Earthworm *Lumbricus terrestris* mediated redistribution of C and N into large macroaggregate-occluded soil fractions in fine-textured no-till soils

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Abstract

By processing large quantities of crop residues, earthworms enhance the mineralization of organic matter but have also been shown to stabilize soil organic carbon (SOC) into soil fractions like microaggregates (53–250 µm) within macroaggregates (>250 µm) especially in no-till soils. Our objective was to find direct evidence on the impact of an anecic, soil surface-feeding earthworm, *Lumbricus terrestris* L., on the redistribution of SOC and soil nitrogen (N) into macroaggregate-occluded soil fractions of boreal soils. We sampled soil (0-5 cm depth) from the middens of *L. terrestris* (mounds of collected residue and surface casts at the openings of its permanent burrows) and the adjacent non-midden (bulk) soil at three no-till sites in southern Finland: two clayey sites (sites 1–2) and one coarse textured site (site 3). Compared to bulk soil, the soil in *L. terrestris* middens featured general increase in aggregate size and content of SOC and N within the large macroaggregates (>2000 µm) at the clayey sites. The microaggregates within the large macroaggregates had accumulated more SOC and N in the midden soil especially at site 1 where 99% of the difference in total SOC between midden and bulk soil was associated with this type of SOC stabilization. At site 2, the increase in SOC found in the large macroaggregates was counteracted by a decrease in SOC in microaggregates within the small macroaggregates (250–2000 µm). No differences in SOC stored in soil fractions were found between midden and non-midden soil at the coarse soil site 3 with higher top soil decomposition rate compared to sites 1 and 2. Across the study sites, the total amount of SOC was 6% higher in midden soil compared to the bulk soil. These results suggest *L. terrestris* mediates the storage of SOC and N into better protected soil fractions in clay soils under boreal conditions.

Keywords: Earthworms; carbon sequestration; nitrogen cycle; soil aggregation; no-till

1. Introduction

Earthworms play a key role in soil organic matter dynamics and the regulation of nutrient cycling (Blouin et al., 2013; Filser et al., 2016). They interact with and impact a multitude of soil processes including soil aggregation, decomposition of residues and formation of macropores, which makes earthworms, and the ecosystem services they offer, of interest when developing sustainable agroecosystems (Jones et al., 1994; Lavelle et al., 1997; Fonte et al., 2009; Giannopoulos et al., 2010; Xiang et al., 2018). Earthworms are often the predominant group of soil animals in terms of biomass (Coleman and Crossley, 2004) and they can consume up to 2 t of litter ha^{-1} yr^{-1} (Whalen and Parmelee, 2000). Earthworm feeding enhances litter decomposition directly through their metabolism (Curry and Schmidt, 2007) and indirectly by fragmenting the coarse organic matter and increasing its surface area (Blouin et al., 2013). Earthworms egest a mixture of metabolized organic material and mineral soil as sub-surface and surface casts. According to a recent meta-analysis, total organic C, total N and total P are 40-48% higher in casts while mineral N and available P are increased by 241% and 84%, respectively (van Groenigen et al. 2019). For the majority of soil fertility relevant properties, the relative difference between casts and the bulk soil ("relative cast fertility") indicates high fertility of casts.

Earthworm species can be categorized in three ecological groups based on their feeding habits and the soil environment they occupy (Bouché, 1977; Lavelle and Spain, 2001). Anecic species, like *L. terrestris*, make permanent, typically close to 1 m deep, vertical burrows (e.g. Nuutinen and Butt, 2003; Don et al., 2008), which open at the soil surface. These species feed on the surface litter which they pull down into their burrows and create litter and cast-made middens within sight on the soil surface (Subler and Kirsch, 1998; Nieminen et al., 2015). Epigeic species live near the soil surface feeding on surface litter while endogeic species mainly reside in the top soil making burrows with varying orientation while feeding on below ground SOM.

Soil macroaggregates (>250 µm) and microaggregates (53–250 µm) protect soil organic carbon (SOC) within them from mineralization. These soil physical fractions increase the residence time of SOC by both offering physical protection from microbial decomposition and by creating conditions of low oxygen content that significantly slow down the decay of organic matter (Six et al., 2002). The turnover rate of SOC is affected by its distribution among the different aggregate fractions (Six et al., 2000; Bossuyt et al., 2002). Microaggregates are more strongly bound together than macroaggregates and thus offer a more stable long-term storage for SOC (Angers et al., 1997; Six et al., 2002). However, macroaggregates play a key role in providing sites for microaggregate formation (Six et al., 2000).

The soils affected by the casting of anecic earthworms are known to have an increased proportion of larger soil aggregates (Arai et al., 2018; Frazão et al., 2019), however, sometimes at the expense of smaller soil aggregates (Alegre et al., 1996; Lavelle et al., 2004). It has also been suggested that the formation rate of microaggregates within macroaggregates is enhanced by passage through the earthworm gut (Bossuyt et al., 2005; Pulleman et al., 2005). This is enabled when processed organic residues, that are often high in SOC due to food selection, and soil particles mix together within the earthworm gut creating new microaggregates that are excreted in casts (Barois et al., 1993). Linings of the earthworm burrows (Don et al., 2008; Leue et al., 2018) and earthworm-affected soil aggregates (Wu et al., 2018) have been found to have higher SOC content compared to bulk soil. However, while earthworms increase soil aggregation and SOC sequestration in the long term, they enhance litter decomposition and losses of SOC as carbon dioxide in the short term; this time dependence in effect has been discussed as the "earthworm dilemma" by Lubbers et al. (2013; 2017).

Effects of earthworms are especially interesting in long-term no-till management which has been found to increase both earthworm numbers and aggregate size (Bai et al., 2018). Enhanced top soil residue availability and low physical disturbance in no-till can increase especially the number of soil surface feeding earthworms, such as *Lumbricus terrestris* L. (Briones and Smith, 2017) which mix the residues into the soil and thus alter the soil structure and nutrient availability (Thevathasan and Gordon, 2004; Whalen and Fox, 2006; Bai et al., 2018).

Direct evidence on macroaggregate formation and SOC stabilization in the presence of earthworms has been gained especially in laboratory incubations (Wu et al., 2017) or field studies in temperate and tropical environments (Blanchart et al., 1999; Fonte and Six, 2010; Arai et al., 2018). Boreal conditions with soil frosting, low carbon input in crop residues (Palosuo et al., 2016) and sufficient soil moisture for decomposition throughout most of the year constrain carbon accrual and relatively modest effects of no-till or reduced tillage on SOC stocks have been observed (Sheehy et al., 2015; Singh et al., 2015). The contribution of deep-burrowing earthworms to the processes leading to SOC stabilization in no-till management in boreal conditions is largely unknown. We studied the impact of the only anecic earthworm species found in the arable soils of Finland, *L. terrestris,* on soil aggregation level and SOC and N division between soil aggregates in three Finnish no-till sites by comparing soil sampled from earthworm middens with the bulk soil. Our aim was to elucidate if the observed no-till induced changes in aggregate size and redistribution of SOC in these soils (Sheehy et al., 2015) could result from earthworm activities. We hypothesized that *L. terrestris* middens would have a higher content of SOC and N in the best protected soil fractions compared to the bulk soil thus providing evidence of the role of earthworms in SOC stabilization in boreal arable soils. As clay soils often are found to favor SOC sequestration, we expected to see more midden-associated SOC in clayey than coarse textured soil.

2. Material and methods

2.1 Study site and management information

This study took place at three long-term no-till fields in southwestern Finland. Two fields (sites 1 and 2) were located in Jokioinen (60°49’N and 23°30’E) with a yearly average precipitation of 627 mm and average temperature of 4.6ºC. Soils at both these sites were classified as Vertic Luvic Stagnosol (IUSS Working Group WRB, 2015). The third field (site 3) was classified as Eutric Regosol (WRB) and was located in Säkylä (60°58’N and 22°31’E) which has a yearly average precipitation of 614 mm and an average temperature of 4.8 ºC. Sites 1–2 were field experiments (randomized complete-block design with four replicates) and the field of site 3 belonged to a private farmer (plot size 100-250 m^2; 4 pseudoreplicates).

