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Soil carbon and nitrogen dynamics in *Lumbricus terrestris*. L. middens in four arable, a pasture, and a forest ecosystems

Received: 1 August 2001 / Published online: 10 July 2002
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Abstract *Lumbricus terrestris*' middens contain large concentrations of organic material and have been characterized as microenvironments distinct from the surrounding soil. The direct and indirect consequences of midden formation on nutrient cycling dynamics and organic matter pools in various ecosystem types have not received much consideration. Therefore, we focused on the differences in C and N dynamics between midden and bulk soil samples in four corn (*Zea mays* L.) agroecosystems, a rotational pasture and a deciduous forest, in June, July and August of 1996, in Ohio, USA. Paired earthworm midden and bulk soil samples were analyzed for mineral N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$), dissolved organic N, microbial biomass N (MBN) and carbohydrate C (CarbC). Additionally, coarse litter, fine litter, particulate organic matter, and soil organic matter fractions were separated and analyzed for total C, total N and C:N ratios. Mineral and dissolved N levels were higher in the midden soil relative to those in the bulk soil for all ecosystem types, except for only $\text{NO}_3^-\text{-N}$ levels in two highly fertilized agroecosystems and in the pasture. MBN, CarbC, and total C and N levels for all organic fractions were significantly greater in the earthworm midden samples relative to these in the bulk samples across all ecosystem types. The plan defined by principal component analysis clearly separated two main groups: (1) includes the forest, the pasture and the less fertilized cornfields and the midden effect is to increase slightly the organic matter content and strongly the inorganic N content, and (2) includes the heavily fertilized agroecosystems and the midden effect is also to in-

crease the organic matter content but to decrease the inorganic N content. We concluded that *L. terrestris*' middens significantly raised overall soil C and N levels relative to the bulk soil, in a variety of ecosystem types, and, given the abundance of earthworm middens, these macrosites should receive important attention when evaluating nutrient cycling processes at the systems level.

Keywords Earthworms · Middens · Casts · Agroecosystems · Carbon · Nitrogen

Introduction

The anecic earthworm species, *Lumbricus terrestris* L. can influence many biological, physical and chemical features of the soil (Satchell 1963; Knollenberg et al. 1985; Laing et al. 1986; Scullion and Ramshaw 1988; Edwards et al. 1990; Binet and Curmi 1992; Edwards and Bater 1992). Laboratory microcosm studies have demonstrated specifically that *L. terrestris* can impact the levels of soil C, soil N, and microbial biomass (Shaw and Pawluk 1986; Binet and Trehen 1992; Devliegher and Verstraete 1995). However, few of the many investigations involving *L. terrestris* have focused on nutrient dynamics and organic matter concentrations in middens compared to the surrounding soil (Nielson and Hole 1964). Yet, middens have been proposed as integral "hotspots" of biological and chemical activity (Subler and Kirsch 1998).

L. terrestris can facilitate interactions between organic litter and mineral soil that create a more palatable food source (King and Heath 1967; Szlavecz 1985), thereby increasing rates of organic matter decomposition (Bohlen et al. 1997). This adaptive feeding habit is involved in the formation of distinct midden microhabitats, which contain mineral soil, shredded organic material and surface casts at the openings to vertical *L. terrestris*' burrows (Shipitalo and Protz 1988). Activity by *L. terrestris* concentrates, fragments, and eventually almost totally consumes midden-associated organic material. Studies comparing nutrients in midden soil to the sur-

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rounding soil have reported elevated levels of organic C, total soil C, C:N ratio, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, dissolved organic N (DON), microbial metabolic activity, total soil N, and coarse litter in middens; however, decreased $\text{NO}_3^-\text{-N}$ levels and C:N ratios have also been reported in midden soil samples (Bohlen et al. 1997; Subler and Kirsch 1998). Middens produced by *L. terrestris* may increase soil nutrient levels through accelerated immobilization-mineralization rates or decrease soil nutrient levels through leaching and gaseous losses.

The studies cited above were made in decreased agroecosystems, although *L. terrestris*' middens have been studied in forests, woodlots, recreational fields, and acid shale spoil banks (described below). These examples demonstrate further the potential importance of *L. terrestris* in altering soil conditions at a systems level, since middens are not merely present in these diverse habitats but are also abundant in natural systems. Midden volumes were determined either indirectly, through assessing the redistribution of organic matter, or directly, through measurements of macropore/midden densities. Hamilton and Sillman (1989) calculated the total percentage of soil surface area covered with midden soil and debris, as 1.8% in a mowed field and 4.3% in a woodlot. Vimmerstedt and Finney (1973) reported that, after 5 years, ten individuals of *L. terrestris* increased in population to a density of 60 middens m^{-2} over a re-vegetated strip-mine. Bohlen et al. (1997) reported that the average number of middens m^{-2} was 48.8 in a reduced-tillage cornfield.

Our investigation focused on the effects of midden formation on nutrient cycling dynamics and organic matter pools in different geographic locations under various

land-uses in Ohio. We focused on differences in soil C and N levels, between midden and paired bulk soil samples in four agroecosystems, a pasture and a forest in June, July and August of 1996. Specifically, we quantified changes in: (1) C pools, i.e., carbohydrate C (CarbC), total C and the C:N ratio; and (2) N pools, i.e., mineral N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$), DON, microbial biomass N (MBN), and total N. The total C and N pools and the C:N ratios, were determined in coarse litter (>6 mm), fine litter (2–6 mm), particulate organic matter (POM; 0.053–2 mm) and soil organic matter (SOM; <2 mm) fractions.

