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Microbial communities of *Lumbricus terrestris* L. middens: structure, activity, and changes through time in relation to earthworm presence

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Abstract
Background, aim, and scope Earthworms make a major contribution to decomposition in ecosystems where they are present, mainly acting in the drilosphere, that is, galleries, burrows, casts, and middens. Earthworm middens are hot-spots of microbial activity and nutrient dynamics and represent a suitable model for studying earthworm-mediated influences on soil microbial communities by alteration of the patch structure of the microbial environment. We studied the structure and activity of the microbial communities in the soil system formed by middens of *Lumbricus terrestris* and the soil below and surrounding them and the role of earthworms in maintaining these structures through time.
Material and methods We set up an experiment in which middens were either left (control) or removed from their original place (translocated) and left in a nearby area free of earthworm activity for 2 months. After 1 and 2 months we sampled middens, soil below them, and surrounding soil. We analyzed the phospholipid fatty acid (PLFA) profiles and measured respiratory fluxes of CO_2 and CH_4.
Results Microbial communities of middens clearly differed from those of soil below and surrounding soil samples, showing higher bacterial and fungal PLFAs ($p<0.0001$ and $p<0.01$, respectively); furthermore, changes in microbial communities were stronger in control middens than in translocated middens. Moreover, gram positive and negative bacterial PLFAs were greater in translocated than control middens ($p<0.0001$ and $p<0.001$, respectively), as well as total organic carbon ($p<0.001$). Microbial activity was higher in middens than in soil below and surrounding soil samples both for CO_2 ($p<0.0001$) and CH_4 ($p<0.0001$).
Discussion Soil bioturbation by the earthworm *L. terrestris* was strong in their middens, but there was not any effect on soil below and surrounding soil. Microbial communities of middens maintain their biomass and activity when earthworms were not present, whereas they decreased their biomass and increased their activity when earthworms were present.
Conclusions Earthworms strongly enhanced microbial activity measured as CO_2 production in middens, which indicates that there are hot spots for soil microbial dynamics and increasing habitat heterogeneity for soil microorganisms. Moreover, our data strongly support the fact that the impact of this earthworm species in this soil is restricted to their middens and increasing soil heterogeneity.
Recommendations and perspectives Our data indicate that it is not clear if earthworms enhance or depress microbial communities of middens since the microbial activity increased, but did not modify their biomass and this was not dependent on soil organic C content. These results indicate no competence for C pools between this anecic earthworm and microorganisms, which has been found for other earthworm species, mainly endogeics. Conversely, they suggest some type of facilitation due to the release of additional nutrient pools in middens when earthworms are

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present, through the digestion of middens' material or the addition of casts produced from other food sources.

Keywords Anecic earthworms · *Lumbricidae* · Methane consumption · Microbial respiration · Microbial transfer

1 Background, aim, and scope

Earthworms make a major contribution to decomposition in ecosystems where they are present, mainly acting in the drilosphere, that is, galleries, burrows, casts, and middens (Lavelle and Spain 2001). One of the most striking components of the drilosphere are the middens built by the earthworm *Lumbricus terrestris* L., which are at the surface of soil, covering the entrance of the burrows. Middens are small mounds of plant litter and casts (Neilson and Hole 1964; Hamilton and Sillman 1989), which are characterized by higher nutrient contents than parent soils (Bohlen et al. 1997; Subler and Kirsch 1998; Wilcox et al. 2002) and support a more diverse microfaunal community (Maraun et al. 1999; Schrader and Seibel 2001). Thus, earthworm middens are hot-spots of microbial activity and nutrient dynamics and represent a suitable model for studying earthworm-mediated influences on soil microbial communities by alteration of the patch structure of the microbial environment (Bohlen et al. 2002).

An important topic in earthworm ecology is the relationship that earthworms establish with microorganisms in decomposing organic matter, because they are microbial-detritivores and may compete for the same resource pools as microorganisms (Scheu and Falca 2000; Tiunov and Scheu 2004). Moreover, microbial communities of soil differ from those of the earthworm gut, casts, and burrows (Tiunov and Scheu 1999; Egert et al. 2004, Sampedro et al. 2006; Sampedro and Whalen 2007), which implies either improved or poorer conditions for decomposition of soil organic matter if microorganisms are stimulated or depressed.

We hypothesized that biomass and activity of microbial communities of middens should be favored by earthworm presence, due to the well-known enhancing effect of earthworms on soil microorganisms in these structures (Bohlen et al. 1997; Subler and Kirsch 1998; Wilcox et al. 2002). Thus, when middens are not maintained by earthworms, we would expect decreases in microbial biomass and activity through time due to depletion of resources already present in the midden and not renewed by the earthworm (i.e., casting and litter addition). We also hypothesized that the effects of the earthworm *L. terrestris* on soil microbial communities should be restricted to their middens and soil below middens due to their behavior, that is, they live in vertical burrows that they only leave for feeding and mating (Butt et al. 2003; Field and Michiels 2006). We studied the effects of *L. terrestris* middens in modifying the structure and activity of soil microbial communities and the role of earthworms in the maintenance of middens. To do this, we broke this earthworm mediated system into three components; (1) the midden, (2) soil below the midden which included burrow, and (3) soil surrounding the midden area. First, we compared the microbial communities in the three components of the system and examined how these communities change through time. Secondly, we studied the role of earthworms in these processes, that is, how microorganisms of middens react to earthworm activity (i.e., presence and active maintenance). To do this, we assessed changes in microbial communities in and around middens, with earthworms and in translocated middens to avoid the active midden maintenance by earthworms, that is, casting and incorporation of litter.