No-till practice, in which the crop was sown without prior soil tillage, had been used at the study sites for eleven (sites 1–2) or twelve (site 3) years (Table 1). Spring barley (*Hordeum vulgare*) was cultivated at sites 1 and 2 and spring turnip rape (*Brassica rapa* subsp. *oleifera*) at site 3. However, at site 3, spring barley had been cultivated during the previous years. Fields were sown and fertilized in May. Seeds were directly sown to 3–5 cm depth with combined drill having triple disc coulters (site 3, row space 15 cm, front single disc coulter is tilling and rear double disc coulter is sowing with roller wheels behind the sowing coulters) or double disc coulters (sites 1 and 2, row space 14.5 cm, packing wheels behind the drill). The direct drills placed the seeds and fertilizer in the same row. The whole annual fertilizer application of 100 kg N ha^{-1} yr^{-1} (sites 1 and 2) or 80 kg N ha^{-1} yr^{-1} (site 3) was made during sowing. Granular ammonium nitrate NPK fertilizer was used at sites 1 and 2 and liquid fertilizer (Urea 32) was used at site 3. Compared to average yield during the past decade, below average yields were harvested in August at all study sites (Table 1).

2.2 Soil sample collection and analysis

Soil for studying the physical soil fractions was sampled in September 2010 about a month after harvest at all study sites. The samples were taken from *L. terrestris* middens and surrounding bulk soil (at least 15 cm from the closest middens) to the depth of 5 cm with a 5 cm diameter soil corer. The midden soil samples included the burrow entrance of *L. terrestris* and were comprised mostly of soil, casts and straw. The bulk soil samples represented the soil without a *L. terrestris* midden-burrow complex. Four midden soil – bulk soil pairs were sampled from four different locations at each study site. The four samples in each location were pooled to form four replicates of large enough samples for soil fractionation.

The aggregate size distribution was analyzed by separating different aggregates by wet sieving according to Elliot et al. (1986) and as described by Sheehy et al. (2015). The field-moist soil samples were sieved through an 8 mm sieve and then air-dried. An 80 g subsample of soil was taken for the wet sieving which was done through a series of three sieves that separated the samples into four different soil fractions; large macroaggregates (LM; >2000 µm), small macroaggregates (sM; 250–2000 µm), microaggregates (m; 53–250 µm) and silt and clay (s+c; <53µm). Prior to wet sieving the samples were submerged into deionized water on top of the 2000 µm sieve for a period of 5 min. The sieving was done by manually moving the sieve up and down 50 times during a 2 min period. The sieve was backwashed and the fraction remaining on top of the sieve was collected in an aluminum pan and oven-dried at 60ºC. Organic material (plant residues) floating on the water after sieving with the 2000 µm sieve was discarded as it is not considered SOM by definition. The sieving was similarly repeated with the remaining sieves.

Microaggregates within LM and sM fractions were isolated according to Six et al. (2000) and as described in Sheehy et al. (2015). The goal of this method was to break down the macroaggregates while avoiding the breakdown of the released microaggregates. A separate subsample of 10, 5 or 3 g, depending on the amount of available material, was taken from LM and sM fractions and was placed on top of a 250 µm mesh. The sample was shaken with 50 stainless steel beads (4 mm diameter) in a reciprocal shaker with a continuous flow of running deionized water until all the macroaggregates were broken down (3–5 min of shaking depending on the soil type). The microaggregates and other released material went through the mesh screen with the running water ending up on a 53 µm sieve and were then sieved as in the wet-sieving method. As a result three different fractions were isolated from the macroaggregates: coarse particulate organic matter (cPOM; >250 µm), microaggregates within macroaggregates (mM; 53–250 µm) and silt and clay (s+cM; < 53 µm).

The mean weight diameter (MWD) of the aggregates, that can be used as an indicator of aggregate stability, was calculated according to van Bavel (1949). Carbon and N content of all fractions from wet sieving and microaggregate isolation were analyzed with a CN-analyzer (ECS 4010, Costech Instruments, USA). The SOC and N content of the different aggregates and the total C and N was calculated using the equivalent soil mass method according to Lee et al. (2009) which takes into account the different bulk densities of the soils sampled.

2.3 Decomposition rate measurements

Decomposition rates of two types of crop residue with different chemical quality, barley (*Hordeum vulgare*) straw and pea (*Pisum sativum*) residue, were measured at all study sites from November 2009 to September 2010. Residue bags were installed to three different depths of the bulk soil: on the top of the soil (0 cm), and buried 10 cm and 20 cm deep. Residue bags (10 x 15 cm) were made out of polyester mesh (1 mm) and 5 g of air dried, untreated barley straw or pea residue as 5 cm long pieces, was put into each bag. Sides of the bags were stitched together with a serger and a plastic tag was attached with a line. The bags with the residue in them were oven dried overnight at 40°C for a final weight. The bags were installed in two rows 4 meters from the end of the study plots and one meter from each side of the plots. The two rows were 50 cm apart from each other. There were four barley straw bags at each depth and two pea residue bags at each depth for a total of 18 residue bags at each study site. Half of these bags were collected at the end of April before the start of the growing season and half were left in the study plots until the end of the growing season (September). Afterwards the residue bags were air dried for a week, the residue samples then moved to paper bags and oven dried at 40°C before grinding the samples for analysis.

Barley straw and pea residue in the residue bags were analysed for mass loss. Due to the 1 mm mesh size, a variable amount of surrounding soil was incorporated into the bags. The mixture of plant residue and soil from the bags was ground and about 2 g of the ground sample was taken for the loss on ignition (LOI) analysis. The samples were incinerated at 550 degree C for 5 h in a high temperature muffle furnace. This enables calculating the content of organic matter in the samples as the ignition leaves the mineral part of the soil as ash while organic matter is lost. The results from the separate LOI analysis from the original barley straw and pea residue, and the surrounding soil samples, were used for correction (ash free dry weight).

2.4 Statistical analysis

Aggregate weights were normally distributed, but distributions of SOC and N were skewed. Linear mixed models with (C & N) and without (aggregate weights) logarithm transformation were used in analysis. The models were fitted by using the residual maximum likelihood (REML) estimation method, having treatment, fraction and field denoted as fixed effects. The effect of treatment (i.e. midden versus surrounding bulk soil (non-midden) was analyzed as repeated measures having heterogeneous compound symmetry (CSH) covariance structure. The model can be expressed in equation form:

$$y_{ijkl} = \mu + T_i + R_j + TR_{ij} + F_k + FB_{kl} + TF_{ik} + TFB_{ikl} + TRF_{ijk} + RF_{jk} + RFB_{jkl} + \varepsilon_{ijkl} \text{ , } (1)$$

where μ is the overall mean, T_i, R_j and F_k are the fixed effects of the treatment, fraction and field, respectively. Two- and three-factor interactions of fixed effects were also included in the model; interaction of the block, B_l, with other factors (FB_{kl}, TFB_{ikl} and RFB_{jkl}) represent the random effects, and ε_{ijkl} is the random error term of the model. The random variables were assumed independent and normally distributed. The mean weight diameter (MWD) was analyzed using the same model without the different fractions.

The data to calculate effects on the decomposition rate consisted of one dependent variable, decomposed plant material, and five independent variables of site, crop management practice, depth, residue type (barley straw vs. pea residue) and date (season). All independent variables and their two- and three-factor interactions were included in the first model. Eventually, all non-significant (α=0.05) fixed effects were removed from the final model. The model takes into account that residue type and date were repeated measures having unstructured covariance structures (un$\otimes$un). The unstructured covariance structure is the most flexible since it imposes no pattern on the covariances. Thus, all variance and covariance components of residue type and date were estimated, unlike simpler structures constraining some components. This structure is constructed by taking the Kronecker product of an unstructured matrix, modeling covariance across the residue types, with an unstructured matrix, modeling covariance across dates (Galecki, 1994).

