Indirect effects of earthworms on microbial assimilation of labile carbon

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Abstract

Interactions between earthworms and microorganisms can be important in regulating the rate of soil carbon turnover and maintaining soil fertility in agroecosystems. Despite the significance of earthworms in nutrient cycling in agroecosystems, the indirect influence of earthworms on C assimilation by microorganisms has not been adequately quantified. We assessed microbial assimilation of 13C-labeled acetate in earthworm (Lumbricus terrestris) middens and surrounding soil collected from maize agroecosystems. Incorporation of 13C into microbial lipids was used as an indicator of microbial growth rates. Earthworm middens had significantly lower concentrations of microbial phospholipid phosphates and lower natural abundance δ13C than the surrounding soil. After incubation with 13C-labeled acetate, microbial communities in earthworm middens had greater 13C/12C ratios of microbial lipids than microbial communities from surrounding soil. The 13C enrichment per unit of microbial phospholipid was much greater in middens than in surrounding soil indicating that: (i) microbial lipid synthesis was significantly higher in the earthworm middens; (ii) microbial assimilation efficiency for 13C-labeled acetate was greater in midden soil; or (iii) assimilation of 13C-labeled acetate relative to other C sources was proportionately greater in middens than in the surrounding soil. Our results suggest that there were functional differences between microbial communities in earthworm middens and surrounding soil, probably due to a combination of physical, chemical, and biological changes in the midden microenvironment. The resulting differences in microbial communities or activity increased microbial growth rates and assimilation of readily available C substrates in middens relative to surrounding soil. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Interactions between earthworms and microorganisms are important for many soil processes in agroecosystems (Edwards and Bohlen, 1996; Lavelle et al., 1992; Lee, 1985). Earthworms facilitate soil carbon and nitrogen transformations through their influence on the soil microflora (Blair et al., 1995; Martin et al., 1992, 1991; Scheu, 1987). Their effects include direct and incidental grazing, dispersal, competition, and potential mutualistic associations (Edwards and Bohlen, 1996; Blair et al., 1995; Daniel and Anderson, 1992; Lee, 1985). Our earlier field and laboratory studies have indicated that earthworm-microbial interactions increase soil carbon evolution, soil nutrient availability and microbial activity, but may reduce microbial biomass (Blair et al., 1995; Bohlen and Edwards, 1995; Schindler-Wessells et al., 1997). These interactions influence soil nutrient availability probably through increasing the activity of the soil microbial
biomass, while reducing its size, thereby increasing overall nutrient availability.

The earthworm, *Lumbricus terrestris*, is unique among earthworms occurring commonly in north temperate agroecosystems, in forming permanent or semi-permanent vertical burrows with small patches of plant litter and casts called middens gathered around the burrow entrance. These middens can comprise a significant proportion of surface crop residues in agroecosystems with large populations of *L. terrestris* and affect the breakdown of crop residues and the spatial heterogeneity of residue microenvironments on the soil surface (Bohlen et al., 1997). Earthworm middens in agroecosystems have been shown to have greater microbial activity than surrounding soil (Bohlen et al., 1997; Subler, 1998), and in forests have been shown to have increased diversity and abundance of soil amoeba (Anderson and Bohlen, 1998). Increased microbial activity coupled with increased abundance of microbial predators suggests the potential for greater turnover of microbial populations. This potential for greater microbial turnover together with alteration of the quality of plant litter in the midden microenvironment (Bohlen et al., 1997) suggests that the carbon assimilation efficiencies of microorganisms in the midden microenvironment may be greater than in the surrounding soil.

Earthworm middens present an ideal model for analyzing animal-mediated influences on soil microbial communities by alteration of the patch structure of the microbial environment. We compared the microbial assimilation of carbon in earthworm middens and surface casts and adjacent undisturbed soils in order to determine the influence of small-scale disturbance by earthworms on the activity and turnover of microbial populations. We analyzed the carbon isotope composition of microbial lipids, obtained from fresh earthworm middens and the adjacent bulk soils, to assess potential differences in the energy sources used by microbes in these different habitats. We also assessed rates of microbial growth and quantified the rates of assimilation of $^{13}$C-labeled acetate into microbial lipids.