2 Material and methods

2.1 Experimental design and field sampling

We conducted this experiment in a mixed deciduous woodland at Lancaster University campus (54° 0′ N, 2° 46′ W); the dominant trees in the woodland are alder (*Alnus glutinosa*), beech (*Fagus sylvatica*), and sycamore (*Acer pseudoplatanus*), and the sparse ground flora is dominated by bramble (*Rubus fruticosus*), with the male fern (*Dryopteris filix-mas*), the wood dock (*Rumex sanguineus*), self-heal (*Prunella vulgaris*), and broad-leaved willowherb (*Epilobium montanum)* being abundant locally. The experimental site is on a silty loam soil, with a mean organic matter content of 15±1% and a pH of 6.3±0.1. On 23 March 2006, we randomly selected and flagged 60 middens from *L. terrestris* L; we flagged more middens than were needed to avoid potential problems due to loss of samples or contamination in the translocation treatment. Thirty of these middens were translocated without disturbing their structure to the same area with no evident signs of earthworm activity (e.g. casts on the soil surface) and placed randomly, these middens constituted the translocated group, and the other thirty remained in their original place as the control group. At sampling, we took the whole midden, the soil below (no more than 5 cm deep and 3 cm wide), and the soil surrounding at a distance of 20 cm from the middens, using PVC corers of 3 cm Ø. Samples (n=10) were destructively and randomly taken after 1 (24 April) and 2 months (23 May) for phospholipid fatty acid (PLFAs) analysis as well as measuring fluxes of CO_2 and CH_4 and soil organic C (n=120, three types of samples (midden, soil below, and soil surrounding), two manipulations (control and translocated) and time (1 and 2 months) and ten

replicates per treatment). Middens, soil below, and surrounding soil samples, as well as, from control samples (not displaced) were checked carefully for earthworms. Middens and soil below from a translocated treatment containing earthworms were discarded (three and seven in the first and second sampling, respectively). Samples were freeze dried, homogenized with a mortar and then carefully sieved (2 mm Ø) prior to analysis to remove any small stone. We include all the organic material comprising the middens. We expect no differences in density of samples (middens, soil below, and soil surrounding), because it has been shown that the passage through the gut of *L. terrestris* did not modify the particle-size distribution of ingested soil (Marhan et al. 2007).Temperature increased significantly during the experiment (*t* test, $p<0.001$), with a mean temperature of 11.2±0.4°C from 23 March to 24 April and a mean temperature of 14.8±0.6°C from 25 April to 23 May. However, there was no difference (*t* test, $p>0.05$) in rainfall between the same time intervals (3.0±0.7 and 3.2± 0.7 mm mean rainfall for first and second interval, respectively).

2.2 Analytical methods

Microbial communities were assessed by PLFA analysis. Total lipids were extracted from 2 g (dry weight) of samples (middens, soil below, and soil surrounding after 1 and 2 months) with methanol and chloroform (2:1 *v*:*v*). The mixture was then filtered and evaporated under an N_2 gas. The total lipid extract was then dissolved with chloroform (3×1 ml). Lipids were separated into neutral, glycol-, and phospholipids using silicic acid columns (Strata SI-1 Silica (55 µm, 70 A), 500 mg/6 ml) with chloroform, acetone, and methanol, respectively. The fraction containing phospholipids was evaporated under an N_2 stream and redissolved in 500 µl of methyl-*tert*-butyl ether. One hundred microliters of this solution were placed in a 1.5 mL vial with 50 µl of the derivatizating agent (trimethylsulfonium hydroxide, TMSH), vortexed for 30 s and allowed to react for 30 min; then 10 µl of nonadecanoic acid methyl ester were added as an internal standard (Lores et al. 2006).

GC–MS analysis of the extracts containing PLFAs was performed on a Varian system: GC 3800C, equipped with a CP-SIL 88 Varian Select FAME FS 50 m×0.25 mm× 0.2 mm capillary column and a MS detector Saturn 2000. One microliter aliquots were injected using splitless mode (2 min split closed and 1:50 split ratio). The oven temperature was programed from 50°C (2 min) to 140°C at 20°C min^{-1} and then to 250°C at 30°C min^{-1}. Helium (1 mL/min) was used as carrier gas. To identify and quantify the fatty acids, retention times and mass spectra were compared to those obtained for known standard mixtures or pure PLFAs.