The appropriateness's of the models were studied by residual analyses. The residuals were checked for normality using boxplot and normal probability plot (Tukey, 1977). The residuals were also plotted against the fitted values. These plots indicated that the assumptions of the models are adequate. Comparison of means was done with the Tukey-Kramer post hoc test. A significance level of α=0.05 was used in all analysis. Degrees of freedom were calculated using Kenward-Roger method. The analyses were performed using the MIXED procedure of the SAS Enterprise Guide 5.1 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1 Aggregate weight and stability

There were more large macroaggregates (LM) in the soil from *L. terrestris* middens than in the surrounding bulk soil at study sites 1 and 2 ($p=0.008$ and <0.001 respectively) but less small macroaggregates (sM) at site 2 ($p<0.0001$) (Fig. 1). The greatest portion of LM was found in the middens of site 2 where they represented 35% of the soil mass. The amount of free microaggregates (m) in the soil was lower ($p=0.006$) in midden soil compared to bulk soil at site 1. No differences were found between midden soil and bulk soil in the different fractions in the coarse soil of site 3.

The proportional weight of coarse particulate organic matter (cPOM) from LM fractions was higher within the midden soil compared to bulk soil at site 2 ($p=0.002$) (Fig. 1). On the other hand, the proportional weight of microaggregates formed within LM fractions (mM) was lower in midden soil than surrounding bulk soil at site 2 ($p=0.005$). Within sM fraction, the proportional weight of cPOM was higher in the middens versus surrounding bulk soil at site 2 ($p=0.012$) and lower at site 1 ($p=0.028$). The proportion of mM fraction within sM fraction was higher in the middens at site 1 ($p<0.001$), lower in the middens at site 2 ($p=0.031$) and without a difference at site 3. As a proportion of the whole soil, the total mM fraction from LM and sM was 6-45% higher in midden soil at all sites (results not shown).

The MWD of aggregates was on average 20% higher across all study sites in *L. terrestris* middens compared to the surrounding soil (site 1: 35%; site 2: 21%; site 3: 3%) (Fig. 2). MWD was significantly higher in the middens versus surrounding bulk soil at the clayey sites 1 and 2 ($p=0.001$ and $p=0.002$ respectively), but not at the coarse textured site 3 (Fig. 2).

3.2 Soil carbon

The total SOC content of the 0-5 cm layer was of the same magnitude at all sites (Table 2). The midden associated soil had higher concentration of SOC than the bulk soil when analyzed across all sites ($p=0.0231$). Within each single field, the total SOC did not differ between midden and non-midden soil.

Enrichment of SOC per area in the soil sampled from the middens was found only in LM fractions at sites 1 and 2 ($p<0.001$ in both) (Table 2). For the fractions isolated from within LM (Fig. 3), there were higher SOC contents in midden soil compared to bulk soil at all study sites in cPOM ($p<0.001$), at sites 1 and 2 in mM fraction ($p=0.014$ and $p<0.001$, respectively) and in s+c fraction ($p=0.011$ and $p<0.001$, respectively). Within sM fractions, a decrease of SOC in middens was found in mM fraction at site 2 ($p=0.025$).

3.3 Soil nitrogen

The amount of total N in midden soil versus bulk soil did not differ at any sites (results not shown). However, higher N content in large macroaggregates in midden soil versus surrounding bulk soil was found at the clayey sites 1 and 2 ($p<0.001$) (Table 3). No changes in N content were found at site 3. Differences were found, however, in the N levels between different fields. For example, site 1 had more N in the top soil than sites 2 or 3 ($p=0.003$ and $p=0.011$ respectively) (results not shown).

Nitrogen content in cPOM within LM fractions was higher in midden soil compared to bulk soil at sites 1 and 2 ($p<0.001$) (Table 3). At these sites the cPOM-N content was 3–6 times higher in middens than bulk soil. More N was also found in midden versus surrounding bulk soil in LM-occluded microaggregates at site 2 ($p=0.005$) and silt and clay fraction at sites 1 and 2 ($p=0.04$ and $p=0.001$ respectively). No differences in soil N content were found in small macroaggregate-occluded fractions between midden and bulk soil.

The C:N ratio of the soil at 0-5 cm depth varied between 11.8 and 13.1 and did not differ between the midden soil and surrounding bulk soil at any study site.

3.4 Decomposition rate of barley straw and pea residue

More residue decomposed from the pea residue bags compared to the barley straw bags at sites 1 and 2 ($p<0.001$) (Fig. 4a). A significant difference in the decomposition rate at all sites was found between the residue bags on the top soil versus under 10 or 20 cm of soil with an increasing trend in decomposition rate deeper in the soil profile ($p<0.001$) (Fig. 4b). Differences between the two deeper layers were smaller but still significant at sites 1 and 3 ($p=0.04$; $p=0.004$). Over 65% of both barley straw and pea residue decomposed at the 20 cm depth within a year. Sites differed from each other in their decomposition rate in the topsoil ($p=0.017$) where site 3 had the highest decomposition rate. No difference in the decomposition rate was found between sites in the other two soil layers indicating that the decomposition rate does not increase as fast with increasing depth at site 3 compared to the other two sites. However, site 1 had a higher decomposition rate of pea residue compared to site 3 ($p=0.042$). Decomposition rate was lower at all depths during the cold winter months with average decomposition rates of only 0.09% per day compared to 0.17% per day during the growing season ($p<0.001$) (Fig. 4c). Lowest decomposition rate was found at 0 cm at site 1 where only 14% of the barley straw was decomposed in the first 6 months after installing the bags.

4. Discussion

4.1 Aggregate stability and aggregate-associated SOC

The observations of increased amount of LM and higher MWD in the midden soil compared to bulk soil point to anecic earthworms having a role in the development of soil aggregation in the clay soils. Our results are supported by several field and laboratory studies where earthworms have enhanced soil aggregation (Blanchart et al., 1999; Bossuyt et al., 2004; Shipitalo and Le Bayon, 2004; Fonte and Six, 2010; Arai et al., 2018; Frazão et al., 2019). The local effect of *L. terrestris* was also seen as higher SOC content in the middens. In a recent meta-analysis this was the case in the casts of *L. terrestris* and the epigeic species *Lumbricus rubellus* unlike the casts of two endogeic species (van Groenigen et al., 2019).

The total SOC content of the midden-associated soil compared to the bulk soil was on average 8% higher at the clayey sites and 3% higher for the coarse textured soil. These results are in accordance with several studies indicating higher SOC sequestration rates in soils with higher clay content (e.g. Leifeld et al., 2005; Heikkinen et al., 2013). Better protection of SOC in clayey soils is often attributed to less accessible soil pores for microbes and SOC binding to mineral surfaces (Strong et al., 2004; von Lützow et al., 2006; Simonetti et al., 2017).

Neither the weights of aggregate fractions nor the SOC content differed between middens and bulk soil of Site 3 with coarse soil texture. The tendency to form aggregates is generally lower in coarse compared to finer textured soils (Simonetti et al., 2017; Schapel et al., 2019). Some studies have shown that rapid breakdown of the newly formed aggregates may occur if increased amount of earthworm activity also enhances the mineralization of polysaccharides and other organic gluing compounds (Guggenberger et al., 1996; Ge et al., 2001). This may be the case with our coarse soil that also had high decomposition rate of top soil litter. Due to the small mesh size of residue bags used, the measured decomposition was obviously not directly driven by earthworm feeding but the observed differences between midden and bulk soil are likely the combined result of *L. terrestris* activity and other biological activity. Middens of *L. terrestris* have been found to accumulate coarse litter and maintain soil moisture (Subler and Kirsch, 1998; Nieminen et al., 2015) as well as favor the activities of microbes (Aira et al., 2009) and fauna (Schrader and Seibel, 2001; Butt and Lowe, 2007; Eisenhauer, 2010; Nieminen et al., 2015; Stroud et al., 2016). On the same three no-till sites, Sipilä et al. (2012) found strong soil fungistasis activity, inhibition of fungal growth related to high microbial biomass, with strongest mean activity at site 3. This together with the high decomposition rate in the top soil points to the possibility that in this coarse textured soil, aggregation is so weak that the enhanced decomposition negates the potential earthworm-induced increase of SOC stabilization.

