

Influence of Earthworm Invasion on Redistribution and Retention of Soil Carbon and Nitrogen in Northern Temperate Forests

Patrick J. Bohlen,^{1*} Derek M. Pelletier,³ Peter M. Groffman,²
Timothy J. Fahey,³ and Melany C. Fisk^{3,4}

¹Archbold Biological Station, Lake Placid, Florida 33852, USA; ²Institute of Ecosystem Studies, Box AB, Millbrook, New York 12545, USA; ³Department of Natural Resources, Cornell University, Ithaca, New York 14853, USA; ⁴Department of Biology, Appalachian State University, Boone, North Carolina 28608-2027, USA

ABSTRACT

We analyzed soil organic matter distribution and soil solution chemistry in plots with and without earthworms at two sugar maple (*Acer saccharum*)–dominated forests in New York State, USA, with differing land-use histories to assess the influence of earthworm invasion on the retention or loss of soil carbon (C) and nitrogen (N) in northern temperate forests. Our objectives were to assess the influence of exotic earthworm invasion on (a) the amount and depth distribution of soil C and N, (b) soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and (c) soil solution chemistry and leaching of C and N in forests with different land-use histories. At a relatively undisturbed forest site (Arnot Forest), earthworms eliminated the thick forest floor, decreased soil C storage in the upper 12 cm by 28%, and reduced soil C:N ratios from 19.2 to 15.3. At a previously cultivated forest site with little forest floor (Tompkins Farm), earthworms did not influence the storage of soil C or N or soil C:N ratios.

Earthworms altered the stable isotopic signature of soil at Arnot Forest but not at Tompkins Farm; the alteration of stable isotopes indicated that earthworms significantly increased the loss of forest floor C but not N from the soil profile at Arnot Forest. Nitrate (NO_3^-) concentrations in tension and zero-tension lysimeters were much greater at Tompkins Farm than Arnot Forest, and earthworms increased NO_3^- leaching at Tompkins Farm. The results suggest that the effect of earthworm invasion on the distribution, retention, and solution chemistry of soil C and N in northern temperate forests may depend on the initial quantity and quality of soil organic matter at invaded sites.

Key words: biological invasions; soil organic matter; stable isotopes; soil solution nitrogen; carbon; forest ecosystems.

INTRODUCTION

It is important to improve our understanding of the factors that control total soil carbon (C) and nitrogen (N) in forest ecosystems because these elements play an important role in forest soil fertility and

global C cycles (Schulze 2000; Johnson and Curtis 2001). Forests contain an estimated half of the total terrestrial C pool, and two-thirds of that pool resides in the soil (Dixon and others 1994). Our knowledge of the factors that control the accumulation or loss of C in forest soils is still incomplete, and changes in the soil pool are more difficult to estimate and predict than changes in aboveground pools (Yanai and others 2000; Johnson and Curtis 2001). Furthermore, links between C and N cycles

Received 13 February 2002; accepted 19 December 2002; published online 12 January 2004.

*Corresponding author; e-mail: pbohlen@archbold-station.org

can affect soil organic matter storage and may have consequences for the response of terrestrial ecosystems to global change (Davidson 1995). Biological factors that influence C and N cycling in forest soils are particularly difficult to evaluate because of the large diversity of organisms in the soil and the complexity of trophic interactions involved in decomposition.

Earthworms (Oligochaeta: Annelida) are an especially important group in terms of their potential to influence C and N cycling in forest soils. They can consume the entire leaf fall in deciduous forests (Neilson and Hole 1964) and can increase C loss from forest soil (Alban and Berry 1994). They can also stimulate N turnover and mobilize and enhance the leaching of N and other nutrients from the forest floor (Haimi and Huhta 1990; Scheu and Parkinson 1994a). Their role in the formation of different humus types has long been recognized (Müller 1884). Humus types have proven to be useful indicators of the quality of organic matter and rates of nutrient transformations, and they may be an important factor in determining the response of forest soils to disturbance, atmospheric pollution, global warming, and other environmental changes (Green and others 1993; Muys and Granval 1997; Groffman and Bohlen 1999; Wilson and others 2001; Prescott and other 2000). Earthworms contribute to the formation of mull humus types in which organic surface layers are mixed into the mineral soil.

The invasion of northern temperate forests in North America by exotic earthworms may effect changes in soil organic matter and nutrient dynamics (Burtelow and others 1998; Groffman and Bohlen 1999). Many northern forests were historically devoid of native earthworms because of slow recolonization of the area after glaciers of the Wisconsinan ice age receded (James 1995). Many exotic earthworm species from Europe and Asia have been introduced into northern forests, and new sites are being colonized across a wide geographic area (Alban and Berry 1994; Scheu and Parkinson 1994b; Dymond and others 1997). One consequence of these invasions is a transformation in the distribution of soil organic matter, including a loss of C from the soil profile (Langmaid 1964; Alban and Berry 1994).

