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

Sheehy, Jatta

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

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6

7 Jatta Sheehy<sup>a</sup>, Visa Nuutinen<sup>a</sup>, Johan Six<sup>b</sup>, Ansa Palojärvi<sup>a</sup>, Ossi Knuutila<sup>c</sup>, Janne Kaseva<sup>a</sup>,  
8 Kristiina Regina<sup>a\*</sup>

9

10 <sup>a</sup>Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland;

11 <sup>b</sup>Department of Environmental Systems Science, Swiss Federal Institute of Technology,  
12 ETH-Zurich, Tannenstrasse 1, 8092 Zurich, Switzerland;

13 <sup>c</sup>Department of Agricultural Sciences, University of Helsinki, Koetilantie 5, P.O.Box 28,  
14 FI-00014 University of Helsinki, Finland

15 \*Corresponding author. Tel.: +358295326474. *E-mail address: kristiina.regina@luke.fi* (K.

16 Regina)

17

18 **Abstract**

19 By processing large quantities of crop residues, earthworms enhance the mineralization of  
20 organic matter but have also been shown to stabilize soil organic carbon (SOC) into soil  
21 fractions like microaggregates (53–250  $\mu\text{m}$ ) within macroaggregates (>250  $\mu\text{m}$ ) especially

1

22 in no-till soils. Our objective was to find direct evidence on the impact of an anecic, soil  
23 surface-feeding earthworm, *Lumbricus terrestris* L., on the redistribution of SOC and soil  
24 nitrogen (N) into macroaggregate-occluded soil fractions of boreal soils. We sampled soil  
25 (0-5 cm depth) from the middens of *L. terrestris* (mounds of collected residue and surface  
26 casts at the openings of its permanent burrows) and the adjacent non-midden (bulk) soil at  
27 three no-till sites in southern Finland: two clayey sites (sites 1–2) and one coarse textured  
28 site (site 3). Compared to bulk soil, the soil in *L. terrestris* middens featured general  
29 increase in aggregate size and content of SOC and N within the large macroaggregates  
30 (>2000  $\mu\text{m}$ ) at the clayey sites. The microaggregates within the large macroaggregates had  
31 accumulated more SOC and N in the midden soil especially at site 1 where 99% of the  
32 difference in total SOC between midden and bulk soil was associated with this type of SOC  
33 stabilization. At site 2, the increase in SOC found in the large macroaggregates was  
34 counteracted by a decrease in SOC in microaggregates within the small macroaggregates  
35 (250–2000  $\mu\text{m}$ ). No differences in SOC stored in soil fractions were found between midden  
36 and non-midden soil at the coarse soil site 3 with higher top soil decomposition rate  
37 compared to sites 1 and 2. Across the study sites, the total amount of SOC was 6% higher  
38 in midden soil compared to the bulk soil. These results suggest *L. terrestris* mediates the  
39 storage of SOC and N into better protected soil fractions in clay soils under boreal  
40 conditions.

41

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

43

44 **1. Introduction**

45

46 Earthworms play a key role in soil organic matter dynamics and the regulation of  
47 nutrient cycling (Blouin et al., 2013; Filser et al., 2016). They interact with and impact a  
48 multitude of soil processes including soil aggregation, decomposition of residues and  
49 formation of macropores, which makes earthworms, and the ecosystem services they offer,  
50 of interest when developing sustainable agroecosystems (Jones et al., 1994; Lavelle et al.,  
51 1997; Fonte et al., 2009; Giannopoulos et al., 2010; Xiang et al., 2018). Earthworms are  
52 often the predominant group of soil animals in terms of biomass (Coleman and Crossley,  
53 2004) and they can consume up to 2 t of litter ha<sup>-1</sup> yr<sup>-1</sup> (Whalen and Parmelee, 2000).  
54 Earthworm feeding enhances litter decomposition directly through their metabolism (Curry  
55 and Schmidt, 2007) and indirectly by fragmenting the coarse organic matter and increasing  
56 its surface area (Blouin et al., 2013). Earthworms egest a mixture of metabolized organic  
57 material and mineral soil as sub-surface and surface casts. According to a recent meta-  
58 analysis, total organic C, total N and total P are 40-48% higher in casts while mineral N and  
59 available P are increased by 241% and 84%, respectively (van Groenigen et al. 2019). For  
60 the majority of soil fertility relevant properties, the relative difference between casts and the  
61 bulk soil ("relative cast fertility") indicates high fertility of casts.

62 Earthworm species can be categorized in three ecological groups based on their  
63 feeding habits and the soil environment they occupy (Bouché, 1977; Lavelle and Spain,  
64 2001). Anecic species, like *L. terrestris*, make permanent, typically close to 1 m deep,  
65 vertical burrows (e.g. Nuutinen and Butt, 2003; Don et al., 2008), which open at the soil  
66 surface. These species feed on the surface litter which they pull down into their burrows  
67 and create litter and cast-made middens within sight on the soil surface (Subler and Kirsch,  
68 1998; Nieminen et al., 2015). Epigeic species live near the soil surface feeding on surface

69 litter while endogeic species mainly reside in the top soil making burrows with varying  
70 orientation while feeding on below ground SOM.