Part of the observed increase in the LM fraction can likely be explained by the smaller fractions growing in size. Indication of this was the earthworm-induced redistribution of SOC from sM fraction to LM fraction in the middens. The SOC content of the middens was 11% higher in the LM fractions and 6% lower in the sM fraction compared to bulk soil. These findings are similar to results presented by Fonte and Six (2010) who found a redistribution of SOC into large macroaggregates and the microaggregates within them by earthworms. Similarly, in Peruvian Amazonia, endogeic earthworms increased the proportion of large macroaggregates (>2 mm) with almost 6% at the expense of smaller aggregates (<0.5mm) that decreased by 8% after six successive crop cycles (Lavelle et al., 2004).

Our results suggest that the presence of *L. terrestris* increases the potential of boreal agricultural soils to store SOC within large macroaggregate-occluded microaggregates. In the clay soils, 44–53% of the observed difference in SOC stored in large macroaggregates between *L. terrestris* midden soil and bulk soil (Table 2) was found in the microaggregates isolated from them (Fig. 3). This highlights the importance of this fraction as a microsite for SOC sequestration (Six and Paustian, 2014). SOC stabilization in large macroaggregate-occluded microaggregates was significant at both clay soil sites but at site 2 the increase was counteracted by a decrease in the amount of SOC in small microaggregate-occluded microaggregates. In contrast, the significance of free microaggregates for SOC storage was small in our study.

Our results represent the situation in soil in autumn, the period of abundant crop residues and high earthworm activity and thus the results do not represent the average annual situation. Although the biological decomposition in winter is half of the rate in summer (Fig. 4c), there are more physical forces breaking down aggregates in the winter time, particularly the frequent freezing and thawing of the soil that can reduce MWD to half of the autumn values (Edwards, 2013). Even though the increased earthworm midden associated aggregation level is potentially beneficial in the short-term it may not be enough to create long-term SOC accumulation. Don et al. (2008) concluded that the *L. terrestris* burrow associated increase in topsoil SOC may be a short-term one, as their study showed that the walls of earthworm-occupied burrows had up to three times higher values of SOC than abandoned earthworm burrows. Fonte and Six (2010) argued that since most of the SOC in earthworm casts is associated with macroaggregate-occluded microaggregates the rate of decomposition in these casts would, with time, possibly decrease to a level below the level of non-ingested soil.

Even though there was an increase in the topsoil SOC content in the middens across all sites, this may not be relevant for the SOC sequestration at field scale. Previous studies have shown that the total density of earthworm burrows in the no-till plots of these study sites were higher in comparison to conventionally tilled plots (Regina and Alakukku, 2010), but the total SOC stocks did not differ between the treatments (Sheehy et al., 2013). This is in line with the meta-analysis by Lubbers et al. (2013) where no earthworm-induced total SOC increase was found. However, the results of this study suggest that the presence of anecic earthworms predicts increased chances to develop relatively stable sites for SOC storage especially as the continuation of no-till enables further increase of earthworm numbers.

4.2 Nitrogen content and aggregate-associated N

Nitrogen allocation in soil fractions followed closely the trends observed for SOC. Nitrogen levels were markedly higher in the middens of the clayey soils, especially within the coarse particulate organic matter and microaggregate fractions within large macroaggregates; this was accompanied by an increase in the amount of the large macroaggregates in midden soil. This underlines *L. terrestris*'s ability to accumulate N within soil particles that are more resistant to decomposition, especially in clayey soils. On the other hand, the total production of mineral N by the earthworm community can be as high as 74 kg ha^{-1} yr^{-1} (Whalen and Parmelee, 2000; Lavelle et al., 2004) which, for instance, almost equals the average amount of mineral N added annually by farmers in Finland. Integration of N into macroaggregate-occluded microaggregates at these sites may counteract the N mineralization effect of earthworms and slow down the N cycle in the top soil. The net effect of earthworms for N cycling in the soil can e.g. determine the nutrient leaching potential of the soil. The presence of earthworms can increase (Dominguez et al., 2004) or decrease (Shuster et al., 2002) N leaching and it has been suggested that there is a threshold value of earthworm density above which the positive effects of increased density turn to increased leaching potential (Shuster et al., 2002).

No *L. terrestris* midden related changes in total N concentration were observed at site 3. This could be partly due to the higher microbial activity and decomposition rate on the top soil at this site compared to the clayey sites as well as a lesser amount of existing fine soil particles. It is also possible that in this coarse textured soil the positive effect of earthworms on N mineralization is accompanied by accelerated rates of denitrification within earthworm casts. This is supported by a study conducted at site 3 in 2008 that found higher flux rates of nitrous oxide, accompanied by increased N mineralization rates, around *L. terrestris* middens compared to surrounding soil (Nieminen et al., 2015).

Accumulation of N in the top-soil of no-till systems has been observed in many studies (Campbell et al., 1996; Spargo et al., 2008). Also, Giannopoulos et al. (2010) found that increased N incorporation into both macroaggregates (>250 µm) and microaggregates

(53–250 µm) was higher when residues were added as a residue layer on the top of the soil instead of incorporating them into the soil. Sheehy et al. (2013) did not, however, observe any consistent increase in the amount of total N in the 0–20 cm layer in no-till versus conventionally tilled soil at any of these sites. Protecting N into macroaggregate-occluded microaggregates would be beneficial, especially in fields with high or moderate N leaching potential. There are indications of higher density of *L. terrestris* individuals and biomass in no-till compared to tilled plots at site 2 (unpublished results). This together with higher levels of large macroaggregate-occluded N in *L. terrestris* middens at the clayey sites found in this study, suggests that N cycling slows down in no-till cultivation.

5. Conclusions

In line with our hypotheses, this study confirmed that *L. terrestris* mediates changes in soil structure and SOC distribution by creating a more opportune environment for enhanced storage of SOC and N into large macroaggregate-occluded fractions in boreal no-tilled clay soils. Even though the measured effects are local and restricted to middens the results suggest that natural *L. terrestris* densities in long-term no-till management eventually have the ability to enhance soil macroaggregation and SOC stock also in field scale. This study corroborated our view that earthworms are essential modifiers of soil aggregate structure and associated carbon storage not only in temperate and tropical but also in boreal arable soils.

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References

Aira, M., McNamara, N.P., Piearce, T.G., Dominguez, J., 2009. Microbial communities of *Lumbricus terrestris* L. middens: structure, activity, and changes through time in relation to earthworm presence. J. Soil Sediment 9, 54–61.

Arai, M., Miura, T., Tsuzura, H., Minamiya, Y., Kaneko, N., 2018. Two-year responses of earthworm abundance, soil aggregates, and soil carbon to no-tillage and fertilization. Geoderma 332, 135-141.

Alegre, J., Pashanasi, B., Lavelle, P., 1996. Dynamics of soil physical properties in Amazonian agroecosystems inoculated with earthworms. Soil Sci. Soc. Am. J. 60, 1522–1529.

Angers, D.A., Bolinder, M.A., Carter, M.R., Gregorich, E.G., Drury, C.F., Liang, B.C., Voroney, R.P., Simard, R.R., Donald, R.G., Beyaert, R.P., Martel, J., 1997. Impact of tillage practices on organic carbon and nitrogen storage in cool, humid soils of eastern Canada. Soil Till. Res. 41, 191–201.