Less is known of how earthworm invasion may affect the retention or loss of other soil nutrients, such as N. Earthworms can influence two important pathways of N loss—N leaching and denitrification—but it is not known whether earthworm invasion will alter these processes significantly.

Earthworms have been shown to increase N availability and leaching in laboratory studies using forest soil from sites with a history of earthworm activity (Haimi and Huhta 1990; Scheu 1987). Other studies have examined the role played by exotic earthworm invasion in promoting nutrient loss from forest soils previously lacking earthworms (Scheu and Parkinson 1994a, 1994b; Steinberg and others 1997; Burtelow and others 1998), and Scheu and Parkinson (1994a) observed greater leaching losses of N from surface soils with earthworms in a laboratory study. In eastern New York State, USA, denitrification activity was shown to be greater in areas where forest soils had been invaded by earthworms than in adjacent areas not colonized by worms (Burtelow and others 1998). Although such studies capture many of the transient effects of earthworm colonization, it is unclear whether these results can be used to predict the influence of earthworms on N retention at larger temporal and spatial scales.

We examined the influence of exotic earthworm invasion on the distribution and quality of soil organic matter and of dissolved organic matter concentrations in soil lysimeters at two forest sites in central and eastern New York State, USA, with differing land-use histories. Our sites included an abandoned farm that was reforested and a continuously forested area that was logged about 100 years ago but never cultivated. At each site, we located areas lacking earthworm populations that were adjacent to areas with populations of invasive exotic earthworms. We set up a comprehensive study of nutrient cycling in plots with and without earthworms at these two forest sites (Bohlen and others 2004). One of our primary goals was to investigate the influence of earthworms on the distribution and retention of soil C and N pools. Our work was motivated by the following questions: (a) How does the presence of invasive exotic earthworm species influence soil C and N pools, and how do these effects differ between a C-rich primary forest and a previously cultivated forest with depleted soil C? (b) Do invading earthworms have differential effects on soil C and N and the retention or loss of these elements from the soil profile? (c) Can stable isotopes of C and N provide insight into the mixing of soil layers by earthworms and the fate of these forest floor elements after such mixing occurs? (d) Do changes in the quantity and quality of soil organic matter after earthworm invasion alter the concentration or potential leaching loss of dissolved forms of soil C and N?

METHODS

Study Sites and Experimental Design

Our study sites included sugar maple–dominated forests in two locations, one in eastern and the other in central New York. Arnot Forest, the site in central New York, is situated on the northern Allegheny Plateau physiographic province (42°16'N, 76°28'W). Annual rainfall is 100 cm; average summer and winter temperatures are 22.0°C and –4.0°C, respectively. Soils are derived from bedrock and glacial till consisting mostly of shales of the Upper Devonian Period. Soils are well-drained acidic Dystrichrepts with a well-developed organic horizon or forest floor (Oe, Oa) averaging about 4 cm thick and overlying an acidic (pH 4.5–5.0) mineral horizon, with pit and mound topography. Soil depths are variable but range from 70 to 100 cm. This site was logged in the late 19th century but has been minimally disturbed since then and has never been cultivated (Fain and others 1994; Volk and Fahey 1994; Fahey 1998). The current forest is dominated by six species characteristic of the Allegheny northern hardwood forests: sugar maple (*Acer saccharum*), red maple (*A. rubrum*), beech (*Fagus grandifolia*), white ash (*Fraxinus americana*), basswood (*Tilia americana*), and hemlock (*Tsuga canadensis*).

Tompkins Farm, the site in eastern New York (41°50'N, 73°45'W), is situated on the northern extension of the Great Appalachian Valley of the Ridge and Valley physiographic province. The site receives annual rainfall of around 98 cm, evenly distributed throughout the year; it has a mean summer temperature of 21.8°C and a winter temperature of –2.4°C. Late Cambrian–early Ordovician shales and slates underlie the upland forests at the site. Soils are principally acidic (pH 4.2–5.0) Dystrichrepts and are silt loams derived from glacial till and outwash ranging in depth from 60 to 100 cm, with very little accumulation of forest floor material. The site has a well-documented and complex history of agricultural cultivation ending around 1920 (Glitzenstein and others 1990).

At each location, we surveyed the site extensively to select three sets of paired plots where areas of forest without any apparent earthworm activity were adjacent to areas with established earthworm populations. In each stand, we established a 20 × 20 m plot that was invaded by earthworms (more than 150 individuals per square meter) and a 20 × 20 m reference plot that lacked earthworms. Subsequent to plot selection, sampling of earthworm populations revealed that some reference plots had

very small earthworm populations that were considered negligible relative to the populations in earthworm plots (fewer than two individuals per square meter) (Bohlen and others 2004). The plots were paired carefully in terms of soils, vegetation, slope position, and topography.