Materials and methods

Field sampling

We selected two corn agroecosystems at the Ohio Management Systems Evaluation Area (MESEA) in southern Ohio and two others at the North Appalachian Experimental Watershed (NAEW) in eastern Ohio for midden and bulk sampling (Table 1). At the time of sampling, three of the corn/soybean (*Glycine max* L.) rotations were under corn, while the corn/soybean/wheat (*Triticum aestivum* L.)/hairy vetch (*Vicia villosa*) winter cover crop rotation was under wheat. The latter rotation was ridge-tilled, and the other agroecosystem at MESEA was a chisel-plowed cornfield. Silt and sandy loam soils dominated these two sites. We sampled at another chisel-plowed cornfield, which was over Rayne silt loam, and a no-till cornfield, which was on Coshocton silt loam, at NAEW. In addition to these four agroecosystems, we selected a silt loam rotational cattle (*Bos taurus*) pasture in northeastern Ohio, and a clay/silty clay loam forest ecosystem in central Ohio (Table 1). Orchard grass (*Dactylis glomerata* L.) was the dominant plant species in the pasture, while the forest consisted of a transitional oak-hickory (*Quercus/Carya*) beech-maple (*Fagus/Acer*) system. The ridge-tilled system was broadcast sprayed with 30 kg N ha^{-1}

Table 1 Midden and bulk soil sampling site information

Ecosystem	Arable				Pasture	Forest
Land use	Corn/soybean/ wheat/cover-crop	Corn/soybean	Corn/soybean	Corn/soybean	Dairy cattle grazing field	Oak-hickory/ beech-maple
Tillage practices	Ridge-till	Chisel-plow	Chisel-plow	No-till	None	None
Soil type	Fine-silty, mixed, mesic Fluventic Hapludolls	Fine-silty, mixed, mesic Fluventic Hapludolls	Fine-loamy, mixed, mesic Typic Hapludult	Fine-loamy, mixed, mesic Aquultic Hapludult	Fine, mixed, mesic Fragiudalf	Fine, illitic, mesic Typic Hapludalf
Fertilizer application rate (Kg N ha^{-1})	30	117	232	232	None	None
Last midden disturbance	12 Months ridge-till	7 Month chisel-plow	1 Month chisel-plow	None no-till	3 Weeks hoof action	None Undisturbed
History	Prior 1991: conventional corn/soybean rotations	Prior 1991: conventional corn/soybean rotations	Prior 1989: high input corn/soybean rotations	Prior 1989: high input corn/soybean rotations	Prior 1993: conventional practices	Mature deciduous forest
Rainfall (cm)						
Summer 1996	21.55	31.24	31.24	30.47	26.72	22.81
Total 1996	102.90	115.85	115.85	113.46	104.72	111.86

Table 2 Chemical properties in middens of *Lumbricus terrestris* and in non-midden soil (composite samples) at the different ecosystems

Ecosystem	Arable				Pasture				Forest			
	Corn/soybean/ wheat/cover-crop		Corn/soybean		Corn/soybean		Corn/soybean		Dairy cattle grazing field		Oak- hickory/ beech- maple	
Fertilization rate (kg N ha ⁻¹)	30		117		232		232		None		None	
Tillage practices	Ridge-till		Chisel-plow		Chisel-plow		No-till		None		None	
	Midden	Bulk	Midden	Bulk	Midden	Bulk	Midden	Bulk	Midden	Bulk	Midden	Bulk
Organic matter (%)	3.65	3.27	3.83	3.74	3.66	2.65	6.29	4.08	5.02	4.55	8.90	7.42
pH	7.0	7.0	6.5	6.5	4.9	4.8	4.9	4.5	5.9	5.9	6.4	6.4
Total P (µg g ⁻¹)	87	84	32	34	31	30	30	41	46	45	47	47
Total K (µg g ⁻¹)	209	163	181	183	175	187	141	142	77	98	205	191
Total Ca (µg g ⁻¹)	1700	1530	1750	1560	810	800	830	730	1290	1150	2610	2280
Total Mg (µg g ⁻¹)	411	423	412	372	150	153	151	144	219	188	285	248

in April, and the chisel-plowed cornfield was broadcast sprayed with 22 kg N ha⁻¹ in May and side-dressed with 95 kg N/ha in June (urea ammonium nitrate solutions). The no-till cornfield and the chisel-plowed cornfield were broadcast fertilized with 232 kg N/ha of NH₄NO₃ in May, which may have magnified naturally occurring acidic soil conditions (Jolley and Pierre 1977; M. J. Shipitalo, personal communication).

In early June 1996, 45 middens were identified and flagged at the six sites described above, providing a total of 270 middens. On the same day in late June, July and August of 1996, ten midden and non-midden pairs were selected randomly at each site. Before sampling, we checked under small to medium sized middens for a 3- to 10-mm-diameter burrow opening, characteristic of *L. terrestris* (Edwards et al. 1988). A 7.5-cm-diameter corer sampler was forced into the soil to 6 cm in depth for the midden and bulk soil samples. Each midden sample therefore included both midden material and the soil directly beneath it. Bulk soil samples were taken at a distance of no more than 20 cm from the middens.

Analytical methods

Two soil and midden cores were mixed into one composite sample and half of this mixture was sieved (2-mm mesh) to be used in mineral N, dissolved N, MBN, and CarbC analyses. The remaining half sample was sieved (6-mm mesh) to separate the coarse litter (>6 mm) and used for measurements of fine litter (2–6 mm), POM (0.053–2 mm) and SOM (<2 mm) total C and N. A portion of each wet soil sample was oven-dried at 60°C for 48 h to determine gravimetric soil moisture content. Samples were stored at 40°C and analyzed within 10 days. Random midden and bulk samples were selected on each sampling date and mixed into one composite sample for pH, organic matter and various other nutrient analyses at the Ohio Agricultural Research and Development Center in Wooster, Ohio (Table 2).

Soil mineral N concentrations (NH₄⁺-N, NO₂⁻-N+NO₃⁻-N) were determined in 0.5 N K₂SO₄ soil extracts (1:5 soil:extractant) using a Lachat AE autoanalyzer. MBN was determined using chloroform fumigation and direct extraction (Brookes et al. 1985). DON was calculated as the difference between mineral N concentrations and the NO₃⁻-N concentrations, determined after alkaline persulfate digestion of the initial soil extracts (Cabrera and Beare 1993). All of these nutrient measurements were expressed as µg g⁻¹. To determine CarbC levels, dry homogenized soil was combined with 1 ml deionized water, 1 ml phenol solution (5%) and 5 ml concentrated sulfuric acid in a small test tube (Brink et al. 1960). These samples were analyzed on a microplate autoreader EL 311SX at 490 and 590 nm and the amount of CarbC was expressed as mg g⁻¹ of glucose.