Bai, Z., Caspari, T., Ruiperez Gonzalez, M., Batjes, N.H., Mäder, P., Bünemann, E.K., de Goede, R., Brussaard, L., Xu, M., Santos Ferreira, C.S., Reintam, E., Fang, H., Mihelič, R., Glavan, M., Tóth, Z., 2018. Effects of agricultural management practices on soil quality: A review of long-term experiments for Europe and China. Agr. Ecosyst. Environ. 265, 1–7.

Barois, I., Villemin, G., Lavelle, P., Toutain, F., 1993. Transformation of the soil structure through Pontosolex corethurus (Oligochaeta) intestinal tract. Geoderma 56, 57–66.

Blanchart, E., Albrecht, A., Alegre, J., Duboisset, A., Gilot, C., Pashanasi, B., Lavelle, P., Brussaard, L., 1999. Effects of earthworms on soil structure and physical properties, in: Lavelle, P., Brussaard, L., Hendrix, P. (Eds.), Earthworm management in tropical agroecosystems. CAB International, Oxford, UK, pp. 149–171.

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendoovern, L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J.-J., 2013. A review of earthworm impact on soil function and ecosystem services. Eur. J. Soil Sci. 64, 161–182.

Bossuyt, H., Six, J., Hendrix, P.F., 2002. Aggregate-protected carbon in no-tillage and conventional tillage agroecosystems using carbon-14 labeled plant residue. Soil Sci. Soc. Am. J. 66, 1965–1973.

Bossuyt, H., Six, J., Hendrix, P.F., 2004. Rapid incorporation of fresh residue-derived carbon into newly formed stable microaggregates within earthworm casts. Eur. J. Soil Sci. 55, 393–399.

Bossuyt, H., Six, J., Hendrix, P.F., 2005. Protection of soil carbon by microaggregates within earthworm casts. Soil Biol. Biochem. 37, 251–258.

Bouché, M.B., 1977. Strategies lombriciennes, in: Lohm, U., Persson, T. (Eds.), Soil Organisms as Components of Ecosystems vol. 25, Stockholm, pp. 122–132.Briones, M.J.I., Schmidt O, 2017. Conventional tillage decreases the abundance and biomass of earthworms and alters their community structure in global meta-analysis. Global Change Biology 23, 4396-4419.

Butt, K.R., Lowe, C.N., 2007. Presence of earthworm species within and beneath *Lumbricus terrestris* L. middens. Eur. J. Soil Biol. 43, Supplement 1, S57–S60.

Campbell, C.A., McConkey, B.G., Zentner, R.P., Sellers, F., Curtin, D., 1996. Long-term effects of tillage and crop rotations on soil organic C and total N in a clay soil in southwestern Saskatchewan. Can. J. Soil Sci. 76, 395–401.

Coleman, D.C., Crossley, Jr., D.A. 2004. Fundamentals of Soil Ecology, second ed. Academic Press, San Diego.

Curry, J.P., Schmidt, O., 2007. The feeding ecology of earthworms – A review. Pedobiologia 50, 463-477.

Don, A., Steinberg, B., Schöning, I., Pritsch, K., Joschko, M., Gleixner, G., 2008. Organic carbon sequestration in earthworm burrows. Soil Biol. Biochem. 40, 1803–1812.

Dominguez, J., Bohlen, P.J., Parmelee, R.W., 2004. Earthworms increase nitrogen leaching to greater soil depths in row crop agroecosystems. Ecosystems 7, 672–685.

Edwards, L.M., 2013. The effects of soil freeze–thaw on soil aggregate breakdown and concomitant sediment flow in Prince Edward Island: A review. Can. J. Soil Sci. 93, 459–472. https://doi.org/10.4141/cjss2012-059.

Eisenhauer, N., 2010. The action of an animal ecosystem engineer: identification of the main mechanisms of earthworm impacts on soil microarhropods. Pedobiologia 53, 343–352.

Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J. 50, 627–633.

Filser, J., Faber, J.H., Tiunov, A.V., Brussaard, L., Frouz, J., De Deyn, G., Uvarov, A.V., Berg, M.P., Lavelle, P., Loreau, M., Wall, D.H., Querner, P., Eijsackers, H., Jiménez, J., 2016. Soil fauna: key to new carbon models. Soil 2, 565–582.

Fonte, S. J., Winsome, T., Six, J., 2009. Earthworm populations in relation to soil organic matter dynamics and management in California tomato cropping systems. Appl. Soil Ecol. 41, 206–214.

Fonte, S.J., Six, J., 2010. Earthworms and litter management contributions to ecosystem services in a tropical agroforestry system. Ecol. Appl. 20, 1061–1073.

Frazao, J., de Goede, R.G.M., Capowiez, Y., Pulleman, M.M., 2019. Soil structure formation and organic matter distribution as affected by earthworm species interactions and crop residue placement. Geoderma 338, 453–463.

Galecki, A.T., 1994. General class of covariance structures for two or more repeated factors in longitudinal data analysis, Commun Stat Theory Methods. 23:11, 3105–3119. https://doi.org/10.1080/03610929408831436.

Ge, F., Shuster, W.D., Edwards, C.A., Parmelee, R.W., Subler, S., 2001. Water stability of earthworm casts in manure- and inorganic-fertilizer amended agroecosystems influenced by age and depth. Pedobiologia 45, 12–26.

Giannopoulos, G., Pulleman, M.M., van Groenigen, J.W., 2010. Interactions between residue placement and earthworm ecological strategy affect aggregate turnover and N_2O dynamics in agricultural soils. Soil Biol. Biochem. 42, 618–625.

Guggenberger, G., Thomas, R.J., Zech, W., 1996. Soil organic matter within earthworm casts of an anecic–endogeic tropical pasture community, Colombia. Appl. Soil Ecol. 3, 263–274.

Heikkinen J., Ketoja E., Nuutinen V., Regina K., 2013. Declining trend of carbon in Finnish cropland soils in 1974–2009. Glob. Change Biol. 19, 1456–1469.

IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, update 2015, International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome.

Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. Oikos 69, 373–386.

Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P., Heal, O.W., Dhillion, S., 1997. Soil function in a changing world: the role of invertebrate ecosystem engineers. Eur. J. Soil Biol. 33, 159–193.

Lavelle, P., Spain, A.V., 2001. Soil Ecology. Kluwer Scientific Publications, Amsterdam.

Lavelle, P., Charpentier, F., Villenave, C., Rossi, J., Derouard, L., Pashanasi, B., Andre, J., Ponge, J., Bernier, N., 2004. Effects of earthworms on soil organic matter and nutrient dynamics at a landscape scale over decades, in: Edwards, C.A. (Eds.), Earthworm Ecology. CRC Press, Boca Raton, pp. 145–160.

Lee, J., Hopmans, J.W., Rolston, D.E., Baer, S.G., Six, J., 2009. Determining soil carbon stock changes: Simple bulk density corrections fail. Agr. Ecosyst. Environ. 134, 251–256.

Leifeld, J., Bassin, S., Fuhrer, J., 2005. Carbon stocks in Swiss agricultural soils predicted by land-use, soil characteristics, and altitude. Agr. Ecosyst. Environ., 105, 255–266.

Leue, M., Wohld, A., Gerke, H.H., 2018. Two-dimensional distribution of soil organic carbon at intact macropore surfaces in BT-horizons. Soil Till. Res. 176, 1-9.

Lubbers, I.M., van Groenigen, K.J., Fonte, S.J., Six, J., Brussaard, L., van Groenigen, J.W., 2013. Greenhouse-gas emissions from soils increased by earthworms. Nat. Clim. Change 3, 187–194.

Lubbers, I.M., Pulleman, M.M., van Groenigen, J.W. 2017. Can earthworms simultaneously enhance decomposition and stabilization of plant residue carbon? Soil Biol. Biochem., 105, 12–24.

Nieminen, M., Hurme T., Mikola J., Regina K., Nuutinen V., 2015. Impact of earthworm *Lumbricus terrestris* living sites on the greenhouse gas balance of no-till arable soil. Biogeosciences 12, 5481–5493.