The PLFAs used as biomarkers were those defined in the literature (Frostegård and Bååth 1996; Zelles 1999; Bååth 2003). Total microbial biomass was determined as the sum of all extracted PLFAs expressed as µg g^{-1} dry weight. Abundances of the different microbial groups (bacteria and fungi) were determined by the abundance of specific biomarkers commonly used for these groups. The sum of PLFAs considered to be predominantly from bacterial origin (c15:0 and c17:0) were sorted into gram positive bacteria (G^+) PLFA (i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0) and gram negative (G^-) PLFAs (c16:1c, cy17:0, c17:1c, 18:1ω7c, and cy19:0) (Frostegård and Bååth 1996); by independently summarizing these PLFAs the G+/G− ratio was calculated. Fungi were determined using c18:1ω9c and c18:2ω6c PLFAs concentration (Frostegård and Bååth 1996; Bååth 2003). The ratio of fungal/bacterial PLFAs was used as an index of the ratio of fungal/bacterial biomass in soil.

We measured the production of CO_2 and the consumption of CH_4 on five samples (five middens and their corresponding five soil below and five soil surrounding samples) per treatment and sampling time. Samples (2 g fresh weight) were placed into 120 ml Wheaton bottles, sealed and incubated at 20°C over 48 h. Gas samples were taken at time 0, 24, and 48 h and analyzed using a gas chromatograph fitted with an FID and methanizer (AutoSystem XL, Perkin Elmer). Gas fluxes were calculated as µg CO_2 and ng CH_4 h^{-1} g^{-1} soil dry weight using standard methods (Holland et al. 1999).

Soil organic C was analyzed following the modified Walkley–Black method (Jackson 1958). Briefly, samples (1 g) were placed in an Erlenmeyer flask (100 ml) and 10 ml of potassium dichromate (5%, *w*/*v*) were added; then 20 ml of sulphuric acid were added. After cooling, 50 ml of barium chloride (0.4%, *w*/*v*) were added and samples stood overnight. An aliquot was read at 600 nm (absorbance) in a microplate reader.

2.3 Statistical analyses

In order to explore the effect of different treatments applied (type of sample, manipulation, and time) data were analyzed under a complete ANOVA factorial design under general linear models in which three factors were fixed: type of sample (midden, soil below, and soil surrounding), manipulation (middens translocated or not), and time (1 and 2 months). Means were separated with Fisher LSD post-hoc tests. We found differences in moisture content between the two sampling dates in middens, but not in soil below or soil surrounding samples. Due to the strong importance that moisture content plays in microbial community dynamics, it was introduced as a covariate in all analysis, and it had no significant effects on any of the variables analyzed.

Temperature also has a significant impact on soil microbes and, despite a marked increase in mean temperature (35°C) between sampling dates, we did not find any effect of time in any microbial biomass parameters analyzed except those regarding microbial activity. In order to analyze the underlying effect of type of sample and manipulation through time, data were subjected to discriminant analysis. All analyses were performed with Statistica 6.0.

3 Results

The discriminant analysis of the 29 identified PLFAs clearly differentiated middens (translocated after 1 and 2 months and control after 2 months) from soil below and surrounding soil (Fig. 1). In addition, microbial communities of middens differed between control and translocated samples and the presence of earthworms also played an important role in modifying microbial communities through time (see Fig. 1). In fact, after 1 month, microbial communities of translocated middens differed from those of control middens, a difference that increased after 2 months. However, there were no differences in microbial communities in soil below and soil surrounding samples, suggesting that earthworms restricted their activity to middens. Total PLFA was significantly affected by type of sample (ANOVA, $F_{2,108}$= 17.95, p<0.0001) and manipulation (ANOVA, $F_{1,108}$=7.39, p<0.001). Thus, midden samples (control and translocated together, 77.90±3.61 μg g^{-1} dw) had 1.4 times more total PLFA than soil below and surrounding soil (55.77±3.40 and 55.52±2.27 μg g^{-1} dw, respectively; Fisher LSD test, p<0.0001). Furthermore, total PLFA (average of 1 and 2 months samples) was higher in translocated (67.82± 2.85 μg g^{-1} dw) than in control middens (58.30± 2.81 μg g^{-1} dw), due to the increase of microbial biomass in translocated middens after 2 months (76.85±6.82 and 98.07±5.83 μg g^{-1} dw for 1 and 2 months, respectively). Fungal PLFAs were only affected by type of sample, with higher contents in middens (3.21±0.26 μg g^{-1} dw) than in soil below and soil surrounding samples (2.08±0.70 and 1.18±0.07 μg g^{-1} dw, respectively; ANOVA, $F_{2,108}$=5.38, p<0.01; Fisher LSD test, p<0.01; Fig. 2a); there were no effects on fungal/bacterial PLFAs. Bacterial PLFAs were significantly higher in midden (53.12±2.73 μg g^{-1} dw) than in soil below and soil surrounding samples (37.06± 1.97 and 39.61±1.83 μg g^{-1} dw, respectively) (ANOVA, $F_{2,108}$=16.86, p<0.0001; Fisher LSD test, p<0.0001; see Fig. 2a), and bacterial PLFAs were more abundant in translocated (46.93±2.13 μg g^{-1} dw) than in control samples (39.59±1.78 μg g^{-1} dw; ANOVA, $F_{1,108}$=9.13, p<0.01). Gram positive (G^+) bacterial PLFAs were significantly higher in middens (14.25±1.03 μg g^{-1} dw) than in soil below and soil surrounding samples (8.12± 0.43 and 8.55±0.34 μg g^{-1} dw, respectively) (ANOVA, $F_{2,108}$=33.85, p<0.0001; Fisher LSD test, p<0.0001; Fig. 2b), with also higher contents in translocated (11.95±0.74 μg g^{-1} dw) than in control samples (8.67± 0.48 μg g^{-1} dw) (ANOVA, $F_{1,108}$=23.29, p<0.0001); this resulted in a significant interaction between type of sample and manipulation (ANOVA, $F_{2,108}$=4.82, p<0.01; see Fig. 2b). Gram negative bacterial PLFAs only were affected by type of sample with higher contents in middens (37.29± 1.80 μg g^{-1} dw) than in soil below and soil surrounding samples (28.12±1.65 and 29.76±1.62 μg g^{-1} dw, respectively) (ANOVA, $F_{2,108}$=8.50, p<0.001; Fisher LSD test, p<0.01; see Fig. 2b). The G^-/G^+ ratio showed a significant effect of type of samples, with higher values in soil below (3.91±0.37) than in middens and soil surrounding samples (2.93±0.17 and 3.51±0.16, respectively) (ANOVA, $F_{2,108}$= 3.68, p<0.05; Fisher LSD test, p<0.01; see Fig. 2b), with also higher values in control (3.86±0.26) than in translocated ones (3.03±0.12; ANOVA, $F_{1,108}$=8.01, p<0.01).