2. Materials and methods

Paired samples of middens and surrounding soil were collected from experimental plots at the Ohio Agricultural Research and Development Center in Wooster, OH, USA (41°N, 82°W) that were cropped annually with maize (*Zea mays*) from 1991 to 1998. Mean monthly temperatures at this location range from −4.8 °C in January to 21.2 °C in July. Mean annual precipitation is 905 mm per year. Soils at the site are moderately well-drained silt loam fragidalfs, of the canfield series (Luvisol), which are deep and gently sloping, and have a relatively impermeable fragipan at a depth of 40–75 cm. The mean percentage organic matter, determined by wet oxidation, was 3.7 ± 0.9%. Soil pH was 6.3 ± 0.4 and cation exchange capacity was 10 ± 2 cmol(+) per 100 g−1 soil.

Soil cores (diameter 8 cm, depth 5 cm) containing fresh surface earthworm middens and equivalent amounts of paired adjacent (within 20 cm) soils were collected in October 1996, when earthworms were very active in the field. Forty-two newly-formed middens with diameters 5–8 cm and 42 paired adjacent soil samples were sampled randomly through-out a corn field that had received 150 kg N ha−1 of NH₄NO₃ fertilizer annually, at the time of planting (May–June) from 1991 to 1996 (Bohlen et al., 1997). Each sample was placed in a plastic bag, stored on ice, and transported to the laboratory within 4 h of collection. Samples were homogenized by hand in the laboratory and stored at 4 °C. Five midden samples and paired surrounding soil samples were chosen randomly for determination of background carbon isotope ratios ($^{13}$C/$^{12}$C) of the microbial lipids. Five additional paired samples of middens and surrounding soil were selected randomly for phospholipid phosphorus determination of microbial biomass.

The remaining midden and paired surrounding soil samples were used to measure microbial growth and C assimilation within 48 h of collection. We incubated samples with $^{13}$C-labeled acetate (99.99% Isotec Inc., Miamisburg, OH) and measured the incorporation of $^{13}$C into microbial lipids. The $^{13}$C enrichment per unit of microbial phospholipid phosphorus was used as the index of microbial growth and C assimilation. For microbial growth determinations, we used a factorial design with two levels of habitat (midden and surrounding soil) and two concentrations of $^{13}$C-labeled acetate (9 and 18 µg $^{13}$C g−1 dry soil), for a total of four treatment combinations. All four treatment combinations were replicated four times for each of four incubation times (0.5, 1, 2, and 4 h) to provide a total...
of 64 samples, 32 each of middens and surrounding soils. Quantities of field moist soil equivalent to 20 g oven-dry wt. (60 °C) were added to 125 ml Erlenmeyer flasks and the isotope solutions were added to bring the samples to approximately 50% of their water-holding capacity. The 13C-labeled acetate concentrations were chosen to achieve enrichments of 1000 and 2000 δ13C units, based on the assumption that the microbial biomass at the field site was about 400 μg C g⁻¹ dry soil (Edwards and Bohlen, 1996). Flasks and samples were incubated at 22 ± 2 °C under free air exchange. Microbial lipids were extracted from incubated samples in a chloroform mixture using the method of Federle et al. (1983). For determination of microbial phospholipid phosphate the lipid extraction was subjected to potassium persulphate digestion and the amount of phospholipid phosphate determined at 610 nm in a microplate reader. The dry lipid extracts of the microbial communities were used for analysis of 13C/12C ratios and 13C enrichment. Differences in the background carbon isotope (13C/12C) ratio and microbial biomass between middens and surrounding soil were analyzed using a one-way analysis of variance (ANOVA) (SAS, 1990, SAS Institute Inc., Cary, NC, USA) after performing Bartlett’s test for homogeneity of variance. The data for microbial biomass were log-transformed prior to statistical analysis. The microbial 13C atom enrichment was analyzed using two between-group factors and one repeated-measures ANOVA (SAS, 1990) with habitat (middens and surrounding soil) and acetate concentrations treated as between group factors, and sampling time serving as a repeated-measures factor. Bartlett’s test for homogeneity of variance was calculated before performing the ANOVA. Percentage 13C enrichment data were arcsine-transformed prior to analysis. If there were significant habitat, concentration and sampling time effects, the least significant difference at $P < 0.05$ was used to separate the means.