Nuutinen, V., Butt, K.R., 2003. Interaction of *Lumbricus terrestris* L. burrows with field subdrains. Pedobiologia, 47, 578–581.

Palosuo T., Heikkinen J., Regina K., 2016. Method for estimating soil carbon stock changes in Finnish mineral cropland and grassland soils. Carbon Management 6, 207-220.

Pulleman, M.M., Six, J., Uyl, A., Marinissen, J.C.Y., Jongmans, A.G., 2005. Earthworms and management affect organic matter incorporation and microaggregate formation in agricultural soils. Appl. Soil Ecol. 29, 1–15.

Regina, K., Alakukku, L., 2010. Greenhouse gas fluxes in varying soil types under conventional and no-tillage practices. Soil Till. Res. 109, 144–152.

Schapel, A., Marschner, P., Churchman, J., 2019. Influence of clay clod size and number for organic carbon distribution in sandy soil with clay addition. Geoderma 335, 123-132.

Schrader, S., Seibel, C., 2001. Impact of cultivation management in an agroecosystem on hot spot effects of earthworm middens. Eur. J. Soil Biol. 37, 309–313.

Sheehy, J., Six, J., Alakukku, L., Regina, K., 2013. Fluxes of nitrous oxide in tilled and no-tilled boreal arable soils. Agr. Ecosyst. Environ. 164, 190–199.

Sheehy, J., Regina, K., Alakukku, L., Six, J., 2015. Impact of no-till and reduced tillage on aggregation and aggregate-associated carbon in Northern European agroecosystems. Soil Till Res., 150, 107–113.

Simonetti, G., Francioso, O., Dal Ferro, N., Nardi, S., Berti, B., Morari, F., 2017. Soil porosity in physically separated fractions and its role in SOC protection. J. Soils Sediments 17, 70-84.

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. Science of the Total Environment 518–519, 337–344.

Sipilä, T. P., Yrjälä, K., Alakukku, L., Palojärvi, A., 2012. Cross-site soil microbial communities under tillage regimes: fungistasis and microbial biomarkers. Appl. Environ. Microb. 78, 8191–8201.

Shipitalo, M.J., Le Bayon, R.C., 2004. Quantifying the effects of earthworms on soil aggregation and porosity, in: Edwards, C.A. (Ed.) Earthworm ecology. CRC Press, Boca Raton, Florida, USA, pp. 183–200.

Shuster, W.D., McDonald, L.P., McCartney, D.A., Stinner, B.R., 2002. Nitrogen source and earthworm abundance affected runoff volume and nutrient loss in a tilled-corn agroecosystem. Biol. Fertil. Soils 35, 320–327.

Six, J., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. 32, 2099–2103.

Six, J., Callewaert, P., Lenders, S., De Gryze, S., Morris, S.J., Gregorich, E.G., Paul E.A., Paustian, K., 2002. Measuring and understanding carbon storage in afforested soils by physical fractionation. Soil Sci. Soc. Am. J. 66, 1981–1987.

Six, J., Paustian, K., 2014. Aggregate-associated soil organic matter as an ecosystem property and measurement tool. Soil Biol. Biochem. 68, A4–A9.

Spargo, J.T., Alley, M.M., Follett, R.F., Wallace, J.V., 2008. Soil nitrogen conservation with continuous no-till management. Nutr. Cycl. Agroecosys. 82, 283–297.

Subler, S. Kirsch, A.S., 1998. Spring dynamics of soil carbon, nitrogen and microbial activity in earthworm middens in a notill cornfield. Biol. Fert. Soils 26, 243–249.

Stroud, J.L., Irons. D., Carter, J.E., Watts, C.W., Murray, P.J., Norris, S.L., Whitmore, A.P., 2016. *Lumbricus terrestris* middens are biological hotspots in a minimum tillage arable ecosystem. Appl. Soil Ecol. 105, 31–35.

Thevathasan, N.V., Gordon, A.M., 2004. Ecology of tree intercropping systems in the North temperate region: experiences from southern Ontario, Canada. Agroforest. Syst. 61–62, 257–268.

Tukey, J.W., 1977. Exploratory data analysis. MA: Addison-Wesley. Reading, UK.

van Bavel, C.H.M., 1949. Mean weight-diameter of soil aggregates as a statistical index of aggregation. Soil Sci. Soc. Am. Pro. 14, 20–23.

van Groenigen, J.W., Lubbers, I.M., Vos, H.M.J., Brown, G.G., De Deyn, G.B., van Groenigen, K.J., 2014. Earthworms increase plant production: a meta-analysis. Sci. Rep. 4, 6365.

van Groenigen, J.W., van Groenigen, K.J., Koopmans, G.F., Stokkermans, L., Vos, H.M.J., Lubbers, I.M., 2019. How fertile are earthworm casts? A meta-analysis. Geoderma 338, 525-535.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. Eur J. Soil Sci. 57, 426–445.

Whalen, J.K., Parmelee, R.W., 2000. Earthworm secondary production and N flux in agroecosystems: a comparison of two approaches. Oecologia 124, 561–573.

Whalen, J.K., Fox, C.A. 2006. Diversity of lumbricid earthworms in temperate agroecosystems, in: Benckiser, G., Schnell, S. (Eds.), Biodiversity in Agricultural Production Systems. CRC, Taylor & Francis, Boca Raton, pp. 249–261.

Wu, Y., Shaaban, M., an Peng, Q., Zhou, A., Hu, R., 2018. Impacts of earthworm activity on the fate of straw carbon in soil: a microcosm experiment. Env. Sci. Poll. Res. 25, 11054–11062.

Xiang, H., Guo, L., Zhang, J., Zhao, B., Wei, H. 2018. In Situ Earthworm Breeding to Improve Soil Aggregation, Chemical Properties, and Enzyme Activity in Papayas. Sustainability 10, 1193; https://doi:10.3390/su10041193www.mdpi.com/journal/sustainability.

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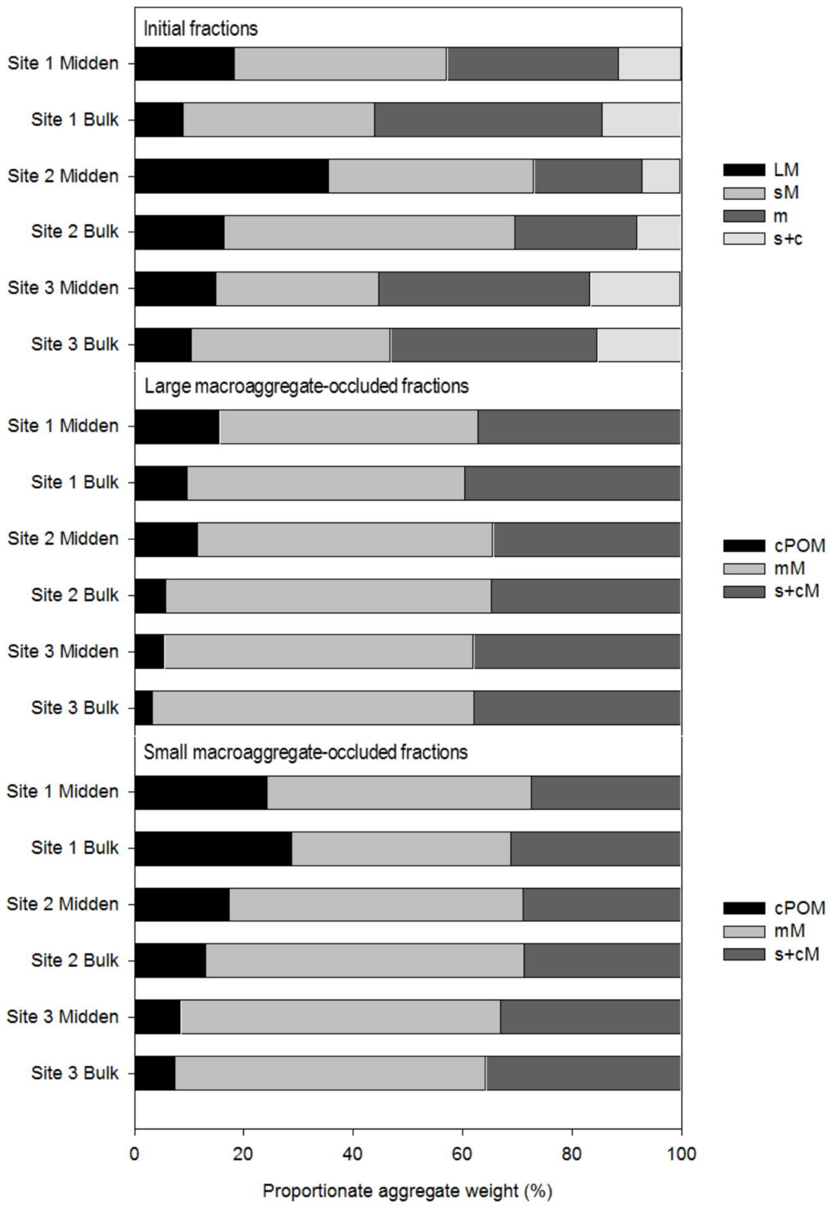