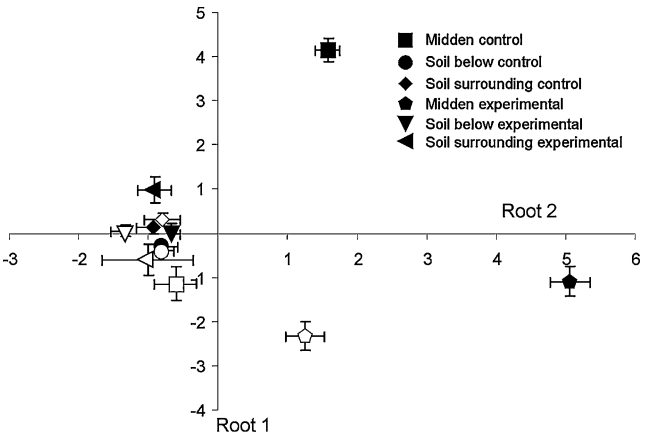

Fig. 1 Discriminant function analysis performed on 29 identified PFLAs of bacterial and fungal origin from samples of middens, soil below, and soil surrounding both control and translocated treatments after 1 (*white symbols*) and 2 months (*black symbols*) (Wilk's lambda 0.004, $F_{319,\ 857}$=1.6651, P<0.0001; *Root 1* represents 35.56% of the variance, *Root 2* 27.8%). Mean±SEM, n=10

Microbial activity, i.e., CO_2 respiration, was strongly affected by type of sample, middens having higher rates of respiration (35.01±6.53 μg CO_2 h^{-1} g^{-1} dw) than soil below and surrounding soil (18.10±2.71 and 12.38± 1.52 μg CO_2 h^{-1} g^{-1} dw, respectively) (ANOVA, $F_{2,108}$= 19.02, p<0.0001; Fisher LSD test, p<0.0001; Fig. 3a). This trend increased with time and with earthworm presence but differentially since middens always respired more CO_2 than soil below and surrounding soil producing a significant triple interaction between sample, manipulation and time (see Fig. 3a, ANOVA, $F_{2,108}$=6.02, p<0.01). Most of the samples were net consumers of CH_4, however, net CH_4 production was observed for two treatments (Fig. 3b).