3. Results and discussion

The natural abundance δ13C of microbial lipids was significantly greater in the earthworm middens than in adjacent soil (Fig. 1). This could reflect differences in the carbon isotope signatures of carbon sources for microorganisms. More maize residues were concentrated in the L. terrestris middens than in the surrounding soil and these maize residues were enriched in 13C because maize is a C₄ plant with the dicarboxylic acid photosynthetic pathway, which allows for greater assimilation of 13C than occurs in C₃ plants. Thus, the microorganisms in the earthworm middens may have assimilated a greater proportion of their total C from maize residues than microbes living in surrounding soil, which lacked surface residues, leading to greater enrichment of microbial phospholipids with 13C in middens. The δ13C of the microbial biomass has been shown to reflect that of maize tissues in continuously-cropped maize agroecosystems (Gregorich et al., 2000). The average δ13C of soil at our study site was −23.6‰, whereas the δ13C of corn residues typically ranges from −12‰ to −13‰ (Arrouays et al., 1995; Gregorich et al., 1995). Although we did not measure the amounts of maize
residues or total organic content of the incubated samples, middens collected from the same site in October 1997 contained 9.2% organic matter, including many coarse fragments of maize residues, whereas surrounding soil contained only 5.9% organic matter, with few maize residues. Furthermore, the maize residues in middens from the same site were shown to have a lower C:N ratio than maize residues that were not in middens, indicating that there were differences in residue quality between earthworm middens and surrounding soil (Bohlen et al., 1997).

Another explanation for the difference in δ13C of microbial phospholipids in middens and surrounding soil is that earthworms may have altered the composition of the microbial community, and that the taxa present in the middens and the surrounding soil differed in the δ13C/12C ratio of their tissues. Lipids have been used as a biomarker to indicate microbial community changes (Tunlid et al., 1985, 1989; Frostegard et al., 1993), and so differences in the δ13C/12C of microbial community lipids may indicate changes in microbial communities. Alternatively, earthworms could have affected the δ13C of assimilable C in the midden environment directly. Investigation of the δ13C enrichment of earthworms, from a variety of habitats, revealed that earthworms were enriched more in 13C than their putative food sources (Neilson et al., 2000). It is possible that such enrichment of earthworm tissue, if it occurred at our site, may have increased the 13C enrichment of readily-assimilable C sources in the midden environment, in the form of mucus and other earthworm excretions.

The total microbial phospholipid content was significantly lower in the *L. terrestris* middens than in the surrounding soil (Fig. 1). These results were unexpected because other studies in the same and a similar agroecosystem (Bohlen et al., 1997; Subler, 1998) and in a temperate forest (Anderson and Bohlen, 1998) reported greater microbial biomass in middens than in surrounding soil. However, earlier studies used chloroform fumigation techniques to assess microbial biomass, which may produce different results than the microbial phospholipid method we used in our investigation. The amounts of microbial phospholipid fatty acids (PLFAs) may not be correlated well with the total microbial biomass. Simultaneous investigation of seasonal patterns in microbial PLFAs and microbial C and N, in managed grasslands, showed that seasonal maxima and minima for microbial C and N occurred at different times of year relative to seasonal maxima and minima of microbial PLFAs (Bardgett et al., 1999). Thus, the lower microbial phospholipid contents we observed in earthworm middens may not have corresponded to a lower microbial biomass in these middens relative to surrounding soils. However, if microbial biomass was lower in our midden samples than in the surrounding soil, the lower biomass may have been due to grazing by earthworms on microorganisms and possible high competition between the earthworms and microflora for carbon sources (Wolters and Joergensen, 1992; Lussenhop, 1992; Bohlen and Edwards, 1995).