71 Soil macroaggregates (>250  $\mu\text{m}$ ) and microaggregates (53–250  $\mu\text{m}$ ) protect soil  
72 organic carbon (SOC) within them from mineralization. These soil physical fractions  
73 increase the residence time of SOC by both offering physical protection from microbial  
74 decomposition and by creating conditions of low oxygen content that significantly slow  
75 down the decay of organic matter (Six et al., 2002). The turnover rate of SOC is affected by  
76 its distribution among the different aggregate fractions (Six et al., 2000; Bossuyt et al.,  
77 2002). Microaggregates are more strongly bound together than macroaggregates and thus  
78 offer a more stable long-term storage for SOC (Angers et al., 1997; Six et al., 2002).  
79 However, macroaggregates play a key role in providing sites for microaggregate formation  
80 (Six et al., 2000).

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

93 short term; this time dependence in effect has been discussed as the “earthworm dilemma”  
94 by Lubbers et al. (2013; 2017).

95 Effects of earthworms are especially interesting in long-term no-till management  
96 which has been found to increase both earthworm numbers and aggregate size (Bai et al.,  
97 2018). Enhanced top soil residue availability and low physical disturbance in no-till can  
98 increase especially the number of soil surface feeding earthworms, such as *Lumbricus*  
99 *terrestris* L. (Briones and Smith, 2017) which mix the residues into the soil and thus alter  
100 the soil structure and nutrient availability (Thevathasan and Gordon, 2004; Whalen and  
101 Fox, 2006; Bai et al., 2018).

102 Direct evidence on macroaggregate formation and SOC stabilization in the presence  
103 of earthworms has been gained especially in laboratory incubations (Wu et al., 2017) or  
104 field studies in temperate and tropical environments (Blanchart et al., 1999; Fonte and Six,  
105 2010; Arai et al., 2018). Boreal conditions with soil frosting, low carbon input in crop  
106 residues (Palosuo et al., 2016) and sufficient soil moisture for decomposition throughout  
107 most of the year constrain carbon accrual and relatively modest effects of no-till or reduced  
108 tillage on SOC stocks have been observed (Sheehy et al., 2015; Singh et al., 2015). The  
109 contribution of deep-burrowing earthworms to the processes leading to SOC stabilization in  
110 no-till management in boreal conditions is largely unknown. We studied the impact of the  
111 only anecic earthworm species found in the arable soils of Finland, *L. terrestris*, on soil  
112 aggregation level and SOC and N division between soil aggregates in three Finnish no-till  
113 sites by comparing soil sampled from earthworm middens with the bulk soil. Our aim was  
114 to elucidate if the observed no-till induced changes in aggregate size and redistribution of  
115 SOC in these soils (Sheehy et al., 2015) could result from earthworm activities. We  
116 hypothesized that *L. terrestris* middens would have a higher content of SOC and N in the

117 best protected soil fractions compared to the bulk soil thus providing evidence of the role of  
118 earthworms in SOC stabilization in boreal arable soils. As clay soils often are found to  
119 favor SOC sequestration, we expected to see more midden-associated SOC in clayey than  
120 coarse textured soil.

121

## 122 **2. Material and methods**

123

### 124 *2.1 Study site and management information*

125

126 This study took place at three long-term no-till fields in southwestern Finland. Two fields  
127 (sites 1 and 2) were located in Jokioinen (60°49'N and 23°30'E) with a yearly average  
128 precipitation of 627 mm and average temperature of 4.6°C. Soils at both these sites were  
129 classified as Vertic Luvisc Stagnosol (IUSS Working Group WRB, 2015). The third field  
130 (site 3) was classified as Eutric Regosol (WRB) and was located in Säkylä (60°58'N and  
131 22°31'E) which has a yearly average precipitation of 614 mm and an average temperature  
132 of 4.8 °C. Sites 1–2 were field experiments (randomized complete-block design with four  
133 replicates) and the field of site 3 belonged to a private farmer (plot size 100-250 m<sup>2</sup>; 4  
134 pseudoreplicates).

135 No-till practice, in which the crop was sown without prior soil tillage, had been used  
136 at the study sites for eleven (sites 1–2) or twelve (site 3) years (Table 1). Spring barley  
137 (*Hordeum vulgare*) was cultivated at sites 1 and 2 and spring turnip rape (*Brassica rapa*  
138 subsp. *oleifera*) at site 3. However, at site 3, spring barley had been cultivated during the  
139 previous years. Fields were sown and fertilized in May. Seeds were directly sown to 3–5  
140 cm depth with combined drill having triple disc coulters (site 3, row space 15 cm, front

141 single disc coulters) or double disc coulters (sites 1 and 2, row space 14.5 cm, packing  
142 the sowing coulters) or double disc coulters (sites 1 and 2, row space 14.5 cm, packing  
143 wheels behind the drill). The direct drills placed the seeds and fertilizer in the same row.  
144 The whole annual fertilizer application of 100 kg N ha<sup>-1</sup> yr<sup>-1</sup> (sites 1 and 2) or 80 kg N ha<sup>-1</sup>  
145 yr<sup>-1</sup> (site 3) was made during sowing. Granular ammonium nitrate NPK fertilizer was used  
146 at sites 1 and 2 and liquid fertilizer (Urea 32) was used at site 3. Compared to average yield  
147 during the past decade, below average yields were harvested in August at all study sites  
148 (Table 1).