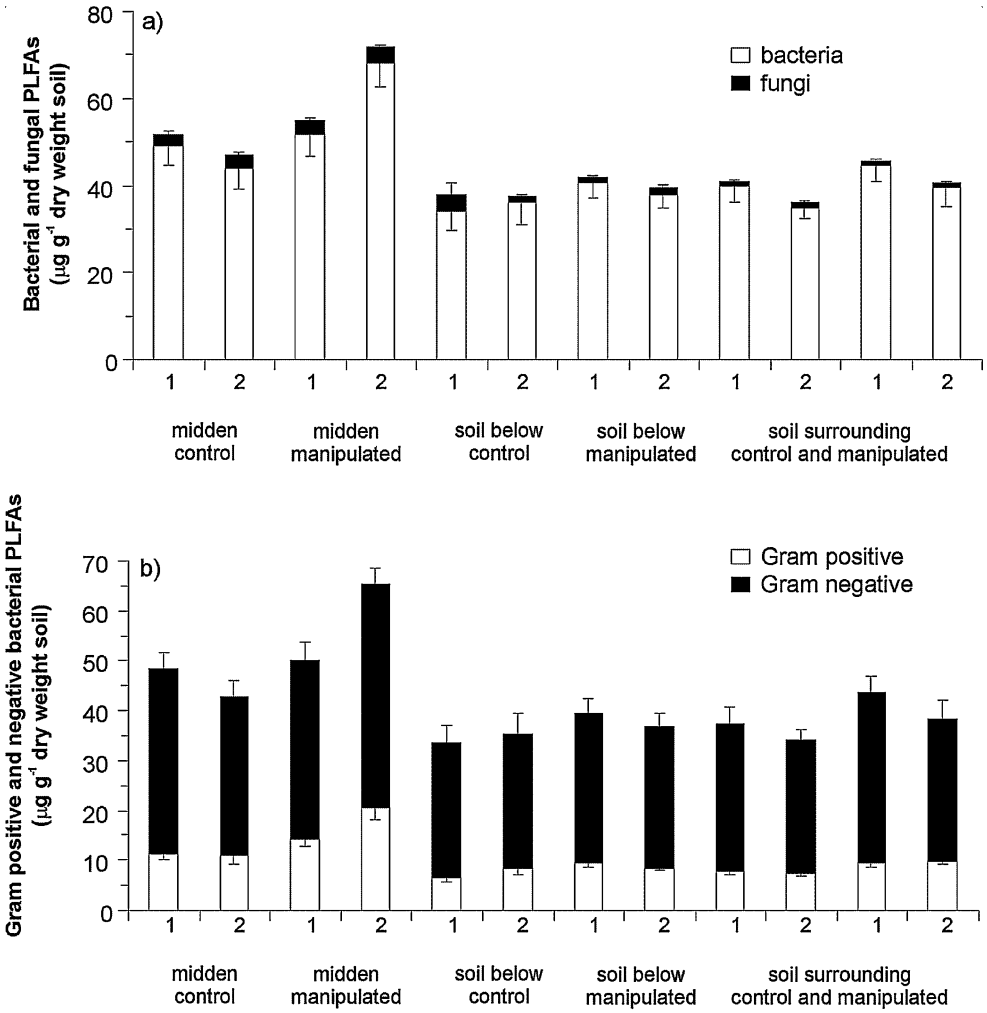

Fig. 2 Changes in **a** bacterial and fungal PLFAs and **b** gram positive and gram negative bacterial PLFAs in middens, soil below, and soil surrounding samples, with and without earthworms after 1 and 2 months. Mean±SEM, n=10

Consumption of CH_4 in control samples was higher (–1.62±0.27 ng CH_4 h^{-1} g^{-1} dw) than in translocated ones (–0.43±0.27 ng CH_4 h^{-1} g^{-1} dw) (ANOVA, $F_{1,48}$=21.06, p<0.0001; see Fig. 3b); time increased consumption of CH_4, which was times higher after 2 months (–1.69± 0.28 ng CH_4 h^{-1} g^{-1} dw) than 1 month (–0.36±0.11 ng CH_4 h^{-1} g^{-1} dw) (ANOVA, $F_{2,48}$=26.24, p<0.0001).

Soil organic C depended strongly on type of sample (ANOVA, $F_{2,108}$=3.34, p<0.05; Fig. 4), being higher in middens (4.52±0.02%) than in soil below and surrounding (4.40±0.03 and 4.41±0.05%) (Fisher LSD test, p<0.05); earthworm presence, higher in absence (4.52±0.02%) than in presence of earthworms (4.36±0.03%) (ANOVA, $F_{2,108}$=14.22, p<0.001), and decreased from 1 month (4.50±0.02%) to 2 months (4.39±0.03%) (ANOVA, $F_{2,108}$=6.68, p<0.05; see Fig. 4). However, the depletion of soil organic C only occurred in samples with earthworms after 2 months. It remained constant in samples without earthworms producing a significant interaction between manipulation and time (ANOVA, $F_{2,108}$=4.50, p<0.05; Fisher LSD test, p<0.01; see Fig. 4).

4 Discussion

To our knowledge this is the first study dealing with changes in microbial communities and their activity (measured as fluxes of CO_2 and CH_4) in earthworm middens and with the role of *L. terrestris* as the active maintainer of these structures (i.e., by adding casts and litter). Earthworms have been shown to play an important role in modifying soil microbial communities since greater changes occurred in middens with than without earthworms as revealed by the discriminant analysis. *L. terrestris* is an anecic earthworm that lives in permanent vertical burrows, which it only leaves for feeding and mating (Butt et al. 2003; Field and Michiels 2006). Thus, we would not expect microbial biomass and activity to be impacted in the soil below and surrounding middens, since the movement, feeding, and other activities of *L. terrestris* are confined to their burrow and midden. The lack of differences between soil below and soil surrounding samples could be due to the fact that the sampling method did not specifically target the drilosphere soil (burrow lining), making, to a certain extent,