In contrast to microbial phospholipid content, the microbial growth rates appeared to be greater in earthworm middens than in surrounding soils as indicated by greater enrichment of microbial lipids with 13C in midden soil (Fig. 2). The application of high concentrations of 13C-labeled acetate (18 μg 13C g⁻¹ dry soil) resulted in the greatest assimilation of 13C into microbial lipids, within 1 h of incubation, in both habitats (Fig. 2). The 13C enrichment per unit of phospholipid
Fig. 3. Assimilation of $^{13}$C-labeled acetate into microbial lipid in the bulk soils and earthworm middens under the concentration of $9/10^{26}$ g $^{13}$C g$^{-1}$ (top panel) or $18/10^{26}$ g $^{13}$C g$^{-1}$ (bottom panel) (mean ± 1 S.E.). Values for middens are significantly higher than values for surrounding soil at all sampling times for both concentrations of $^{13}$C ($P < 0.001$).

was much greater in these phospholipids from middens than in those from surrounding soils (Fig. 3), which indicates that: (i) microbial lipid synthesis was significantly higher in the earthworm middens; (ii) microbial assimilation efficiency for $^{13}$C-labeled acetate was greater in earthworm middens; or (iii) assimilation of $^{13}$C-labeled acetate relative to other C sources was proportionately greater in earthworm middens than in the surrounding soil. Acetate is a readily-assimilable C source and it is unlikely that microbial assimilation efficiency for acetate was three times greater in middens than in the surrounding soil, as is indicated in Fig. 3; so it is likely that there was increased microbial growth or lipid synthesis in middens, not just a greater assimilation efficiency. The addition of readily-available carbon compounds such as acetate may have increased microbial activities and growth significantly because normally most microorganisms remain in a relatively inactive state in soil, due to constraints in carbon availability (Paul and Clark, 1989).

The indirect influence of earthworms on microbial growth and assimilation of C in the midden environment might have resulted from a combination of physical, chemical and biological modifications of the microbial habitat. Microbial communities in earthworm middens may have been primed to be more responsive to additions of readily assimilable C and may have responded more rapidly to the acetate addition than microorganisms in surrounding bulk soils. The greater amounts of organic matter in the earthworm midden environment and possibly, the increased mineral N content (Suhler, 1998) could have influenced assimilation of carbon. Modification of the physical structure of soil in the midden environment may have influenced microbial uptake and/or assimilation of carbon. Earthworms can increase the proportion of macroaggregates in soil and middens (Shaw and Pawluk, 1986) and microbial biomass has been shown to assimilate more, but mineralize less, $^{13}$C-labeled glucose in macroaggregates than in microaggregates (Aoyama et al., 2000). Collectively, these indirect influences of earthworms on the microhabitat of the midden environment may have created favorable conditions for microbial growth and assimilation of new carbon sources.

The results of this experiment suggest that earthworms mediate small-scale patchiness of the soil microenvironment by redistributing soil microbial biomass and activity in space and thereby contribute to spatial heterogeneity in soil nutrient processes (Culver and Beattie, 1980, 1983). Further evidence for the influence of earthworms on spatial heterogeneity of microbial processes comes from analysis of denitrification rates, which were consistently 4–10 times greater in middens than in the surrounding soil in samples from the same field site used in this study (Bohlen, unpublished data). These earthworm-mediated controls on the spatial patchiness of soil processes in agroecosystems may be important where there are large populations of *L. terrestris* and may be especially significant in minimum or no-tillage row crop ecosystems where crop residues are maintained on the soil surface (Suhler, 1998).

Isotopically-labeled substrates continue to be useful for investigating animal-microbial interactions in soil and microbial growth and assimilation efficiencies. Others workers have used $^{14}$C-labeled acetate to quantify microbial growth rates under different environmental conditions (Parmelee et al., 1993; Tate, 1985; Tate et al., 1991). Using $^{13}$C-labeled substrates obviates concerns over handling of radioactive $^{14}$C.
and provides an accurate way of assessing assimilation efficiencies. Labeling of soil with 2000 13C units with a 1 h incubation was optimal for incorporating 13C into the microbial lipids in our experimental setting. Use of more realistic substrates, such as labeled plant material or earthworms, could provide more ecologically relevant results. Examining incorporation of 13C-labeled substrates into particular microbial ecologically relevant results. Examining incorporation of 13C-labeled substrates into particular microbial PLFAs, that are specific to different microbial groups, could help elucidate the influences of earthworms on fungal and bacterial communities (Arao, 1999). Our experiment focused on the indirect effects of earthworms on microbial communities via modification of the soil environment; direct effects of earthworms on microbial C assimilation could be examined by incubating soil with or without earthworms and different isotopically labeled substrates.

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References