Soil Sampling and Analysis

Forest floor samples were collected in July of 1998 using the “pin block” method, in which a 15 × 15 cm wooden block is placed over the soil surface and held in place by large nails passed through the corner of the block. A knife is then used to cut through the soil around the block. The forest floor was carefully removed from the sample, and the underlying soil was sampled quantitatively in 3-cm depth increments to a depth of 12 cm. This depth corresponded approximately to the depth of the mixed A horizon in plots with earthworms. Samples were hand-sorted to remove fine roots and coarse fragments larger than 2 mm in diameter, and a subsample was air-dried and stored at 4°C for phosphorus (P) analysis (Suarez and others 2004). Additional samples from deeper in the soil (15, 25, and 35 cm) were obtained by taking horizontally oriented soil cores (5 × 15 cm) from the inner wall of soil pits excavated during installation of zero-tension lysimeters in the plots. Two samples were taken from each depth from two pits per plot and processed as described above.

All samples were dried (100°C) and finely ground for nutrient analysis, which was done by the Stable Isotope Facility at the University of California at Davis. Total C and N content and stable isotope ratios of C and N were measured by continuous flow isotope ratio mass spectrometry (IRMS) (20-20 mass spectrometer; PDZEuropa, Sandbach, England, UK) after sample combustion to CO₂ and N₂ at 1000°C by an on-line elemental analyzer (PDZEuropa ANCA-GSL). The gases were separated on a Carbosieve G column (Supelco, Bellefonte, PA, USA) before introduction to the IRMS. Sample isotope ratios were compared to those of standard gases injected directly into the IRMS before and after the sample peaks, and the δ¹⁵N (Air) and δ¹³C (Pee Dee Belemnite) values were calculated.

Lysimeter Sampling and Analysis

We used zero-tension pan lysimeters and ceramic cup tension lysimeters in our plots to sample different fractions of soil water (Landon and others 1999). Zero-tension lysimeters have been found to sample preferentially the most mobile water in the soil, whereas tension lysimeters preferentially sam-

ple micropore water that is closer to equilibrium with the surrounding soil (Schaffer and others 1979; Haines and others 1982; Magid and others 1992; Magid and Christensen 1993; Marques and others 1996). However, it is likely that tension lysimeters sample some mixture of more mobile and less mobile water (Landon and others 1999).

In June 1998, we positioned two tension lysimeters and two zero-tension lysimeters at 15- and 40-cm depth below the soil surface in each plot. We waited for two full growing seasons (summer 1998 and summer 1999) after installation of the lysimeters before initiating our sampling to allow for recovery from the soil disturbance associated with installation. The sampling period at Arnot Forest extended from October 1999 through October 2000, with no sampling in January and February. Tompkins Farm lysimeters were sampled from April to October 2000.

Tension lysimeters were maintained under tension (30 cm Hg) and were sampled every 2 weeks throughout the sampling period, except during the winter. Zero-tension lysimeters were sampled every 4 weeks, except during the winter, to ensure sufficient volume for chemical analysis. Samples were returned to the laboratory and filtered (0.45 μm) the same day they were collected and stored at 4°C until analysis. Samples from a subset of the dates were analyzed for total dissolved N (TN) (3 dates) at both sites and for dissolved organic C (DOC) (4 dates) at Arnot Forest only. Samples from Tompkins Farm were not preserved at time of collection and thus could not be analyzed for DOC. Dissolved organic N (DON) was determined as the difference between TN and the sum of dissolved inorganic N ($\text{NO}_3^- + \text{NH}_4^+$).

Nitrate and NH_4^+ were analyzed colorimetrically on a Perstorp series 3000 autoanalyzer (Alpkem, Clackamas, OR, USA). Persulfate digestion, followed by NO_3^- analysis, was employed to determine TN (D'Elia and others 1977). Samples for DOC analysis were oxidized in sealed ampules with an acid persulfate digestion (Huffman 1977) and analyzed using coulometric titration (Coulometrics, Wheatridge, CO, USA).

Data Analysis

Soil C and N data were analyzed using a general linear model with field site, presence of earthworms, soil depth, and their interactions as main effects using SAS version 1988, statistical analysis software. Soil C:N ratios were arcsine-transformed prior to analysis. In the event that assumptions of normality or homogeneity of variances (Levene's test of equality of error variances) were not met for

a given variable, the data were rank-transformed prior to analysis. Where significant interactions occurred between earthworm effects and site or soil depth, earthworm effects were analyzed by site or depth. Mean values for total C and N storage and C:N ratio of the top 12 cm of soil plus forest floor were compared using one-way chi-square tests.