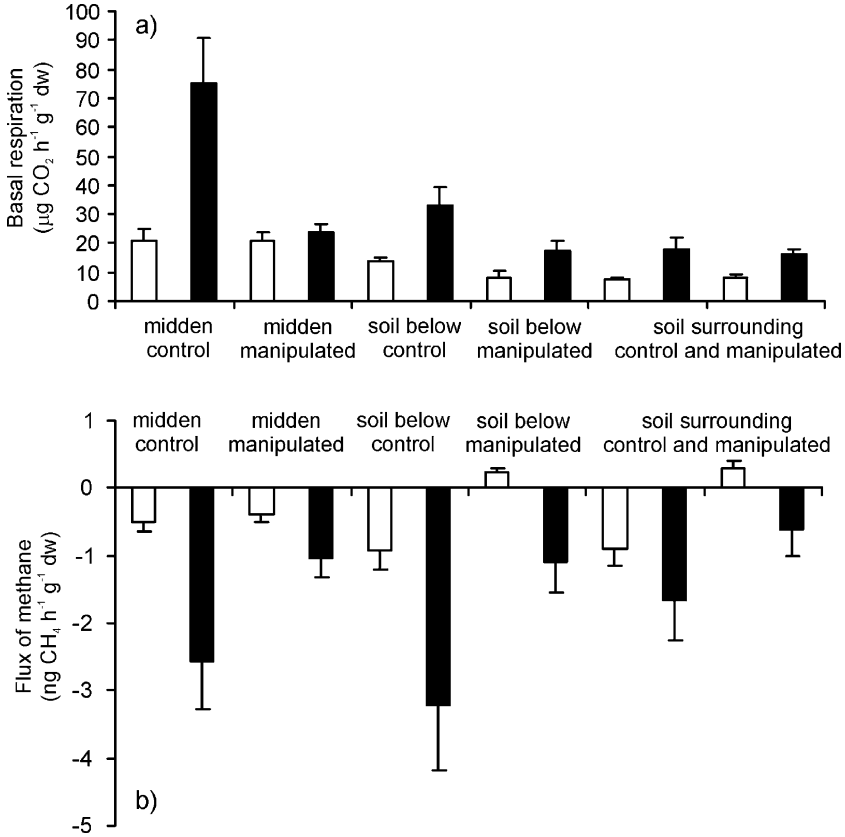

Fig. 3 Changes in **a** production of CO_2 and **b** consumption and release of CH_4, in middens, soil below, and soil surrounding samples, with and without earthworms after 1 (*white symbols*) and 2 months (*black symbols*). Negative values of CH_4 fluxes mean methane consumption. Mean±SEM, $n=5$

some dilution effect. Our results correspond with the increase in overall microbial biomass (Bohlen et al. 1997; Anderson and Bohlen 1998; Subler and Kirsch 1998; Wilcox et al. 2002) and microbial activity (Bohlen et al. 1997; Schrader and Seibel 2001) observed in middens of different forest and agricultural soils.

Casts of *L. terrestris* have different microbial communities (microfungi, archaea, and bacteria) from uningested soil (Egert et al. 2004; Tiunov and Scheu 2000a) and are also enriched with nutrients (Le Bayon and Binet 2006; Marhan et al. 2007), and therefore the mixture of casts and litter characteristic of middens build by earthworms may support different microbial communities than the bulk soil. In accordance with this, we found that organic C was always higher in middens than in soils. A lower C–N ratio and coarse–fine litter ratio in middens than in the

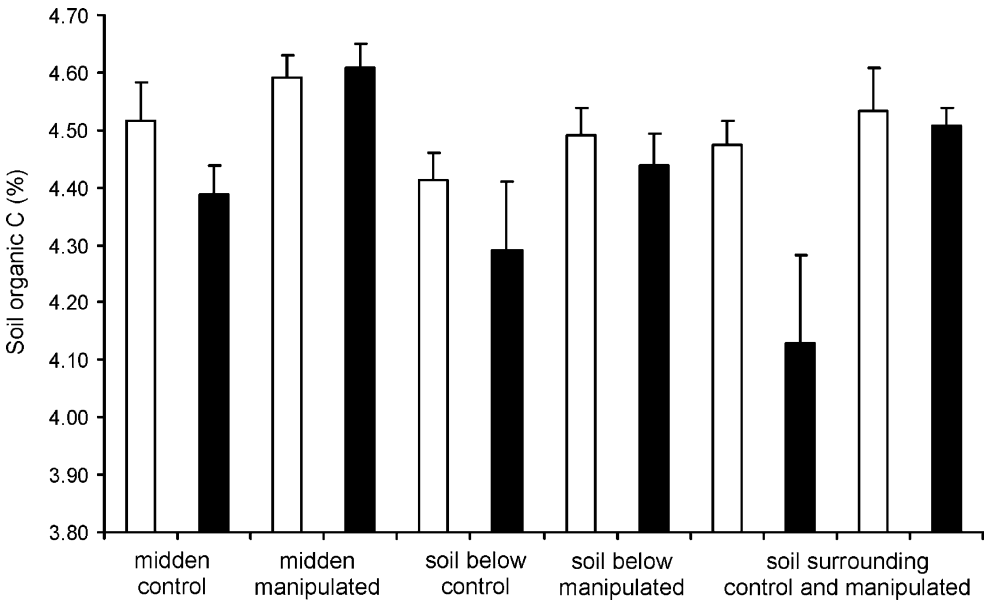

Fig. 4 Soil organic C (%) in middens, soil below, and soil surrounding samples, with and without earthworms after 1 (*white symbols*) and 2 months (*black symbols*). Mean±SEM, $n=10$

