013. Permanent and new arable field margins support large earthworm communities but do not increase in-field populations. Agric. Ecosyst. Environ. 170, 45–55.
Rossi, J.P., Nuutinen, V., 2004. The effect of sampling unit size on the perception of the spatial pattern of earthworm (*Lumbricus terrestris* L.) middens. Appl. Soil Ecol. 27, 189–196.
Simonsen, J., Posner, J., Rosemeyer, M., Baldock, J., 2010. Endogeic and anecic earthworm abundance in six Midwestern cropping systems. Appl. Soil Ecol. 44, 147–155.
Singh, P., Heikkinen, J., Ketoja, E., Nuutinen, V., Palojärvi, A., Sheehy, J., Esala, M., Mitra, S., Alakukku, L., Regina, K., 2015. Tillage and crop residue management methods had minor effects on the stock and stabilization of topsoil carbon in a 30-year field experiment. Sci. Total Environ. 518–519, 337–344.
Stobart, R.M., Morris, N.L., 2014. The impact of cover crops on yield and soils in the New Farming Systems programme. Aspects Appl. Biol. 127, 223–231.
Stobart, R., Hallet, P., George, T., Morris, N., Newton, A., Valentine, T., Mckenzie, B., 2014. Platforms to test and demonstrate sustainable soil management: integration of major UK field experiments. Aspects Appl. Biol. 127, 233–240.
Stroud, J.L., Irons, D., Watts, C.W., Whitmore, A.P., 2016a. *Lumbricus terrestris* abundance is not enhanced after three years of compost amendments on a reduced tillage wheat cultivation conversion. Appl. Soil Ecol. 98, 282–284.
Stroud, J.L., Irons, D.E., Carter, J.E., Watts, C.W., Murray, P.J., Norris, S.L., Whitmore, A. P., 2016b. *Lumbricus terrestris* middens are biological and chemical hotspots in a minimum tillage arable ecosystem. Appl. Soil Ecol. 105, 31–35.
Subler, S., Kirsch, A.S., 1998. Spring dynamics of soil carbon, nitrogen, and microbial activity in earthworm middens in a no-till cornfield. Biol. Fertil. Soils 26, 243–249.
Valckx, J., Pina, A.C., Govers, G., Hermy, M., Muys, B., 2011. Food and habitat preferences of the earthworm *Lumbricus terrestris* L. for cover crops. Pedobiologia 54 (Supplement), S139–S144.
van Capelle, C., Schrader, S., Brunotte, J., 2012. Tillage-induced changes in the functional diversity of soil biota—a review with a focus on German data. Eur. J. Soil Biol. 50, 165–181.