Lysimeter samples within each of the study areas were analyzed using the mixed linear model (PROC MIXED) routine in SAS (SAS 1988). This method was chosen because of its ability to handle unbalanced data and to account for the fixed effects of earthworm presence, lysimeter depth, sampling date, and their interactions, as well as the random effects of the three sites within each study area, replicates within each plot, and their interactions. Significant fixed effects ($P < 0.05$) were evaluated using least square means comparisons with Tukey's adjustment to control the family-wise error rate. Data that were not normally distributed were log-transformed prior to analysis. All figures show non-transformed data and SE. We also repeated all analyses on the total amount of each nutrient in the lysimeter samples (concentration times the volume of water in the lysimeter) to account for possible dilution effects.

RESULTS

Soil Organic Matter and Stable Isotopes

Soils at the two study sites differed in the total amount, vertical distribution, and quality of soil organic matter. The total amount of C in the forest floor and upper 12 cm of mineral soil was significantly greater at Arnot Forest than at Tompkins Farm ($P = 0.025$), but the total amount of N in these combined layers did not differ between the two study areas (Table 1). As a result, the overall C:N ratio of surface soil was greater at Arnot than at Tompkins, particularly in plots without earthworms ($P = 0.049$) (Table 1, Figure 1). At both sites, total soil C and N and soil C:N declined with soil depth. Soils at Arnot Forest were more enriched in ^{13}C ($P = 0.011$) and ^{15}N ($P < 0.001$) than soils at Tompkins Farm (Figures 2,3).

Earthworms significantly ($P < 0.001$) reduced the forest floor at Arnot forest (Figures 4,5). At Tompkins Farm, where the mass of the forest floor was much less than at Arnot, the total amount of C in the forest floor did not differ significantly between plots with and without earthworms, but total N in the forest floor was about half as much in plots with worms as in plots without worms ($P = 0.08$). At both sites the C:N ratio of forest floor material

Table 1. Total Soil Carbon (C) and Nitrogen (N) and Soil C:N Ratio in the Upper 12 cm of Soil in Plots with and without Earthworms at Arnot Forest and Tompkins Farm

Site	Earthworms Present or Absent	Total C (g C/m ²)	Total N (g N/m ²)	C:N Ratio
Arnot Forest	No-worm	5610 ± 994 ^a	289 ± 15 ^a	19.2 ± 2.7 ^a
	Worm	4060 ± 263 ^b	265 ± 15 ^a	15.3 ± 0.1 ^b
Tompkins Farm	No-worm	3543 ± 245 ^c	270 ± 6.7 ^a	13.1 ± 0.7 ^c
	Worm	3537 ± 579 ^c	270 ± 39 ^a	13.1 ± 0.3 ^c

Values are means ± SE for three plots of each type at each site (n = 3).

^{a,b,c}Values within a column followed by a different letter are significantly different (P < 0.05, Chi-square test)