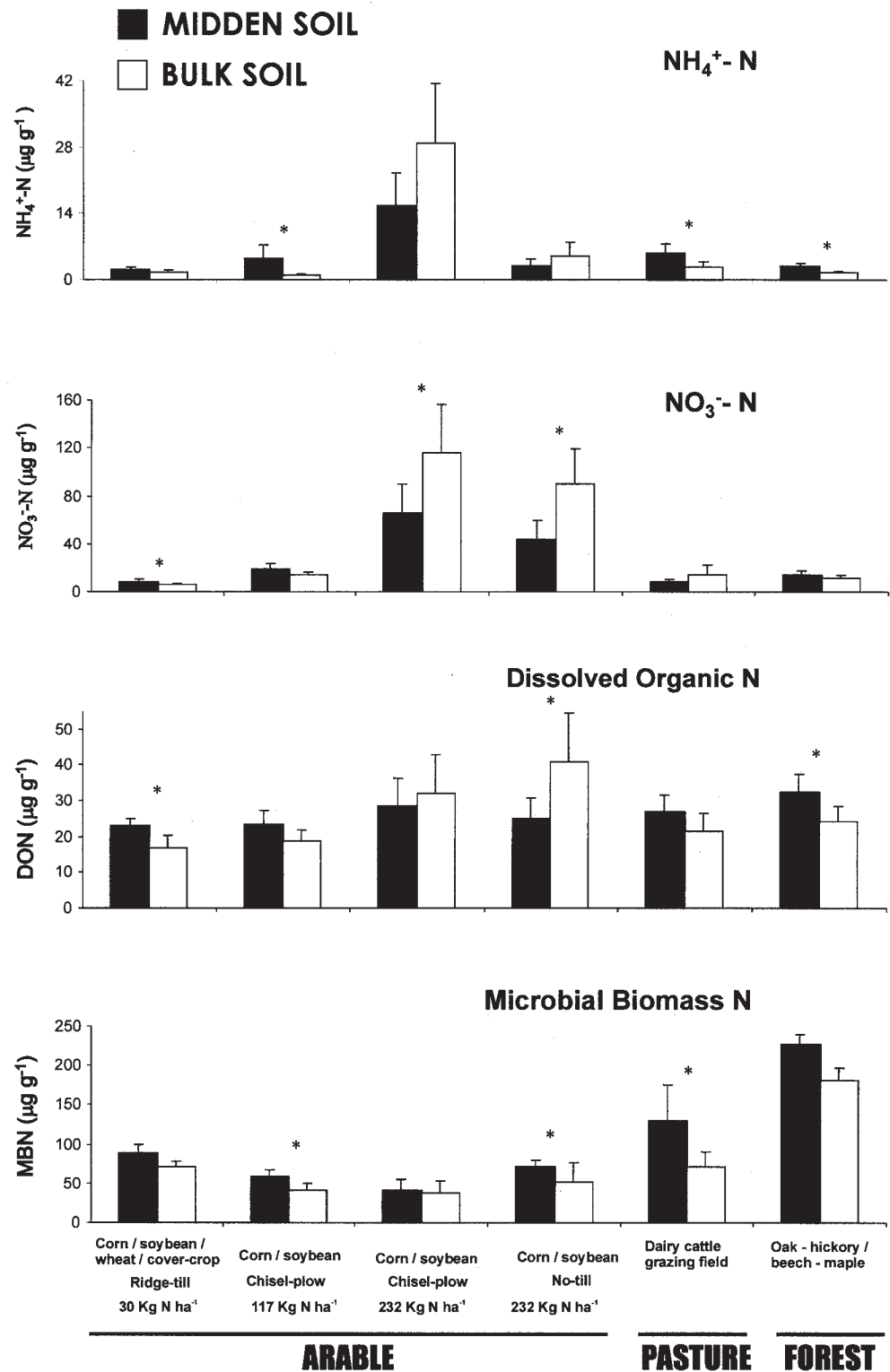
Table 3 Microbial biomass, carbohydrates and C and N content of different organic matter fractions in middens of *L. terrestris* and in non-midden soil across ecosystem types. POM Particulate organic matter, SOM soil organic matter

	Midden soil	Bulk soil
Microbial biomass (µg N g ⁻¹ dry weight)	102.44±2.99	75.84±3.14
Carbohydrates (mg g ⁻¹)	11.57±0.18	9.87±0.18
Total C (g m ⁻²)		
Coarse litter	220±9.60	78.21±11
Fine litter	232.76±8.37	81.54±10.94
POM	883.5±15.91	636.2±15.91
SOM	3220±39.07	2710±38.80
Total N (g m ⁻²)		
Coarse litter	5.30±0.26	1.94±0.31
Fine litter	8.95±0.37	3.22±0.47
POM	50.38±0.93	36.53±0.92
SOM	264.7±3.61	239.8±3.61
C:N ratio		
SOM	11.95±0.09	11.16±0.09

All significant; *P*<0.0001

Organic material was separated into four organic matter fractions by dry or wet sieving and dispersion by sodium metaphosphate (5%). Coarse litter (>6 mm) from the entire sample had been collected previously from the 6-mm sieve and dispersed with sodium metaphosphate, placed on a rotational shaker (250 revolutions min⁻¹ for 15 h), and rinsed with deionized water to wash away excess sodium metaphosphate and soil particles. Fine litter (2–6 mm) and POM samples (0.053–2 mm) were obtained from 50-g subsamples of field-moist soil, by dispersing with sodium metaphosphate and washing through a stack of 2-mm and 0.053-mm sieves. SOM (<2 mm) was obtained by passing dry soil samples through a 2-mm sieve. All the fractions were dried at 60°C for 48 h, weighed and homogenized in a centrifugal mill (0.2-mm screen). Total C and N levels were determined on a Carlo Erba NA1500 C/N analyzer and expressed as g m⁻².