surrounding litter and soil has been reported (Bohlen et al. 1997; Wilcox et al. 2002) which would increase the rate of decomposition, favoring the contribution of bacterial communities, especially gram negative bacteria, which flourish under high availability of organic C substrates (Melillo et al. 1982; Holland and Coleman 1987; Díaz-Raviña et al. 2006), as occurred in translocated middens. This suggests that when earthworms are present they ingest the organic C that they collect reducing the availability of substrates for microbial growth and activity, although respiration rates were higher in control than in translocated middens.

It has been stated that the activity of earthworms increases the ratio of bacteria–fungi (Brown 1995), however, we found more fungal PLFAs in middens than in the soil below and surrounding middens, possibly due to the concentration of lignocellulosic material in middens which stimulated fungal growth (Lynd et al. 2002). This greater content of fungal PLFA occurred despite the fact that earthworms are able to break and digest fungal hyphae (Wolter and Scheu 1999; Bonkowski et al. 2000; Butenschoen et al. 2007). Nevertheless, we found that fungal growth in middens did not depend on earthworm presence, which suggests that earthworms did not feed on fungal populations or did not modify the optimal conditions of middens for fungal development. Although not significant, fungal PLFAs in middens tended to increase with time, regardless of earthworm presence. Thus, ageing of casts included in the middens may be responsible for this slight increase because cast ageing enhances nutrient availability and abundance of certain fungal species (Tiunov and Scheu 2000b).

Microbial activity was higher in control middens than in translocated ones, indicating that earthworms provide support to the microbial activity of middens, as shown by the peak in respiration of middens with earthworms after 2 months. The overall increase in microbial activity in all treatments after 2 months could be due to a net increase in temperature between sampling dates, which could have favored microbial metabolism. However, the net increase was stronger in middens than in soil below and soil surrounding samples, indicating the significance of earthworms in enhancing microbial activity. Net consumption of CH_4 increased in samples without earthworms and with ageing. These data suggest that either CH_4 oxidation rates increased with time, or that CH_4 production processes decreases or that both occurred. As net CH_4 fluxes are the sum of the combination of CH_4 production and oxidation processes, it is hard to elucidate which processes changed.

5 Conclusions

The earthworm *L. terrestris* showed a limited impact in the studied area, mainly modifying the soil properties in middens. We did not find any evidence of microbial transfer from middens to the soil below; furthermore, microbial community structure and activity of soil below and soil surrounding samples did not differ at all, even through time and with earthworm presence. Our results suggest that, in soils supporting large populations of *L. terrestris*, earthworm middens are hot spots for soil microbial dynamics, increasing habitat heterogeneity for soil microorganisms. Our data indicate that the relationships that earthworms and microorganisms establish during decomposition of organic matter are far from being well understood.

6 Recommendations and perspectives

Our data indicate that it is not clear if earthworms enhance or depress microbial communities of middens since they increased microbial activity but did not modify their biomass and this was not dependent on soil organic C content (analysis of covariance, $F_{1,107}=1.50$, $p=0.22$) (analysis of covariance, $F_{1,107}=0.17$, $p=0.68$). These results indicate no competence for C pools between this anecic earthworm and microorganisms, which has been found for other earthworm species, mainly endogeics (Tiunov and Scheu 2004). Conversely, they suggest some type of facilitation due to the release of additional nutrient pools in middens when earthworms are present, through the digestion of middens material or the addition of cast produced from other food sources.

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References

Anderson OR, Bohlen PJ (1998) Abundances and diversity of gymnamoebae associated with earthworm (*Lumbricus terrestris*) middens in a northeastern U.S. forest. Soil Biol Biochem 30:1213–1216

Bååth E (2003) The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. Microbl Ecol 45:373–383

Bohlen PJ, Parmelee RW, McCartney DA, Edwards CA (1997) Earthworm effects on carbon and nitrogen dynamics of surface litter in corn agroecosystems. Ecol Appl 7:1341–1349

Bohlen PJ, Edwards CA, Zhang Q, Parmelee RW, Allen M (2002) Indirect effects of earthworms on microbial assimilation of labile carbon. Appl Soil Ecol 20:255–261

Bonkowski M, Griffiths BS, Ritz K (2000) Food preferences of earthworms for soil fungi. Pedobiologia 44:666–676

Brown GG (1995) How do earthworms affect microflora and faunal diversity? Plant Soil 170:233–239

Butenschoen O, Poll C, Langel R, Kandeler E, Marhan S, Scheu S (2007) Endogeic earthworms alter carbon translocation by fungi at the soil–litter interface. Soil Biol Biochem 39:2854–2864