149

## 150 2.2 Soil sample collection and analysis

151

152 Soil for studying the physical soil fractions was sampled in September 2010 about a month  
153 after harvest at all study sites. The samples were taken from *L. terrestris* middens and  
154 surrounding bulk soil (at least 15 cm from the closest middens) to the depth of 5 cm with a  
155 5 cm diameter soil corer. The midden soil samples included the burrow entrance of *L.*  
156 *terrestris* and were comprised mostly of soil, casts and straw. The bulk soil samples  
157 represented the soil without a *L. terrestris* midden-burrow complex. Four midden soil –  
158 bulk soil pairs were sampled from four different locations at each study site. The four  
159 samples in each location were pooled to form four replicates of large enough samples for  
160 soil fractionation.

161 The aggregate size distribution was analyzed by separating different aggregates by  
162 wet sieving according to Elliot et al. (1986) and as described by Sheehy et al. (2015). The  
163 field-moist soil samples were sieved through an 8 mm sieve and then air-dried. An 80 g  
164 subsample of soil was taken for the wet sieving which was done through a series of three



165 sieves that separated the samples into four different soil fractions; large macroaggregates  
166 (LM; >2000  $\mu\text{m}$ ), small macroaggregates (sM; 250–2000  $\mu\text{m}$ ), microaggregates (m; 53–  
167 250  $\mu\text{m}$ ) and silt and clay (s+c; <53 $\mu\text{m}$ ). Prior to wet sieving the samples were submerged  
168 into deionized water on top of the 2000  $\mu\text{m}$  sieve for a period of 5 min. The sieving was  
169 done by manually moving the sieve up and down 50 times during a 2 min period. The sieve  
170 was backwashed and the fraction remaining on top of the sieve was collected in an  
171 aluminum pan and oven-dried at 60°C. Organic material (plant residues) floating on the  
172 water after sieving with the 2000  $\mu\text{m}$  sieve was discarded as it is not considered SOM by  
173 definition. The sieving was similarly repeated with the remaining sieves.

174         Microaggregates within LM and sM fractions were isolated according to Six et al.  
175 (2000) and as described in Sheehy et al. (2015). The goal of this method was to break down  
176 the macroaggregates while avoiding the breakdown of the released microaggregates. A  
177 separate subsample of 10, 5 or 3 g, depending on the amount of available material, was  
178 taken from LM and sM fractions and was placed on top of a 250  $\mu\text{m}$  mesh. The sample was  
179 shaken with 50 stainless steel beads (4 mm diameter) in a reciprocal shaker with a  
180 continuous flow of running deionized water until all the macroaggregates were broken  
181 down (3–5 min of shaking depending on the soil type). The microaggregates and other  
182 released material went through the mesh screen with the running water ending up on a 53  
183  $\mu\text{m}$  sieve and were then sieved as in the wet-sieving method. As a result three different  
184 fractions were isolated from the macroaggregates: coarse particulate organic matter (cPOM;  
185 >250  $\mu\text{m}$ ), microaggregates within macroaggregates (mM; 53–250  $\mu\text{m}$ ) and silt and clay  
186 (s+cM; < 53  $\mu\text{m}$ ).

187         The mean weight diameter (MWD) of the aggregates, that can be used as an indicator  
188 of aggregate stability, was calculated according to van Bavel (1949). Carbon and N content

189 of all fractions from wet sieving and microaggregate isolation were analyzed with a CN-  
190 analyzer (ECS 4010, Costech Instruments, USA). The SOC and N content of the different  
191 aggregates and the total C and N was calculated using the equivalent soil mass method  
192 according to Lee et al. (2009) which takes into account the different bulk densities of the  
193 soils sampled.

194

### 195 *2.3 Decomposition rate measurements*

196

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

213 Barley straw and pea residue in the residue bags were analysed for mass loss. Due to the 1  
214 mm mesh size, a variable amount of surrounding soil was incorporated into the bags. The  
215 mixture of plant residue and soil from the bags was ground and about 2 g of the ground  
216 sample was taken for the loss on ignition (LOI) analysis. The samples were incinerated at  
217 550 degree C for 5 h in a high temperature muffle furnace. This enables calculating the  
218 content of organic matter in the samples as the ignition leaves the mineral part of the soil as  
219 ash while organic matter is lost. The results from the separate LOI analysis from the  
220 original barley straw and pea residue, and the surrounding soil samples, were used for  
221 correction (ash free dry weight).