Fig. 1 Concentrations of inorganic and organic forms of N in midden and bulk soil samples for ecosystem effects (mean+SE). Asterisks indicate significant differences between midden soil and bulk soil for each individual site ($P \leq 0.05$)

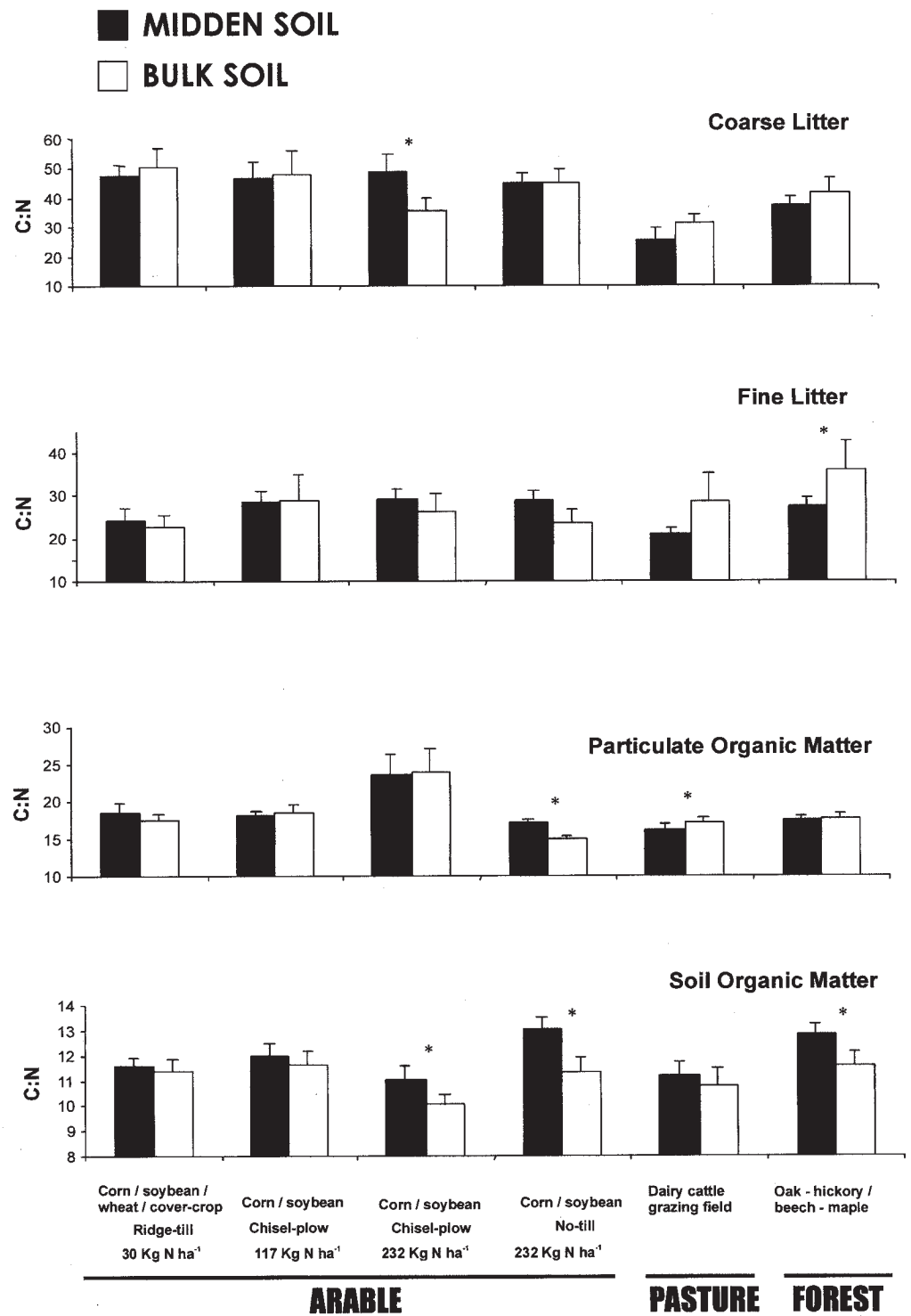


Analysis of results

This experiment was designed to evaluate overall effects of *L. terrestris*' middens that occurred consistently across ecosystem types and also within each individual ecosystem. The ecosystem types were treated as replicates in analyzing across-ecosystem effects and the ecosystem×midden term was the error term for significance testing of the overall midden effects. The midden×date

interaction was tested by using the sum of the ecosystem×midden and ecosystem×midden×date terms as the error term. Overall midden effects are summarized in Table 3, and ecosystem×midden and midden×date effects are given in Figs. 1, 2, and 3. Individual ecosystems were evaluated for midden effects with the residual error terms used for testing of significance. Log transformations were done on all data to normalize the variance. Means were separated using Fisher's LSD (0.05). In order to analyze the

Fig. 2 C:N ratios for coarse, fine, particulate and soil organic matter fractions in midden and bulk soil samples for ecosystem effects (mean±SE). *Asterisks* indicate significant differences between midden and bulk soil samples for each individual site ($P \leq 0.05$)