Butt KR, Nuutinen V, Siren T (2003) Resource distribution and surface activity of adult *Lumbricus terrestris* L. in an experimental system. Pedobiologia 47:548–553

Diaz-Raviña M, Bååth E, Martin A, Carballas T (2006) Microbial community structure in forest soils treated with a fire retardant. Biol Fertil Soils 42:465–471

Egert M, Marhan S, Wagner B, Scheu S, Friedrich MW (2004) Molecular profiling of 16S rRNA genes reveals diet-related differences of microbial communities in soil, gut, and casts of *Lumbricus terrestris* L. (Oligochaeta: Lumbricidae). FEMS Microbiol Ecol 48:187–197

Field SG, Michiels NK (2006) Does the acephaline gregarine *Monocystis* sp. modify the surface behaviour of its earthworm host *Lumbricus terrestris*? Soil Biol Biochem 38:1334–1339

Frostegård A, Bååth E (1996) The use of phospholipid analysis to estimate bacterial and fungal biomass in soils. Biol Fertil Soils 22:59–65

Hamilton WE, Sillman DY (1989) Influence of earthworm middens on the distribution of soil microarthropods. Biol Fertil Soils 8:279–284

Holland EA, Coleman DC (1987) Litter placement effects on microbial and organic matter dynamics in an agroecosystem. Ecology 68:425–433

Holland EA, Boone R, Greenberg J, Groffman PM, Robertson GP (1999) Measurement of soil CO_2, N_2O and CH_4 exchange. In: Robertson GP et al (ed) Standard soil methods for long term ecological research. Oxford University Press, New York, pp 185–201

Jackson ML (1958) Soil chemical analysis. Constable, London

Lavelle P, Spain AV (2001) Soil ecology. Kluwer Academic, London

Le Bayon RC, Binet F (2006) Earthworms change the distribution and availability of phosphorus in organic substrates. Soil Biol Biochem 38:235–246

Lores M, Gomez-Brandon M, Perez-Diaz D, Dominguez J (2006) Using FAME profiles for the characterization of animal wastes and vermicomposts. Soil Biol Biochem 38:2993–2996

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS (2002) Microbial cellulose utilization: fundamentals and biotechnology. Microbiol Mol Biol Rev 66:506–577

Maraun M, Alphei J, Bonkowski M, Buryn R, Migge S, Peter M, Schaefer M, Scheu S (1999) Middens of the earthworm *Lumbricus terrestris* (Lumbricidae): microhabitats for micro- and mesofauna in forest soil. Pedobiologia 43:276–287

Marhan S, Kandeler E, Scheu S (2007) Phospholipid fatty acid profiles and xylanase activity in particle size fractions of forest soil and casts of *Lumbricus terrestris* L. (Oligochaeta, Lumbricidae). Appl Soil Ecol 35:412–422

Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63:621–626

Neilson GA, Hole FE (1964) Earthworms and the development of coprogenous A1 horizons in forest soils of Wisconsin. Soil Sci Soc Am Proc 28:426–430

Sampedro L, Whalen J (2007) Changes in the fatty acid profiles through the digestive tract of the earthworm *Lumbricus terrestris* L. Appl Soil Ecol 35:226–236

Sampedro L, Jeannote R, Whalen J (2006) Trophic transfer of fatty acids from gut microbiota to the earthworm *Lumbricus terrestris* L. Soil Biol Biochem 3:2188–2198

Scheu S, Falca M (2000) The soil food web of two beech forests (*Fagus sylvatica*) of contrasting humus type: stable isotope analysis of a macro- and a mesofauna-dominated community. Oecologia 2:285–296

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

Subler S, Kirsch AS (1998) Spring dynamics of soil carbon, nitrogen, and microbial activity in earthworm middens in a no-till cornfield. Biol Fertil Soils 26:243–249

Tiunov AV, Scheu S (1999) Microbial respiration, biomass, biovolume and nutrient status in burrow walls of *Lumbricus terrestris* L. (Lumbricidae). Soil Biol Biochem 31:2039–2048

Tiunov AV, Scheu S (2000a) Microfungal communities in soil, litter and casts of *Lumbricus terrestris* L. (Lumbricidae): a laboratory experiment. Appl Soil Ecol 14:17–26

Tiunov AV, Scheu S (2000b) Microbial biomass, biovolume and respiration in *Lumbricus terrestris* L. cast material of different age. Soil Biol Biochem 32:265–275

Tiunov AV, Scheu S (2004) Carbon availability controls the growth of detritivores (Lumbricidae) and their effect on nitrogen mineralization. Oecologia 138:83–90

Wilcox CJ, Domínguez J, Parmelee RW, McCartney DA (2002) Soil carbon and nitrogen dynamics in *Lumbricus terrestris* L. middens in four arable, a pasture, and a forest ecosystem. Biol Fertil Soils 36:26–34

Wolter C, Scheu S (1999) Changes in bacterial numbers and hyphal lengths during the gut passage through *Lumbricus terrestris* (Lumbricidae Oligochaeta). Pedobiologia 43:891–900

Zelles L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol Fertil Soils 29:111–129