222

#### 223 *2.4 Statistical analysis*

224

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

232

$$233 y_{ijkl} = \mu + T_i + R_j + TR_{ij} + F_k + FB_{kl} + TF_{ik} + TFB_{ikl} + TRF_{ijk} + RF_{jk} + RFB_{jkl} + \epsilon_{ijkl}, (1)$$

234

235 where  $\mu$  is the overall mean,  $T_i$ ,  $R_j$  and  $F_k$  are the fixed effects of the treatment, fraction and  
236 field, respectively. Two- and three-factor interactions of fixed effects were also included in

237 the model; interaction of the block,  $B_i$ , with other factors ( $FB_{kl}$ ,  $TFB_{ikl}$  and  $RFB_{jkl}$ )  
238 represent the random effects, and  $\varepsilon_{ijkl}$  is the random error term of the model. The random  
239 variables were assumed independent and normally distributed. The mean weight diameter  
240 (MWD) was analyzed using the same model without the different fractions.

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

253 The appropriateness's of the models were studied by residual analyses. The residuals  
254 were checked for normality using boxplot and normal probability plot (Tukey, 1977). The  
255 residuals were also plotted against the fitted values. These plots indicated that the  
256 assumptions of the models are adequate. Comparison of means was done with the Tukey-  
257 Kramer post hoc test. A significance level of  $\alpha=0.05$  was used in all analysis. Degrees of  
258 freedom were calculated using Kenward-Roger method. The analyses were performed

259 using the MIXED procedure of the SAS Enterprise Guide 5.1 (SAS Institute Inc., Cary,  
260 NC, USA).

261

### 262 **3. Results**

263

#### 264 *3.1 Aggregate weight and stability*

265

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

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

283           The MWD of aggregates was on average 20% higher across all study sites in *L.*  
284 *terrestris* middens compared to the surrounding soil (site 1: 35%; site 2: 21%; site 3: 3%)  
285 (Fig. 2). MWD was significantly higher in the middens versus surrounding bulk soil at the  
286 clayey sites 1 and 2 ( $p=0.001$  and  $p=0.002$  respectively), but not at the coarse textured site  
287 3 (Fig. 2).

288

### 289 *3.2 Soil carbon*

290

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

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

301

### 302 *3.3 Soil nitrogen*

303

304           The amount of total N in midden soil versus bulk soil did not differ at any sites (results not  
305 shown). However, higher N content in large macroaggregates in midden soil versus  
306 surrounding bulk soil was found at the clayey sites 1 and 2 ( $p<0.001$ ) (Table 3). No

307 changes in N content were found at site 3. Differences were found, however, in the N levels  
308 between different fields. For example, site 1 had more N in the top soil than sites 2 or 3  
309 ( $p=0.003$  and  $p=0.011$  respectively) (results not shown).

310 Nitrogen content in cPOM within LM fractions was higher in midden soil compared  
311 to bulk soil at sites 1 and 2 ( $p<0.001$ ) (Table 3). At these sites the cPOM-N content was 3–  
312 6 times higher in middens than bulk soil. More N was also found in midden versus  
313 surrounding bulk soil in LM-occluded microaggregates at site 2 ( $p=0.005$ ) and silt and clay  
314 fraction at sites 1 and 2 ( $p=0.04$  and  $p=0.001$  respectively). No differences in soil N content  
315 were found in small macroaggregate-occluded fractions between midden and bulk soil.

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

318

#### 319 *3.4 Decomposition rate of barley straw and pea residue*

320

321 More residue decomposed from the pea residue bags compared to the barley straw bags at  
322 sites 1 and 2 ( $p<0.001$ ) (Fig. 4a). A significant difference in the decomposition rate at all  
323 sites was found between the residue bags on the top soil versus under 10 or 20 cm of soil  
324 with an increasing trend in decomposition rate deeper in the soil profile ( $p<0.001$ ) (Fig. 4b).  
325 Differences between the two deeper layers were smaller but still significant at sites 1 and 3  
326 ( $p=0.04$ ;  $p=0.004$ ). Over 65% of both barley straw and pea residue decomposed at the 20  
327 cm depth within a year. Sites differed from each other in their decomposition rate in the  
328 topsoil ( $p=0.017$ ) where site 3 had the highest decomposition rate. No difference in the  
329 decomposition rate was found between sites in the other two soil layers indicating that the  
330 decomposition rate does not increase as fast with increasing depth at site 3 compared to the

331 other two sites. However, site 1 had a higher decomposition rate of pea residue compared to  
332 site 3 ( $p=0.042$ ). Decomposition rate was lower at all depths during the cold winter months  
333 with average decomposition rates of only 0.09% per day compared to 0.17% per day during  
334 the growing season ( $p<0.001$ ) (Fig. 4c). Lowest decomposition rate was found at 0 cm at  
335 site 1 where only 14% of the barley straw was decomposed in the first 6 months after  
336 installing the bags.