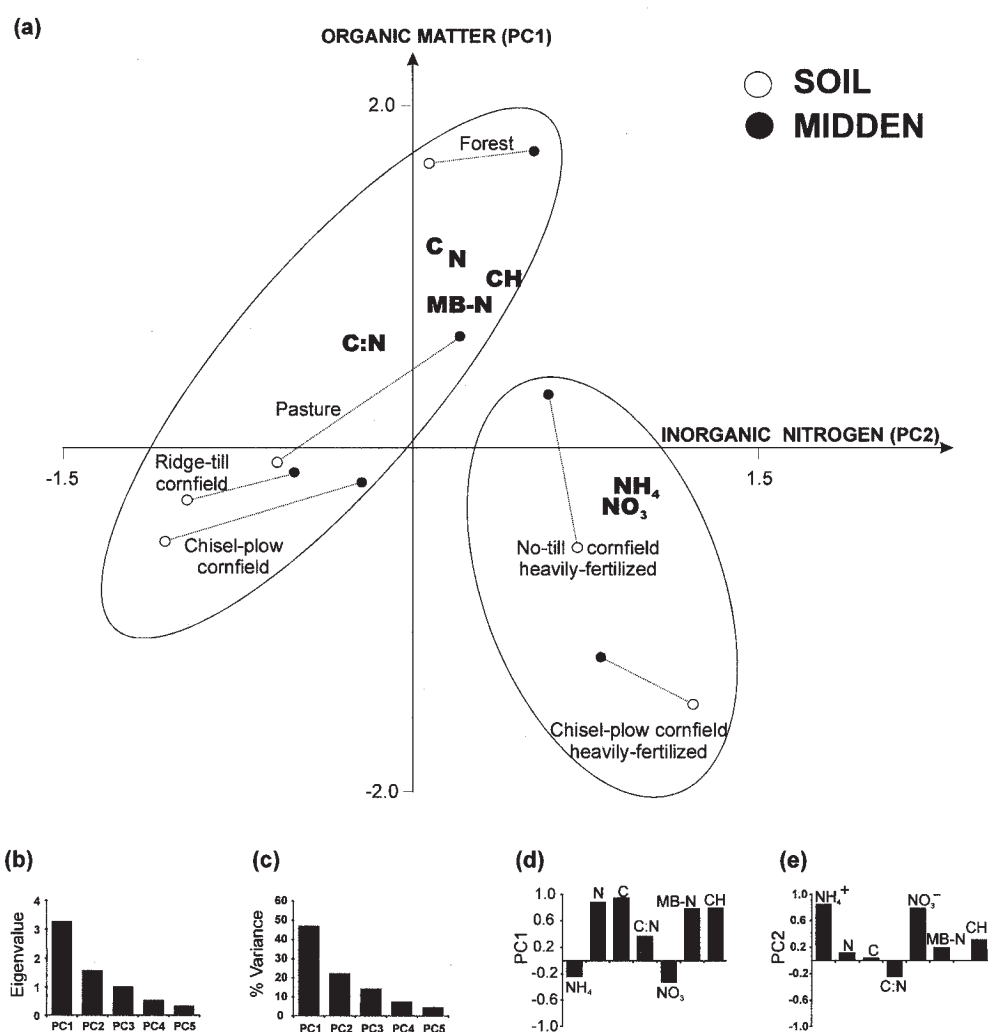


underlying effect of earthworm middens in different ecosystems, the data were subjected to principal component analysis (PCA). The PCA was carried out using each soil and midden sample as a case and the natural logarithm of every chemical parameter as variables.

Results

Compared to bulk soil, the earthworm midden samples had more overall soil C and N levels in all different ecosystem types. The concentration of organic matter into earthworm middens also raised other nutrient levels, such as Ca^{2+} (Table 2). Across the ecosystem types, midden samples had significantly more MBN, CarbC, C:N

Fig. 3 **a** Midden effect and position of the different ecosystems in the plan defined by principal component (*PC*) axes. PC1 represents 69.35% of total inertia and PC2 46.87%. **b** Graph of relative eigenvalues of the PC axes. **c** Percentage of variance of the PC axes. **d** Factor loadings of the PC1. **e** Factor loadings of the PC2.



ratios for SOM, and total C and N levels for all organic matter fractions. In other words, all measurements except the mineral N (NH₄⁺-N and NO₃⁻-N), DON and the C:N ratios for the organic matter coarser fractions had significant differences between middens and surrounding soil (Table 3). Mineral and DON levels were greater in the midden soil compared to the surrounding soil on all sites, except for the heavily fertilized (232 kg N ha⁻¹) NAEW agroecosystems and the pasture, for NO₃⁻-N levels (Fig. 1). The less fertilized MESEA chisel-plowed cornfield, the pasture, and the forest middens soils had significantly greater NH₄⁺-N levels than bulk soil, while the least fertilized (30 kg N ha⁻¹) MESEA ridge-till cornfield had significantly greater NO₃⁻-N levels in midden soil relative to bulk soil. The heavily fertilized agroecosystems had significantly less NO₃⁻-N in the midden soil, compared to the bulk soil, but differences in NH₄⁺-N concentrations between middens and bulk soils were not significant. DON levels were correlated to trends in mineral N levels, in comparing midden and bulk soil for ecosystem types. The least fertilized ridge-till cornfield and the forest had significantly higher DON levels in the midden samples, while the no-till cornfield had signifi-

cantly lower DON levels in the midden soil relative to bulk soil. All sites had greater MBN concentrations in midden soils compared to bulk soil (Fig. 1).

Trends in mineral N levels between middens and bulk soils at all six sites were consistent for each sampling date. As with the mineral and dissolved N measurements, half of the individual sites had significant differences in MBN concentrations between the midden and bulk soil: the chisel-plowed and the no-till cornfields, and the pasture had significantly higher MBN levels in midden soil (Fig. 1).

Other than overall differences between middens and bulk soils, there were no other significant effects for CarbC and the midden samples had higher total C and N levels, for all organic fractions compared to the bulk soil samples (Table 3). From June to August, total C and N levels in the coarse litter fraction decreased in the midden soils and increased in the bulk soils, while total C and N levels for SOM decreased in both midden and bulk soils (data not shown).

Depending on the ecosystem type, C:N ratios were greater or smaller in the midden samples relative to those in the bulk samples for the coarse, fine and particulate

(POM) fractions (Fig. 2). In the coarse litter, the fine litter and the POM fractions, the heavily fertilized agroecosystems had greater or nearly equal C:N ratios in the midden soil samples, while the pasture and the forest had lower or nearly equal C:N ratios. Few of the individual sites had significant differences between midden and bulk samples for these coarser fractions. For the SOM fractions, all sites had significantly greater C:N ratios in the midden relative to bulk soils (Fig. 2). The heavily fertilized agroecosystems and the forest had significantly greater C:N ratios in the midden soil compared to bulk soil. The fewer fertilized agroecosystems had only small differences in C:N ratios between midden and bulk soil samples for all organic fractions.

PCA (Fig. 3) revealed the presence of two main factors accounting for 69.35% of the total variance observed. The first factor (PC1) of the PCA explains 46.87% of the environmental variance; it represents the organic matter content in the soil and is highly correlated with the total C, total N, MBN and carbohydrates C (Fig. 3d). PC1 separates ecosystems with a high SOM content (forest and pasture) from the different agroecosystems. The second factor (PC2) of the PCA accounts for 22.49% of the environmental variance and it was defined as the effect of inorganic N, explained by the $\text{NH}_4^+\text{-N}$ and the $\text{NO}_3^-\text{-N}$ content, which are high and positively correlated with PC2 (Fig. 3d). It separates the forest, the heavily fertilized no-till cornfield and the heavily fertilized chisel-plow cornfield from the pasture and the less fertilized cornfields. The plan defined by both principal components clearly separates two main groups: (1) includes the forest, the pasture and the less fertilized cornfields; the midden effect is to increase slightly the organic matter content and strongly the inorganic N content; and (2) includes the heavily fertilized agroecosystems and the midden effect is also to increase the organic matter content but to decrease the inorganic N content.