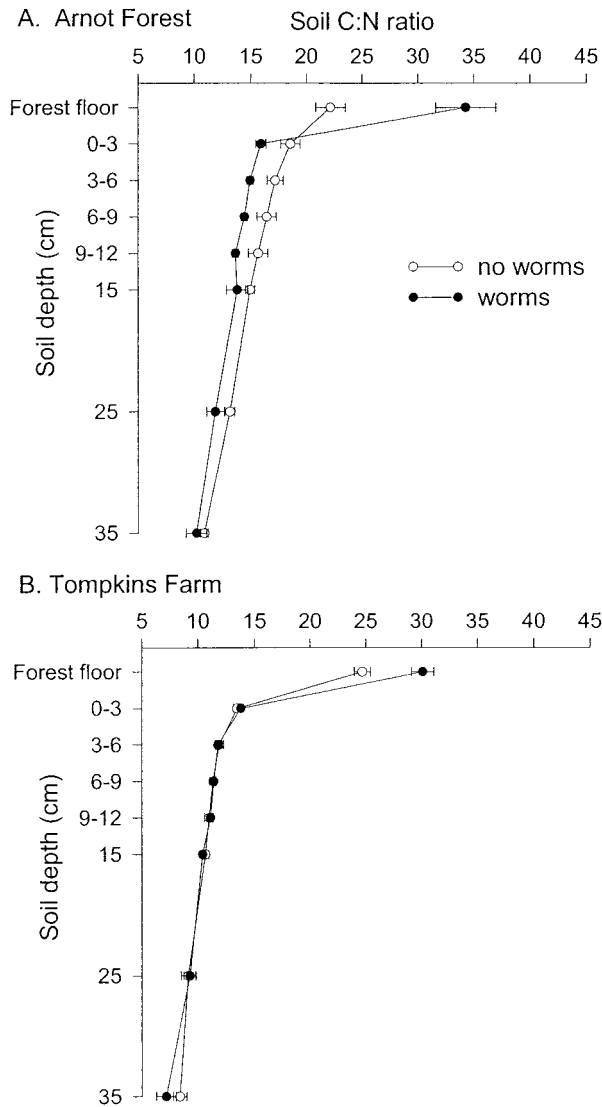


Figure 1. Soil carbon–nitrogen (C:N) ratio at different soil depths in plots with (filled circles) or without (open circles) earthworms at **A** Arnot Forest and **B** Tompkins Farm. Error bars are SE (n = 3).

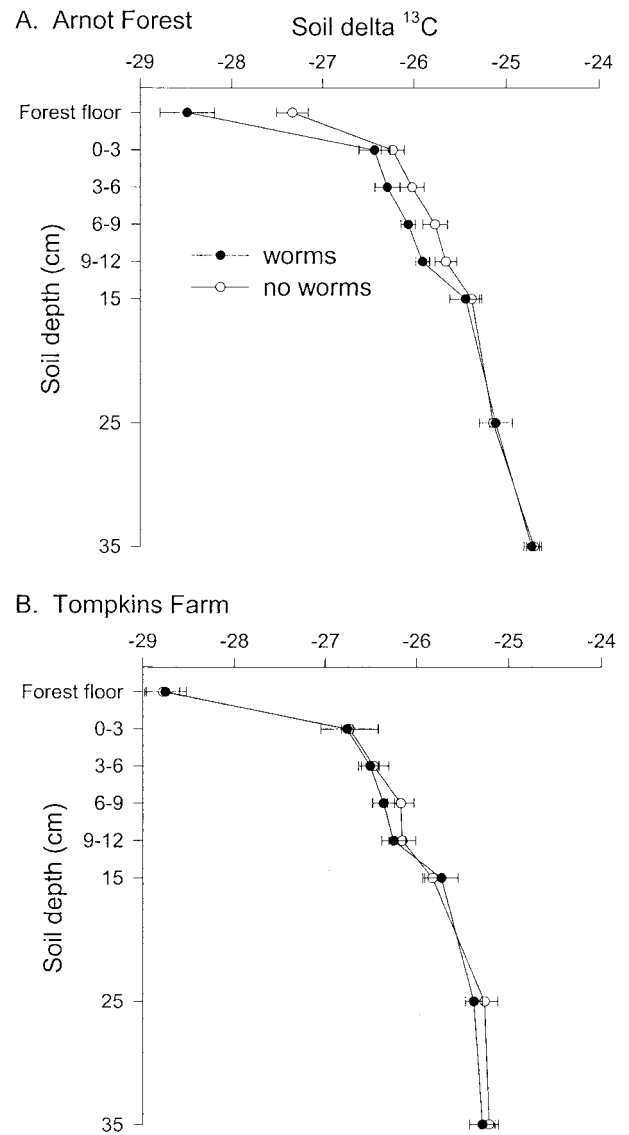


Figure 2. Soil $\delta^{13}\text{C}$ values at different soil depths in plots with (filled circles) or without (open circles) earthworms at **A** Arnot Forest and **B** Tompkins Farm. Error bars are SE (n = 3).