337

## 338 **4. Discussion**

339

### 340 *4.1 Aggregate stability and aggregate-associated SOC*

341

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

351 The total SOC content of the midden-associated soil compared to the bulk soil was on  
352 average 8% higher at the clayey sites and 3% higher for the coarse textured soil. These  
353 results are in accordance with several studies indicating higher SOC sequestration rates in  
354 soils with higher clay content (e.g. Leifeld et al., 2005; Heikkinen et al., 2013). Better



355 protection of SOC in clayey soils is often attributed to less accessible soil pores for  
356 microbes and SOC binding to mineral surfaces (Strong et al., 2004; von Lützow et al.,  
357 2006; Simonetti et al., 2017).

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

377 Part of the observed increase in the LM fraction can likely be explained by the  
378 smaller fractions growing in size. Indication of this was the earthworm-induced

379 redistribution of SOC from sM fraction to LM fraction in the middens. The SOC content of  
380 the middens was 11% higher in the LM fractions and 6% lower in the sM fraction  
381 compared to bulk soil. These findings are similar to results presented by Fonte and Six  
382 (2010) who found a redistribution of SOC into large macroaggregates and the  
383 microaggregates within them by earthworms. Similarly, in Peruvian Amazonia, endogeic  
384 earthworms increased the proportion of large macroaggregates (>2 mm) with almost 6% at  
385 the expense of smaller aggregates (<0.5mm) that decreased by 8% after six successive crop  
386 cycles (Lavelle et al., 2004).

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

397 Our results represent the situation in soil in autumn, the period of abundant crop  
398 residues and high earthworm activity and thus the results do not represent the average  
399 annual situation. Although the biological decomposition in winter is half of the rate in  
400 summer (Fig. 4c), there are more physical forces breaking down aggregates in the winter  
401 time, particularly the frequent freezing and thawing of the soil that can reduce MWD to half  
402 of the autumn values (Edwards, 2013). Even though the increased earthworm midden

403 associated aggregation level is potentially beneficial in the short-term it may not be enough  
404 to create long-term SOC accumulation. Don et al. (2008) concluded that the *L. terrestris*  
405 burrow associated increase in topsoil SOC may be a short-term one, as their study showed  
406 that the walls of earthworm-occupied burrows had up to three times higher values of SOC  
407 than abandoned earthworm burrows. Fonte and Six (2010) argued that since most of the  
408 SOC in earthworm casts is associated with macroaggregate-occluded microaggregates the  
409 rate of decomposition in these casts would, with time, possibly decrease to a level below  
410 the level of non-ingested soil.

411 Even though there was an increase in the topsoil SOC content in the middens across  
412 all sites, this may not be relevant for the SOC sequestration at field scale. Previous studies  
413 have shown that the total density of earthworm burrows in the no-till plots of these study  
414 sites were higher in comparison to conventionally tilled plots (Regina and Alakukku,  
415 2010), but the total SOC stocks did not differ between the treatments (Sheehy et al., 2013).  
416 This is in line with the meta-analysis by Lubbers et al. (2013) where no earthworm-induced  
417 total SOC increase was found. However, the results of this study suggest that the presence  
418 of anecic earthworms predicts increased chances to develop relatively stable sites for SOC  
419 storage especially as the continuation of no-till enables further increase of earthworm  
420 numbers.

421

#### 422 *4.2 Nitrogen content and aggregate-associated N*

423

424 Nitrogen allocation in soil fractions followed closely the trends observed for SOC. Nitrogen  
425 levels were markedly higher in the middens of the clayey soils, especially within the coarse  
426 particulate organic matter and microaggregate fractions within large macroaggregates; this

427 was accompanied by an increase in the amount of the large macroaggregates in midden  
428 soil. This underlines *L. terrestris*'s ability to accumulate N within soil particles that are  
429 more resistant to decomposition, especially in clayey soils. On the other hand, the total  
430 production of mineral N by the earthworm community can be as high as 74 kg ha<sup>-1</sup> yr<sup>-1</sup>  
431 (Whalen and Parmelee, 2000; Lavelle et al., 2004) which, for instance, almost equals the  
432 average amount of mineral N added annually by farmers in Finland. Integration of N into  
433 macroaggregate-occluded microaggregates at these sites may counteract the N  
434 mineralization effect of earthworms and slow down the N cycle in the top soil. The net  
435 effect of earthworms for N cycling in the soil can e.g. determine the nutrient leaching  
436 potential of the soil. The presence of earthworms can increase (Dominguez et al., 2004) or  
437 decrease (Shuster et al., 2002) N leaching and it has been suggested that there is a threshold  
438 value of earthworm density above which the positive effects of increased density turn to  
439 increased leaching potential (Shuster et al., 2002).