Discussion

Depending on the type of ecosystem, middens had either more or less $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and DON compared to the surrounding soil (Figs. 1, 3). Larger mineral and dissolved N levels in middens relative to bulk soil resulted from the concentrating of organic material and associated soil resources by *L. terrestris* above their burrow entrances. However, the lower mineral and DON levels in the midden soil may have indicated a potential loss of soil N in midden soils, as recorded in the heavily fertilized agroecosystems. Subler and Kirsch (1998) reported more $\text{NH}_4^+\text{-N}$ and DON in midden soil than in bulk soil, and suggested that mineralization was accelerated in the midden soil relative to bulk. Greater mineralization rates in midden soils appeared to have caused elevated mineral and dissolved N concentrations in the ridge-till cornfield, the chisel-plowed cornfield, the pasture and the forest soils, separated as a group by the PCA analysis.

Earthworm mucus and excretions in middens would have also contributed to increased $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and DON levels in midden/burrow soil (Curry et al. 1995). Additionally, Gorres et al. (1997) hypothesized that *L. terrestris*' burrows produced favorable conditions for nematodes, which may have also produced greater mineral N levels in midden soil relative to bulk soil. Maraun et al. (1999) concluded that the increased biomass of bacterial feeding nematodes and euryoecious Collembola was caused by an increased microbial biomass in the middens. Although Subler and Kirsch (1998) reported greater $\text{NH}_4^+\text{-N}$ and DON concentrations, they also reported lower $\text{NO}_3^-\text{-N}$ levels in the midden soil relative to those in bulk soil due to reduced nitrification or increased leaching and gaseous loss.

Land-use differences (i.e., N fertilization rates) mainly and possibly differences in geography (i.e., soil type, climate, topography), seemed to have produced an opposite effect on mineral and dissolved N levels in midden soil relative to bulk at the heavily fertilized agroecosystems (Fig. 3). Deep, vertical, *L. terrestris*' burrows beneath midden macrosites, could have increased the potential for leaching of nutrient compared to the surrounding soil (Zachmann et al. 1987; Lachnicht et al. 1997). Therefore, more precipitation combined with higher application levels of N fertilizer, may have increased the leaching of $\text{NO}_3^-\text{-N}$ (Edwards et al. 1989, 1992; Blair et al. 1997; Subler and Kirsch 1998), $\text{NH}_4^+\text{-N}$ (Stevenson 1982; Scheu and Parkinson 1994) and DON (Subler et al. 1997) from the midden soil at the heavily fertilized agroecosystems. Additionally, the high concentrations of available $\text{NO}_3^-\text{-N}$ and organic C in middens may have established favorable conditions for denitrification (Parkin 1987; P. J. Bohlen, personal communication), assuming this process was not inhibited by acidic soil conditions (Gilliam and Gambrell 1978; Knowles 1981). However, the nitrification of $\text{NH}_4^+\text{-N}$ can occur readily under acidic soil conditions and may have occurred predominantly in the middens at the heavily fertilized agroecosystems because of increased nutrient availability, soil fertility and aeration (Verstraete 1981a), leading to an increased potential for the leaching of the oxidized $\text{NH}_4^+\text{-N}$ (Verstraete 1981b). High nitrification rates may have explained the significantly lower amounts of $\text{NH}_4^+\text{-N}$ at the no-till cornfield with larger and less disturbed middens, when compared to the chisel-plowed cornfield.

MBN levels were consistently greater in midden soils relative to bulk soils at all sites, including the heavily fertilized agroecosystems (Table 3). Increased concentrations of readily decomposable organic material in middens compared to those in the surrounding soil could have resulted from the redistribution and partial processing of organic matter by *L. terrestris*. Bohlen et al. (1997) and Subler and Kirsch (1998) also reported high microbial activity in earthworm middens and the latter study also reported significantly more soil moisture in earthworm middens compared to those in the surrounding soil. Although soil moisture has been specified as a primary factor influencing microbial populations (Doran

1980), our investigation did not find significant differences in soil moisture levels between middens and bulk soils. However, significantly more CarbC (Jansson and Persson 1982), total C and N in all organic fractions and especially in the SOM fraction (Schnurer et al. 1985), and greater aeration occurred in midden soil (Gorres et al. 1997), and appeared to have increased immobilization. Although microbial populations did not appear to be inhibited by the increased mineral and dissolved N levels in the midden samples at the no-till cornfield and the chisel-plowed cornfield they may have been affected because differences in MBN levels between midden and bulk soil were the least pronounced ($\pm 3.48 \text{ mg g}^{-1}$) compared with all other sites. High mineral N levels in the midden soil at the chisel-plowed cornfield when compared to those in the other sites, may have decreased microbial activity by altering microbial catabolism (Verstraete 1981b).

Coarse litter, fine litter, POM and SOM amounts were significantly greater in the midden samples relative to the bulk samples, across ecosystem types, as measured by total C and N (Table 3). The bulk soil surrounding middens often had little coarse and fine litter due to the feeding activities of *L. terrestris*, since most of the available organic material was incorporated into the midden. Together with the large quantities of coarse and fine litter in middens, earthworm casts were also integral components of middens and may have contributed to increased POM and SOM levels. Shipitalo and Protz (1988) reported that *L. terrestris*' casts contained more fine-textured organic material and significantly greater concentrations of clay than the surrounding soil. Clay associations with SOM, including interactions within casts, may have caused greater rates of microaggregate or macroaggregate formation in the middens (Sorensen 1981; Tisdall and Oades 1982). These aggregates would have protected organic nutrients from biodegradation, leading to increased total C and N levels in midden samples relative to bulk.