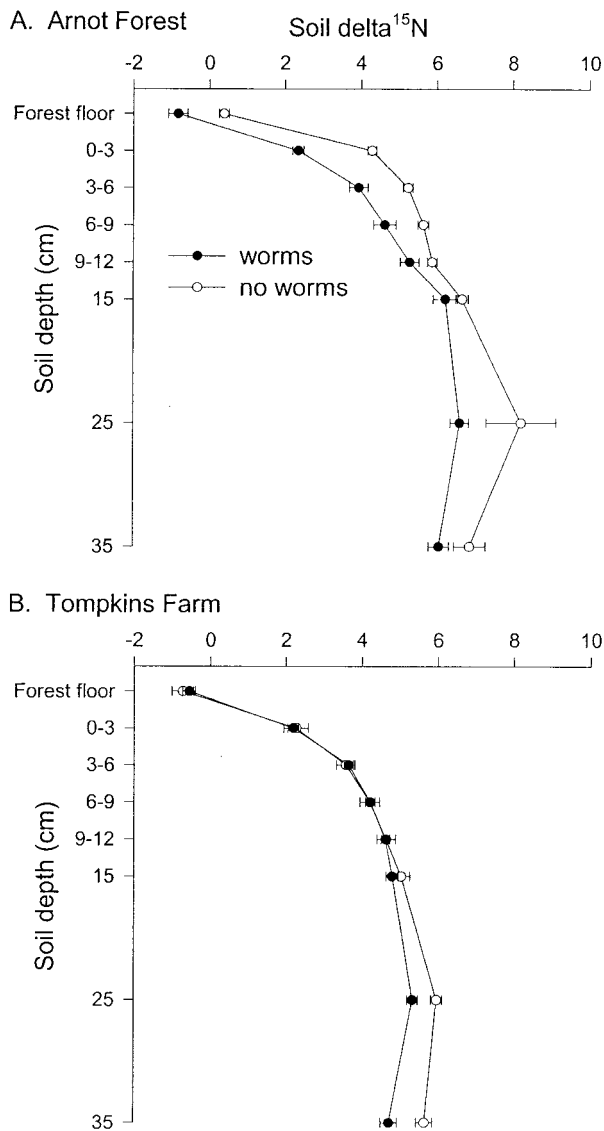


Figure 3. Soil $\delta^{15}\text{N}$ values at different soil depths in plots with (filled circles) or without (open circles) earthworms at A Arnot Forest and B Tompkins Farm. Error bars are SE ($n = 3$).

was greater in plots with worms than in plots without worms ($P = 0.03$) (Figure 1).

The effects of earthworms on the distribution of C and N in the mineral soil also differed between sites. At Arnot, the mineral soil in plots with earthworms was enriched with C and N relative to plots without earthworms, although the mineral soil in plots with worms was relatively more enriched in N than C (compare Figure 4 and 5). Consequently, the C:N ratio of mineral soil was significantly lower in plots with worms than in plots without worms at Arnot ($P = 0.002$) (Figure 1). There was variability in soil C content of mineral soil among plots at Arnot. In

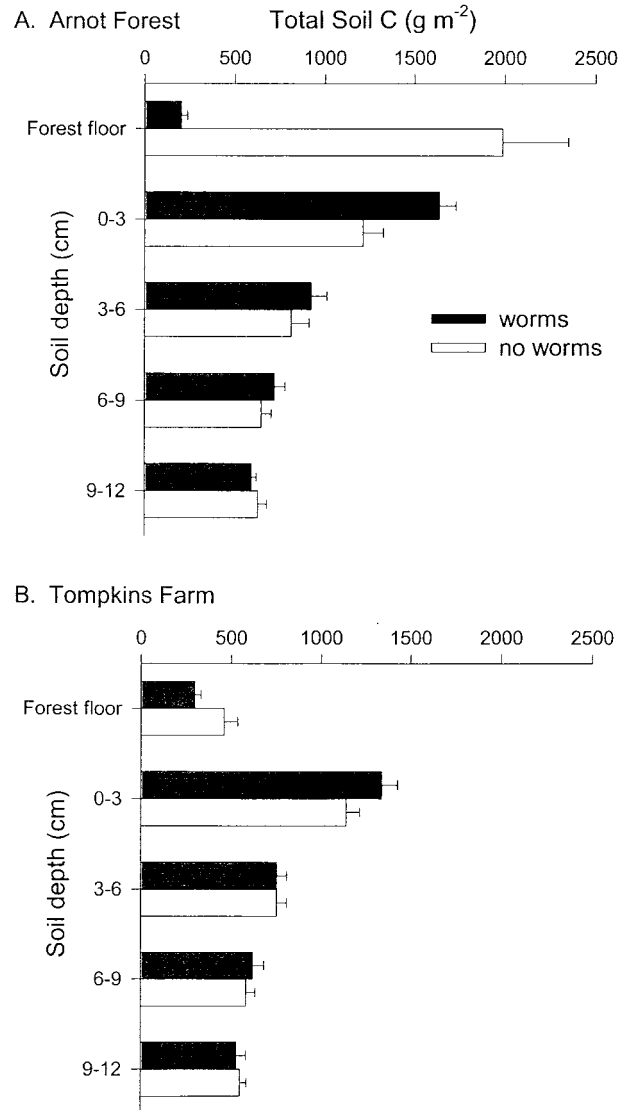


Figure 4. Depth distribution of total soil carbon (C) in the forest floor and upper 12 cm of mineral soil in 3-cm increments in plots with (dark shading) or without (light shading) earthworms at A Arnot Forest and B Tompkins Farm. Error bars are SE ($n = 3$).

particular, plot 3 had less mineral soil C in plots with earthworms than in plots without earthworms in all but the 0–3-cm soil layer, whereas plots 1 and 2 had more soil C throughout the upper 12 cm in plots with earthworms. By contrast, at Tompkins Farm, the presence of earthworms had no effect on total soil C or N or soil C:N throughout the mineral soil profile (Figure 1).