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

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

451 (53–250  $\mu\text{m}$ ) was higher when residues were added as a residue layer on the top of the soil  
452 instead of incorporating them into the soil. Sheehy et al. (2013) did not, however, observe  
453 any consistent increase in the amount of total N in the 0–20 cm layer in no-till versus  
454 conventionally tilled soil at any of these sites. Protecting N into macroaggregate-occluded  
455 microaggregates would be beneficial, especially in fields with high or moderate N leaching  
456 potential. There are indications of higher density of *L. terrestris* individuals and biomass in  
457 no-till compared to tilled plots at site 2 (unpublished results). This together with higher  
458 levels of large macroaggregate-occluded N in *L. terrestris* middens at the clayey sites found  
459 in this study, suggests that N cycling slows down in no-till cultivation.

460

## 461 **5. Conclusions**

462

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

472

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474

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482

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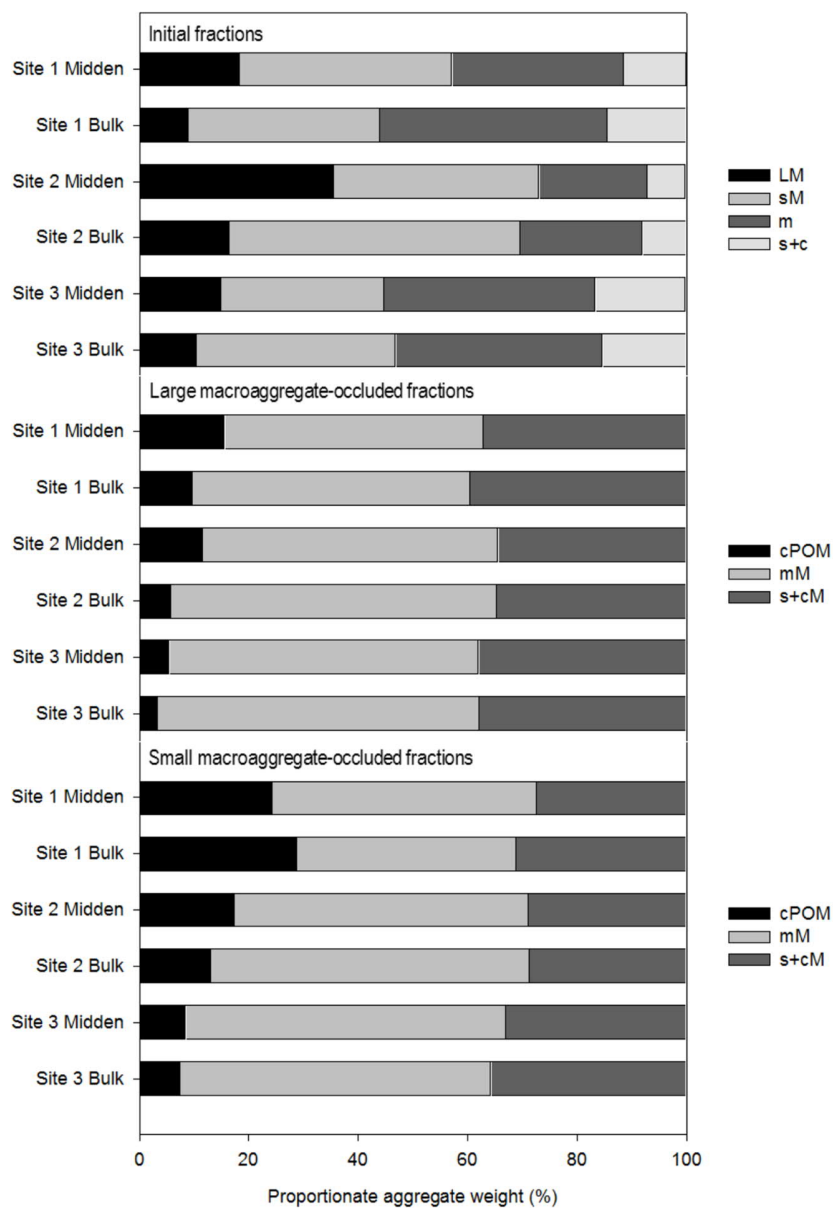
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648



649

650

**Fig. 1** Proportional weights (%) of the aggregate fractions ( LM = large macroaggregates, sM =

651

small macroaggregates, m = microaggregates, s+c = silt and clay) and microaggregate isolation

652

from large and small macroaggregates ( fractions within the aggregates: cPOM=particulate organic