Fig. 1 Proportional weights (%) of the aggregate fractions (LM = large macroaggregates, sM = small macroaggregates, m = microaggregates, s+c = silt and clay) and microaggregate isolation from large and small macroaggregates (fractions within the aggregates: cPOM=particulate organic matter, mM=microaggregates, s+cM=silt and clay) in soil from earthworm middens and surrounding bulk soil at three study sites under no-till management.

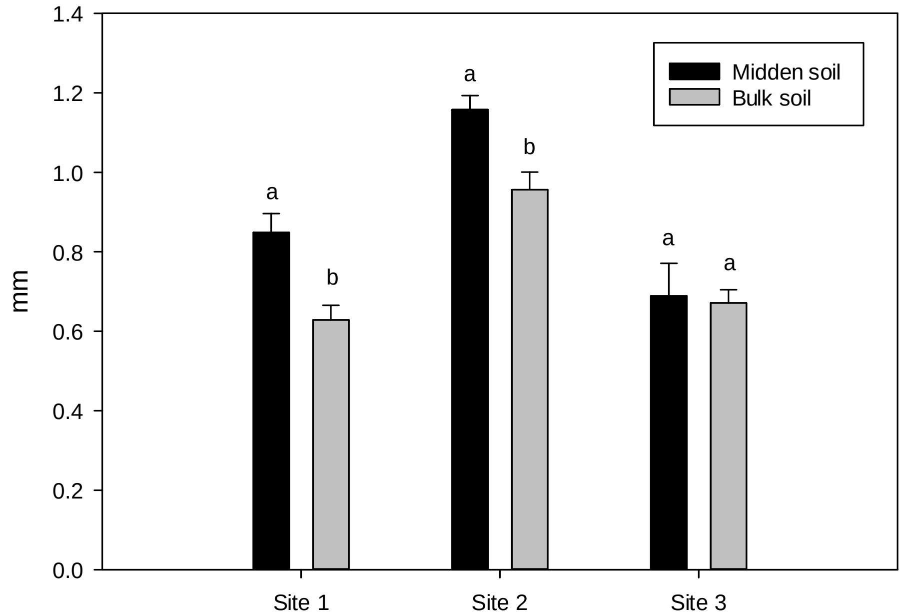

Fig. 2 Mean weight diameter (MWD; mm) of earthworm middens and surrounding bulk soil at three study sites under no-till management (mean ± standard error). Different letters denote statistical differences between midden and bulk soil (linear mixed model).

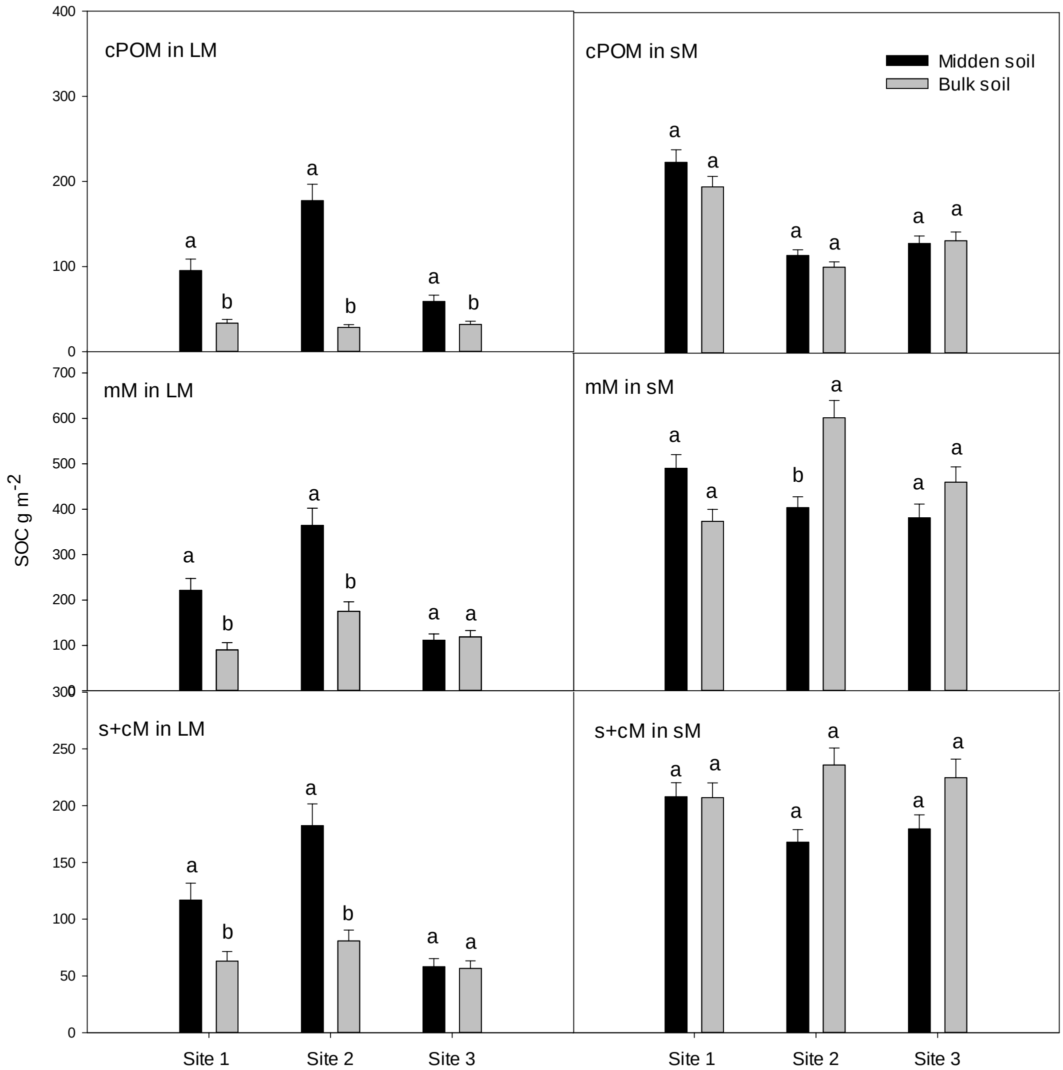

Fig. 3 Amount of soil organic carbon (SOC; g C m^{-2}) in coarse particulate organic matter (cPOM), microaggregates (mM) and silt and clay (s + cM) within large (LM) and small macroaggregates (sM) in earthworm middens versus surrounding bulk soil at three study sites under no-till management (mean ± standard error). Different letters denote statistical differences between midden and bulk soil (linear mixed model).