Soil $\delta^{13}\text{C}$ values increased with depth at both sites, but soils at Tompkins Farm were more depleted in ^{13}C than soils at Arnot ($P = 0.01$) (Figure 2). Average mineral soil $\delta^{13}\text{C}$ values were virtually

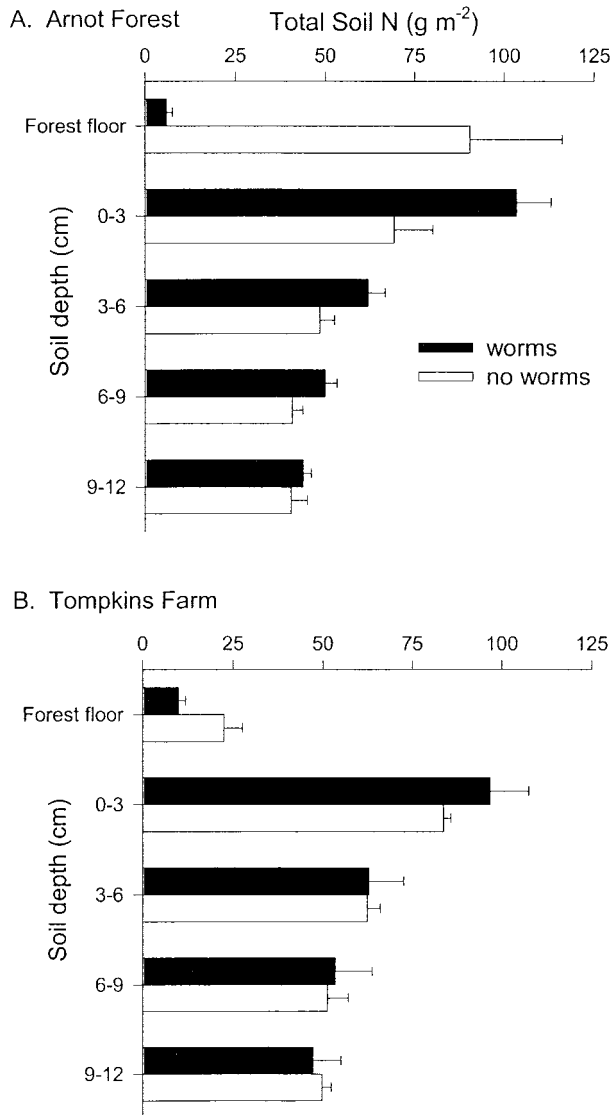


Figure 5. Depth distribution of total soil nitrogen (N) in the forest floor and upper 12 cm of mineral soil in 3-cm increments in plots with (*dark shading*) or without (*light shading*) earthworms at **A** Arnot Forest and **B** Tompkins Farm. Error bars are SE ($n = 3$).

identical in plots with and without earthworms at Tompkins Farm. By contrast, soil $\delta^{13}\text{C}$ values were lower in the upper mineral soil in plots with earthworms than in plots without earthworms at Arnot Forest ($P = 0.024$). Soil $\delta^{15}\text{N}$ values increased with depth at both sites and were lower throughout the soil profile at Tompkins Farm than at Arnot Forest. There was a strong interaction between the influence of earthworms and site on $\delta^{15}\text{N}$ values ($P = 0.02$). At Tompkins Farm, there was no difference between plots with or without worms in $\delta^{15}\text{N}$ values in the upper 15 cm of soil, but $\delta^{15}\text{N}$ values

deeper in the soil profile (25 and 35 cm) were significantly lower in plots with earthworms than in plots without earthworms ($P < 0.01$) (Figure 3). At Arnot Forest, $\delta^{15}\text{N}$ values were significantly lower in plots with worms than in plots without worms throughout the soil profile ($P < 0.001$) (Figure 3).

Soil Solutions

Dissolved N. Nitrate concentrations in both zero-tension and tension lysimeters were much lower at Arnot Forest than at Tompkins Farm ($P < 0.001$) (Table 2). Our sampling season at Tompkins Farm was several months shorter than at Arnot, and lysimeters there did not yield water as reliably as those at Arnot. Therefore, our data from Tompkins Farm were unbalanced and should be interpreted cautiously; however, they provide useful comparisons with the more complete data from Arnot Forest.

The presence of earthworms had different effects on NO_3^- concentrations in zero-tension lysimeters at Arnot Forest and Tompkins Farm. At Arnot Forest, NO_3^- concentrations in zero-tension lysimeters tended to be lower in the earthworm plots than in the reference plots ($P = 0.067$) (Figure 6). Distinct peaks occurred in NO_3^- concentrations in the fall across all three earthworm plots. In contrast, NO_3^- concentrations in zero-tension lysimeters at Tompkins Farm were significantly greater in earthworm plots than in reference plots ($P = 0.015$) (Figure 7). Average NO_3^- concentrations did not differ between depths in either treatment at Tompkins Farm.