Increased C:N ratios in midden soils relative to bulk soils for the organic matter fraction at all sites may have been caused by lower concentrations of soil N, available for decomposition, due to the direct and indirect effects of *L. terrestris*' feeding activities. If *L. terrestris* selectively consumed material with a low C:N ratio (Satchell and Lowe 1967; Daniel 1991; Binet and Trehen 1992), then the remaining litter could have had a higher C:N ratio. However, the ingestion of low C:N ratio litter may have resulted in casts containing a low C:N ratio (Syers et al. 1979). Lower quantities of soil N available for decomposition, due to high microbial activity and/or a loss of soil N from middens, may have worked to raise C:N ratios. Other studies have found a greater proportion of organic N was mineralized as the particle size decreased (Chichester 1969; Ladd et al. 1977; Cameron and Posner 1979), which could explain why an overall effect of middens was expressed clearly in the SOM fraction. C:N ratios in the midden and bulk soil samples depended on the amount of C and N constituents of the organic matter

available for decomposition and on the chemical composition of the litter originally incorporated into the middens (Jansson and Person 1982; Sinsabaugh et al. 1993).

In conclusion, our results demonstrated that, in different ecosystem types, *L. terrestris* concentrated soil resources within midden macromolecules, which are hotspots of biological and chemical activity. Although there were some differences in midden nutrient pools depending on the ecosystem types, most middens contained significantly more soil C and N overall, compared to the surrounding soil. Our investigation and related work (Subler and Kirsch 1998) revealed how *L. terrestris*' middens seemed to have increased N immobilization-mineralization and correspondingly increased the potential for loss of mineral N through leaching and gaseous losses. The variability in organic matter distribution due to midden formation, may have altered not only N transformations, but also the rates of decomposition of organic materials, and the accessibility of soil C and N for associated plant/animal utilization. Furthermore, high midden density may affect nutrient cycling dynamics at the ecosystem level; however, extrapolating results from small-scale investigations, will require further research in field manipulations (Blair et al. 1995; Parmelee et al. 1998).

Acknowledgements The comments and suggestions of S. Subler during the development of this work and the critical reviews of M. Fisher, W. Shuster and M. Allen are gratefully acknowledged. We also thank M. Shipitalo, J. Dickinson, J. Van Arsdale, D. McHugh, C. Baranski, A. Kirsch, M. McCoy, D. Brazis, C. Lucht, R. Atiyeh, and J. Whalen for their assistance and A. Velando for helping with the statistics.

References

- Binet F, Curmi P (1992) Structural effects of *Lumbricus terrestris* (Oligochaeta: Lumbricidae) on the soil-organic matter system: micromorphological observations and autoradiographs. *Soil Biol Biochem* 24:1519-1523
- Binet F, Trehen P (1992) Experimental microcosm study of the role of *Lumbricus terrestris* (Oligochaeta: Lumbricidae) on N dynamics in cultivated soils. *Soil Biol Biochem* 24:1501-1506
- Blair JM, Parmelee RW, Lavelle P (1995) Influences of earthworms on biogeochemistry. In: Hendrix PF (ed) *Earthworm ecology and biogeography in North America*. Lewis, Boca Raton, Fla. pp 127-158
- Blair JM, Parmelee RW, Allen MF, McCartney DA, Stinner BR (1997) Changes in soil N pools in response to earthworm population manipulations in agroecosystems with different N sources. *Soil Biol Biochem* 29:361-367
- Bohlen PJ, Parmelee RW, McCartney DA, Edwards CA (1997) Earthworm effects on C and N dynamics of surface litter in corn agroecosystems. *Ecol Appl* 7:1341-1349
- Brink RH Jr, Dubach P, Lynch DL (1960) Measurement of carbohydrates in soil hydrolyzates with anthrone. *Soil Sci* 89: 157-166
- Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil N: a rapid direct extraction method to measure microbial biomass N in soil. *Soil Biol Biochem* 17:837-842
- Cabrera ML, Beare MH (1993) Alkaline persulfate oxidation for determining total N in microbial biomass extracts. *Soil Sci Soc Am J* 57:1007-1012
- Cameron RS, Posner AM (1979) Mineralisable organic N in soil fractionated according to particle size. *J Soil Sci* 30:565-577