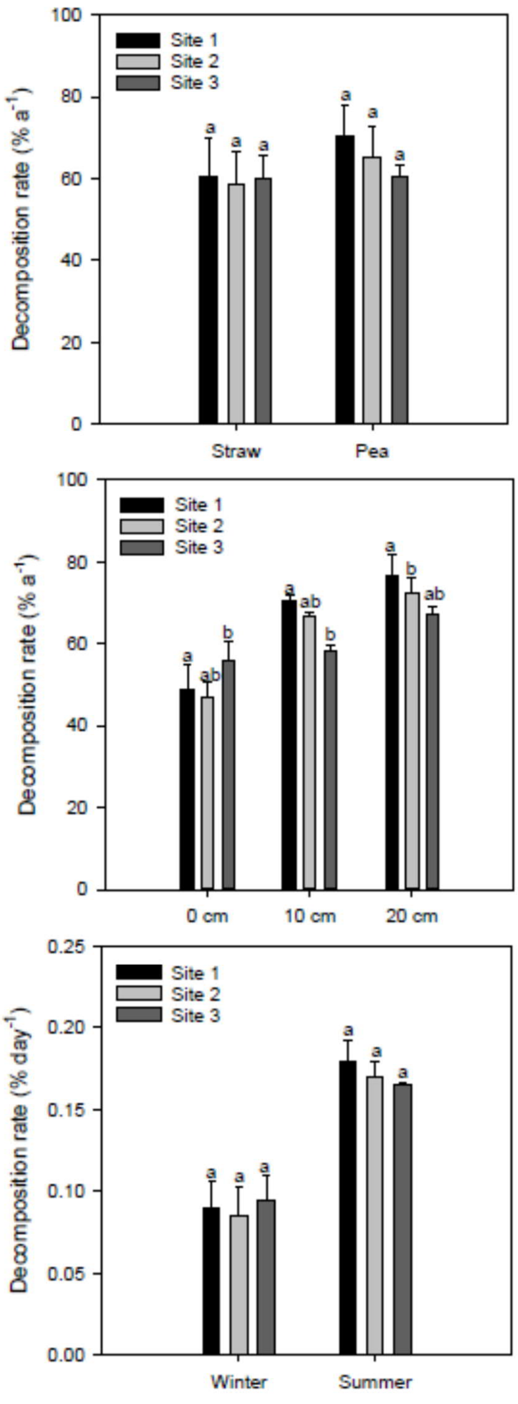

Fig. 4 Residue decomposition (± standard error) under no-till as % of the original mass a) of barley straw and pea, b) in different depths of the soil profile, c) in winter and summer. Different letters denote statistical differences between sites (linear mixed model).

Table 1

Site management and top soil properties (ND=not determined).

	Site 1	Site 2	Site 3
Years under no-till	11	11	12
Fertilizer (kg N ha^{-1} yr^{-1})	100	100	80
Crop 2010	Spring barley	Spring barley	Turnip rape
Yield 2010 (kg ha^{-1})	2609	1562	1000
Particle fractions (%)			
Clay (< 2 µm)	46	62	19
Silt (2–20 µm)	29	19	30
Fine sand (20–200 µm)	14	11	34
Coarse sand (> 200 µm)	11	8	17
Bulk density 0–5 cm (g cm^{-3})	1.33	1.02	1.37
L. terrestris density (ind. m^{-2})†	ND‡	12	27
L. terrestris biomass (g m^{-2})†	ND‡	25	67

†Data from Sep 2009; combination of hand sorting and formalin extraction methods (International Organization for Standardization 2006)

‡ND = Not Determined

Table 2

Amount of soil organic carbon (g C m^{-2}) within the soil fractions acquired from wet sieving of whole soil of the top 5 cm layer from earthworm middens and surrounding bulk soil (mean ± standard error) at three study sites under no-till management.

	Site 1		Site 2		Site 3	
	Midden soil	Bulk soil	Midden soil	Bulk soil	Midden soil	Bulk soil
Total soil	2020±50^{a}	1900±48^{b}	1800±51^{a}	1660±25^{b}	1730±85^{a}	1690±77^{b}
LM	**440 ± 45^{a}**	**196 ± 17^{b}**	**737 ± 59^{a}**	**305± 30^{b}**	229 ± 21^{a}	207 ± 20^{a}
sM	859 ± 31^{a}	761 ± 72^{a}	657 ± 48^{a}	895 ± 34^{a}	663 ± 53^{a}	834 ± 56^{a}
m	555 ± 44^{a}	758 ± 85^{a}	304 ± 24^{a}	329 ± 32^{a}	519 ± 42^{a}	492 ± 48^{a}
s+c	177 ± 14^{a}	226 ± 22^{a}	98 ± 8^{a}	112 ± 11^{a}	215 ± 20^{a}	220 ± 22^{a}

Statistically significant differences between the treatments (linear mixed model) within a study site are denoted by different lower case letters (^{a,b}) and bold font

LM = large macroaggregates

sM = small macroaggregates

m = microaggregates

s+c = silt and clay

Table 3

Amount of N (g N m^{-2}) within soil fractions acquired from wet sieving of whole soil and fractions isolated from large and small macroaggregates of the top 5 cm layer from earthworm middens and surrounding bulk soil at three study sites under no-till management (mean ± standard error).

		Site 1		Site 2		Site 3	
		Midden soil	Bulk soil	Midden soil	Bulk soil	Midden soil	Bulk soil
Whole soil	LM	**36.0 ± 3.2^{a}**	**18.8 ± 1.1^{b}**	**61.5 ± 5.4^{a}**	**27.7 ± 3.3^{b}**	17.4 ± 1.8^{a}	18.3 ± 2.2^{a}
	sM	69.7 ± 4.3^{a}	58.5 ± 6.8^{a}	55.6 ± 4.9^{a}	70.4 ± 8.5^{a}	48.8 ± 1.6^{a}	66.2 ± 8.0^{a}
	m	45.7 ± 4.0^{a}	69.6 ± 9.7^{a}	24.5 ± 2.2^{a}	27.2 ± 3.3^{a}	39.3 ± 3.5^{a}	36.2 ± 4.4^{a}
	s+c	16.5 ± 1.5^{a}	20.8 ± 2.5^{a}	8.4 ± 0.7^{a}	10.5 ± 1.3^{a}	17.8 ± 1.8^{a}	19.0 ± 2.3^{a}
Within LM	cPOM	**6.9 ± 1.1^{a}**	**2.2 ± 0.3^{b}**	**9.8 ± 1.4^{a}**	**1.5 ± 0.2^{b}**	2.9 ± 0.4^{a}	1.7 ± 0.2^{a}
	mM	18.4 ± 2.7^{a}	9.8 ± 1.4^{a}	**31.9 ± 4.2^{a}**	**15.6 ± 1.8^{b}**	9.4 ± 1.3^{a}	9.7 ± 1.1^{a}
	s+cM	**12.4 ± 1.8^{a}**	**6.1 ± 0.8^{b}**	**18.0 ± 2.4^{a}**	**7.8 ± 0.9^{b}**	6.0 ± 0.9^{a}	5.0 ± 0.6^{a}
Within sM	cPOM	11.7 ± 0.9^{a}	9.8 ± 0.7^{a}	6.1 ± 0.4^{a}	6.4 ± 0.4^{a}	7.7 ± 0.6^{a}	7.6 ± 0.6^{a}
	mM	42.0 ± 2.7^{a}	33.1 ± 2.6^{a}	33.6 ± 2.2^{a}	49.7 ± 3.4^{a}	28.7 ± 2.6^{a}	33.5 ± 2.6^{a}
	s+cM	18.7 ± 1.2^{a}	18.1 ± 1.2^{a}	14.2 ± 1.1^{a}	19.9 ± 1.3^{a}	15.2 ± 1.2^{a}	19.5 ± 1.5^{a}

Statistically significant differences between different treatments (linear mixed model) within a study site are denoted by different lower case letters (^{a,b}) and bold font

LM = large macroaggregates

sM = small macroaggregates

m(M) = microaggregates (within macroaggregates)

s+c(M) = silt and clay (within macroaggregates)

cPOM = coarse particulate organic matter