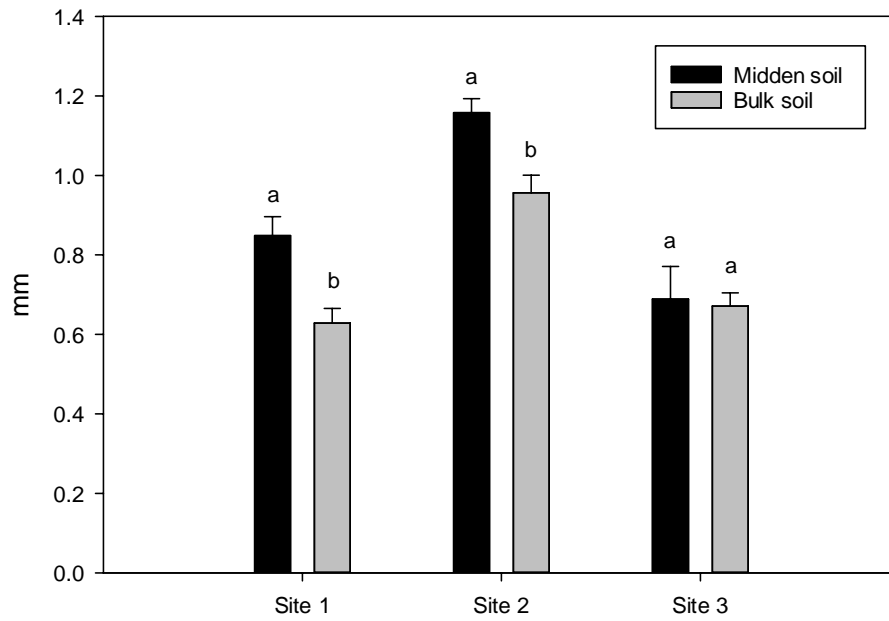
653

matter, mM=microaggregates, s+cM=silt and clay) in soil from earthworm middens and

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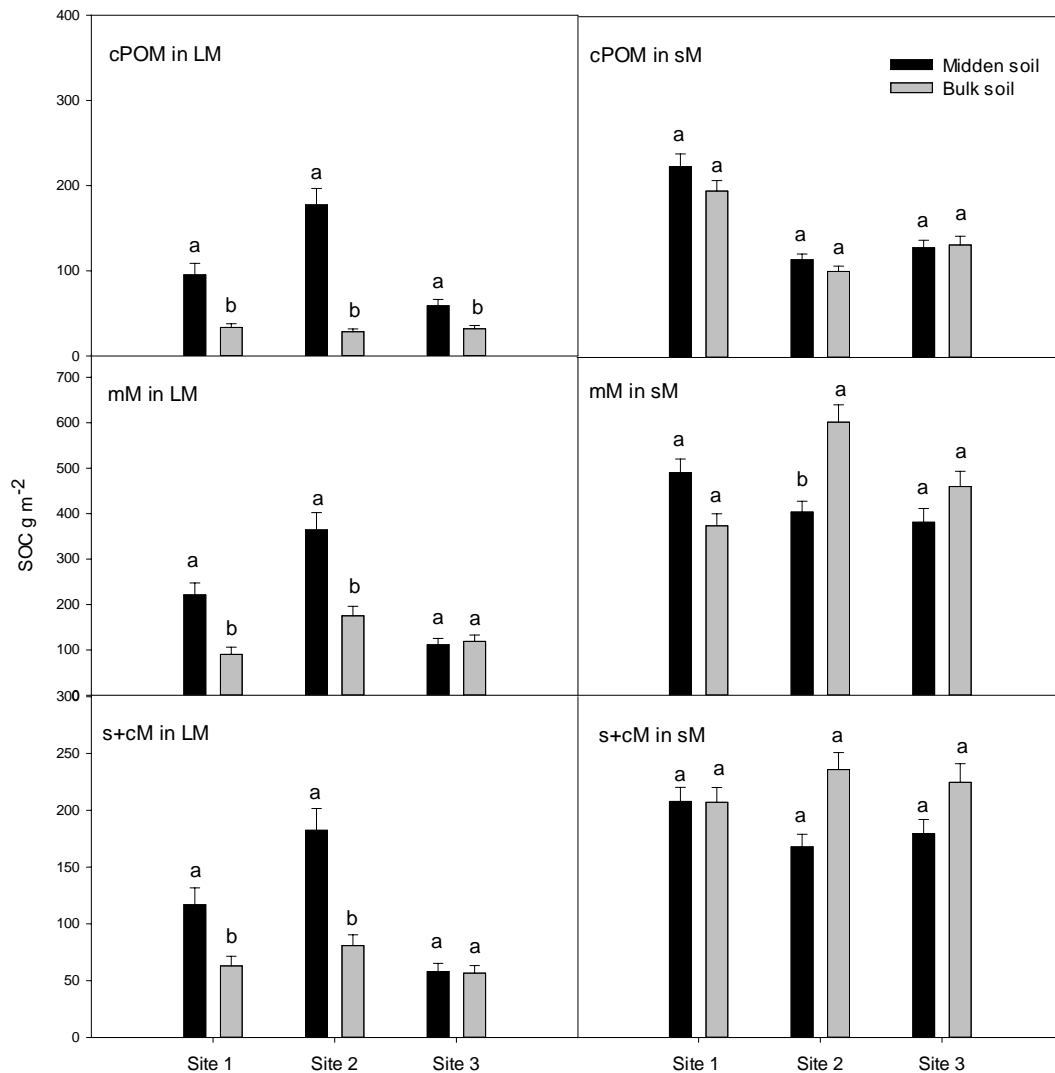
surrounding bulk soil at three study sites under no-till management.