- Chichester FW (1969) Nitrogen in soil organo-mineral sedimentation fractions. *Soil Sci* 107:356–363
- Curry JP, Byrne D, Boyle KE (1995) The earthworm population of a winter cereal field and its effects on soil and N turnover. *Biol Fertil Soils* 19:166–172
- Daniel O (1991) Leaf-litter consumption and assimilation of juveniles of *Lumbricus terrestris* L. (Oligochaeta, Lumbricidae) under different environmental conditions. *Biol Fertil Soils* 12:202–208
- Devliegher W, Verstraete W (1995) *Lumbricus terrestris* in a soil core experiment: nutrient-enrichment processes (NEP) and gut-associated processes (GAP) and their effect on microbial biomass and microbial activity. *Soil Biol Biochem* 27:1573–1580
- Doran JW (1980) Soil microbial and biochemical changes associated with reduced tillage. *Soil Sci Soc Am J* 44:765–771
- Edwards CA, Bajer JE (1992) The use of earthworms in environmental management. *Soil Biol Biochem* 24:1683–1689
- Edwards WM, Shipitalo MJ, Norton LD (1988) Contributions of macroporosity to infiltration into a continuous corn no-tilled watershed: implications for contaminant movement. *J Contamin Hydrol* 3:193–205
- Edwards WM, Shipitalo MJ, Owens LB, Norton LD (1989) Water and nitrate movement in earthworm burrows within long-term no-till cornfields. *J Soil Water Conserv* 44:240–243
- Edwards WM, Shipitalo MJ, Owens LB, Norton LD (1990) Effect of *Lumbricus terrestris* L. burrows on hydrology of continuous no-till corn fields. *Geoderma* 46:73–84
- Edwards WM, Shipitalo MJ, Dick WA, Owens LB (1992) Rainfall intensity affects transport of water and chemicals through macropores in no-till soil. *Soil Sci Soc Am J* 56:52–58
- Gilliam JW, Gambrell RP (1978) Temperature and pH as limiting factors in loss of nitrate from saturated Atlantic coastal plain soil. *J Environ Qual* 7:526–532
- Gorres JF, Savin MC, Amador JA (1997) Dynamics of C and N mineralization, microbial biomass, and nematode abundance within and outside the burrow walls of anecic earthworms (*Lumbricus terrestris*). *Soil Sci* 162:666–671
- Hamilton WE, Sillman DY (1989) Influence of earthworm middens on the distribution of soil microarthropods. *Biol Fertil Soils* 8:279–284
- Jansson SL, Persson J (1982) Mineralization and immobilization of soil N. In: Stevenson FJ (ed) Nitrogen in agricultural soils. ASA-CSSA-SSSA agronomy monographs vol 22. ASA, CSSA, SSSA, Madison, Wis. pp 229–253
- Jolley VD, Pierre WH (1977) Soil acidity from long-term use of N fertilizer and its relationship to recovery of the N. *Soil Sci Soc Am J* 41:368–373
- King HGC, Heath GW (1967) The chemical analysis of small samples of leaf material and the relationship between the disappearance and composition of leaves. *Pedobiologia* 7:192–197
- Knollenberg WG, Merritt RW, Lawson DL (1985) Consumption of leaf litter by *Lumbricus terrestris* (Oligochaeta) on a Michigan woodland floodplain. *Am Midl Nat* 113:1–6
- Knowles R (1981) Denitrification. In: Clark F, Rosswall T (ed) Terrestrial nitrogen cycles. Processes, ecosystem strategies and management impacts. *Ecol Bull [Stockh]* 33:315–329
- Lachnicht SL, Parmelee RW, McCartney D, Allen M (1997) Characteristics of macroporosity in a reduced tillage agroecosystem with manipulated earthworm populations: implications for infiltration and nutrient transport. *Soil Biol Biochem* 29:494–498
- Ladd JN, Parsons JW, Amato M (1977) Studies of N immobilization and mineralization in calcareous soils. II. Mineralization of immobilized N from soil fractions of different particle size and density. *Soil Biol Biochem* 9:319–325
- Laing JE, Heraty JM, Corrigan JE (1986) Leaf burial by the earthworm, *Lumbricus terrestris* (Oligochaeta: Lumbricidae), as a major factor in the population dynamics of *Phyllonorycter blancardella* (Lepidoptera: Gracillariidae) and its parasites. *Environ Entomol* 15:321–326
- Maraun M, Alpehi 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
- Nielson GA, Hole FD (1964) Earthworms and the development of coprogenous A1 horizons, in forest soils of Wisconsin. *Soil Sci Soc Am Proc* 28:426–430
- Parkin TB (1987) Soil microsites as a source of denitrification variability. *Soil Sci Soc Am J* 51:1194–1199
- Parmelee RW, Bohlen PJ, Blair JM (1998) Earthworms and nutrient cycling processes: integrating across the ecological hierarchy. In: Edwards CA (ed) earthworm ecology. St. Lucie Press, Boca Raton, Fla. pp 123–143
- Satchell JE (1963) Nitrogen turnover by a woodland population of *Lumbricus terrestris*. In: Doeksen J, Van Der Drift J (ed) Soil Organisms. North Holland Press, Amsterdam, pp 60–66
- Satchell JE, Lowe DG (1967) Selections of leaf litter by *Lumbricus terrestris*. In: Graff O, Satchell JE (eds) Progress in soil biology. Vieweg, Braunschweig, pp 102–120
- Scullion J, Ramshaw GA (1988) Factors affecting surface casting behavior in several species of earthworm. *Biol Fertil Soils* 7:39–45
- Scheu S, Parkinson D (1994) Effects of earthworms on nutrient dynamics, C turnover and microorganisms in soils from cool temperate forests of the Canadian Rocky Mountains – laboratory studies. *Appl Soil Ecol* 1:113–125
- Schnurer J, Clarholm M, Rosswall T (1985) Microbial biomass and activity in an agricultural soil with different organic matter contents. *Soil Biol Biochem* 17:611–618
- Shaw C, Pawluk S (1986) Faecal microbiology of *Octolasion tyrtaeum*, *Aporrectodea turgida* and *Lumbricus terrestris* and its relation to the C budgets of three artificial soils. *Pedobiologia* 29:377–389
- Shipitalo MJ, Protz R (1988) Factors influencing the dispersability of clay in worm casts. *Soil Sci Soc Am J* 52:764–769
- Sinsabaugh RL, Antibus RK, Linkins AE, McLaugherty CA, Rayburn L, Repert D, Weiland T (1993) Wood decomposition: N and phosphorus dynamics in relation to extracellular enzyme activity. *Ecology* 74:1586–1593
- Sorensen LH (1981) Carbon-N relationships during the humification of cellulose in soils containing different amounts of clay. *Soil Biol Biochem* 13:313–321
- Stevenson FJ (1982) Origin and distribution of N in soil. In: Stevenson FJ (ed) nitrogen in agricultural soils. ASA-CSSA-SSSA agronomy monographs vol 22. ASA, CSSA, SSSA, Madison, Wis. pp 1–44
- Subler S, Kirsch AS (1998) Spring dynamics of soil C, N, and microbial activity in earthworm middens in a no-till corn field. *Biol Fertil Soils* 26:243–249
- Subler S, Baranski CM, Edwards CA (1997) Earthworm additions increased short-term N availability and leaching in two grain-crop agroecosystems. *Soil Biol Biochem* 29:413–421
- Syers JK, Sharpley AN, Keeney DR (1979) Cycling of N by surface-casting earthworms in a pasture ecosystem. *Soil Biol Biochem* 11:181–185
- Szlavec K (1985) The effect of microhabitats on the leaf litter decomposition and on the distribution of soil animals. *Holarct Ecol* 8:33–38
- Tisdall JM, Oades JM (1982) Organic matter and water-stable aggregates in soils. *J Soil Sci* 33:141–163
- Verstraete W (1981a) Nitrification. In: Clark FE, Rosswall T (ed) Terrestrial nitrogen cycles. Processes, ecosystem strategies and management impacts. *Ecol Bull [Stockh]* 33:303–314
- Verstraete W (1981b) Nitrification in agricultural systems: call for control. In: Clark FE, Rosswall T (ed) Terrestrial nitrogen cycles. Processes, Ecosystem strategies and management impacts. *Ecol Bull [Stockh]* 33:565–572
- Vimmerstedt JP, Finney JH (1973) Impact of earthworm introduction on litter burial and nutrient distribution in Ohio strip-mine spoil banks. *Soil Sci Soc Am Proc* 37:388–391
- Zachmann JE, Linden DR, Clapp CE (1987) Macroporous infiltration and redistribution as affected by earthworms, tillage, and residue. *Soil Sci Soc Am J* 51:1580–1586