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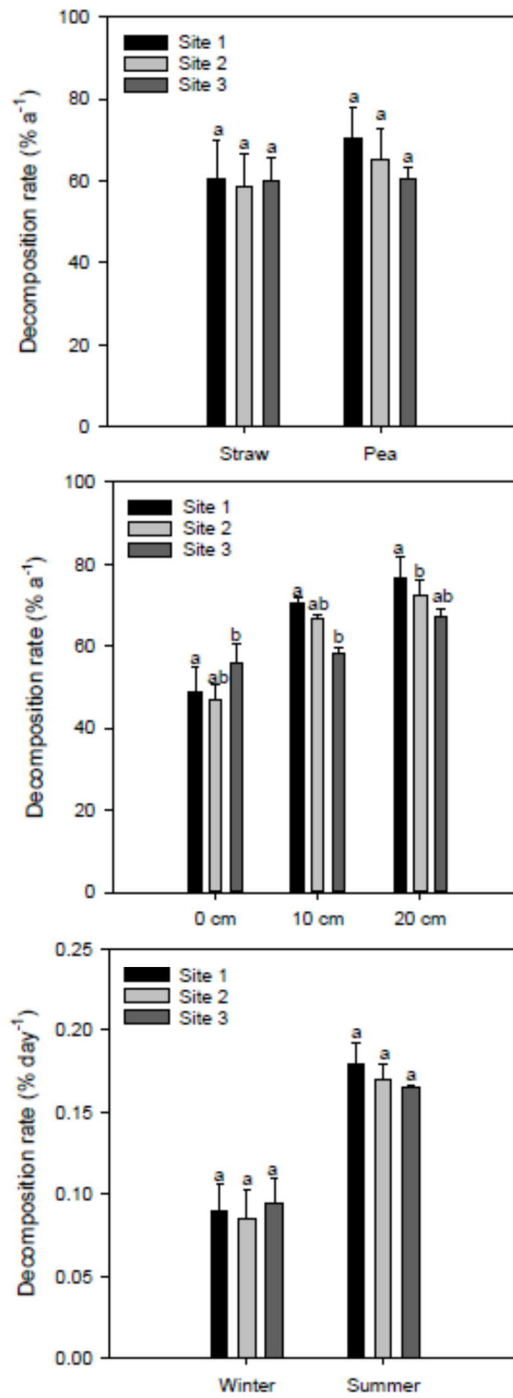
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657 **Fig. 2** Mean weight diameter (MWD; mm) of earthworm middens and surrounding bulk soil at  
 658 three study sites under no-till management (mean  $\pm$  standard error). Different letters denote  
 659 statistical differences between midden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



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



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



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**Table 1**

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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 <sup>-1</sup> yr <sup>-1</sup> )	100	100	80
Crop 2010	Spring barley	Spring barley	Turnip rape
Yield 2010 (kg ha <sup>-1</sup> )	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 <sup>-3</sup> )	1.33	1.02	1.37
<i>L. terrestris</i> density (ind. m <sup>-2</sup> )†	ND‡	12	27
<i>L. terrestris</i> biomass (g m <sup>-2</sup> )†	ND‡	25	67

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†Data from Sep 2009; combination of hand sorting and formalin extraction methods (International

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Organization for Standardization 2006)

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‡ND = Not Determined

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**Table 2**

Amount of soil organic carbon (g C m<sup>-2</sup>) 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 <sup>a</sup>	1900±48 <sup>b</sup>	1800±51 <sup>a</sup>	1660±25 <sup>b</sup>	1730±85 <sup>a</sup>	1690±77 <sup>b</sup>
LM	<b>440 ± 45<sup>a</sup></b>	<b>196 ± 17<sup>b</sup></b>	<b>737 ± 59<sup>a</sup></b>	<b>305 ± 30<sup>b</sup></b>	229 ± 21 <sup>a</sup>	207 ± 20 <sup>a</sup>
sM	859 ± 31 <sup>a</sup>	761 ± 72 <sup>a</sup>	657 ± 48 <sup>a</sup>	895 ± 34 <sup>a</sup>	663 ± 53 <sup>a</sup>	834 ± 56 <sup>a</sup>
m	555 ± 44 <sup>a</sup>	758 ± 85 <sup>a</sup>	304 ± 24 <sup>a</sup>	329 ± 32 <sup>a</sup>	519 ± 42 <sup>a</sup>	492 ± 48 <sup>a</sup>
s+c	177 ± 14 <sup>a</sup>	226 ± 22 <sup>a</sup>	98 ± 8 <sup>a</sup>	112 ± 11 <sup>a</sup>	215 ± 20 <sup>a</sup>	220 ± 22 <sup>a</sup>

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Statistically significant differences between the treatments (linear mixed model) within a study site are denoted by different lower case letters (<sup>a,b</sup>) and bold font

LM = large macroaggregates  
sM = small macroaggregates  
m = microaggregates  
s+c = silt and clay

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**Table 3**

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

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Statistically significant differences between different treatments (linear mixed model) within a study site are denoted by different lower case letters (<sup>a,b</sup>) 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

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