Ammonium and DON concentrations in zero-tension lysimeters were not different in plots with or without earthworms at Arnot or Tompkins (Table 2). However, TN concentrations (dissolved inorganic N [DIN] + DON) in zero-tension lysimeters at Arnot were significantly lower in earthworm plots than in reference plots ($P = 0.026$), due mainly to differences in NO_3^- concentration. However, there was no difference between earthworm and reference plots at Arnot Forest in TN or NO_3^- in zero-tension lysimeters when the concentration data were volume-weighted to account for the greater amount of water collected in plots with earthworms.

Tension lysimeter NO_3^- and NH_4^+ concentrations did not differ between earthworm and reference plots at Arnot Forest or Tompkins Farm (Table 2). At Arnot Forest, tension lysimeter DON concentrations tended to be lower in earthworm plots than in reference plots ($P = 0.100$); TN concentrations also tended to be lower ($P = 0.075$) in the earthworm plots ($0.514 \text{ mg N/L} \pm 0.163$) than in the

Table 2. Average Inorganic Nitrogen (N) and Dissolved Organic N (DON) Concentrations (mg N/L) in Tension and Zero-tension Lysimeters at Arnot Forest and Tompkins Farm

	Tension			Zero-tension		
	NO ₃ ⁻	NH ₄ ⁺	DON ^a	NO ₃ ⁻	NH ₄ ⁺	DON ^a
<i>Arnot Forest</i>						
Reference	0.869 (0.482)	0.181 (0.088)	0.335 (0.068)	1.390 (0.390)	0.375 (0.144)	0.264 (0.092)
Worm	0.139 (0.061)	0.094 (0.036)	0.218 (0.098)	0.469 (0.105)	0.297 (0.181)	0.248 (0.091)
<i>Tompkins Farm</i>						
Reference	2.444 (0.907)	0.288 (0.131)	0.750 (0.820)	2.199 (0.610)	0.699 (0.410)	0.558 (0.325)
Worm	2.033 (0.665)	0.124 (0.063)	0.938 (0.656)	3.740 (0.842)	0.510 (0.240)	1.637 (0.623)

Values are means and (SE) for 18 and 9 sample dates between October 1999 and October 2000 in tension and zero-tension lysimeters at Arnot, respectively, and 9 and 8 sampling dates between April 2000 and October 2000 in tension and zero-tension lysimeters at Tompkins Farm, respectively. Values are average concentrations over two sample depths (15 and 40 cm) because there were no significant differences between depths. ^aDON data are means from 3 sample dates only.

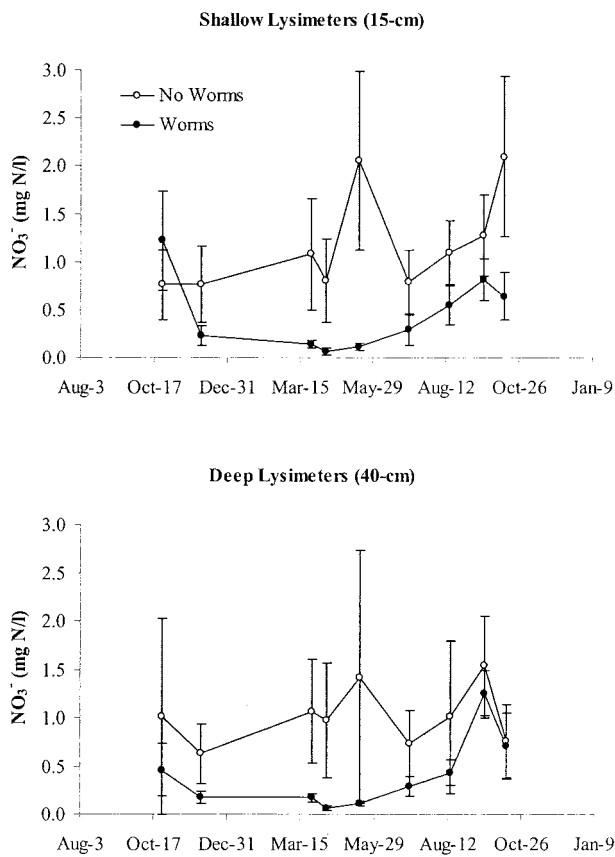


Figure 6. Average dissolved nitrate concentrations (mg NO₃⁻/L) in zero-tension lysimeters at the Arnot Forest site. Each point represents the mean from the three plots in each treatment. Error bars represent SE.

reference plots (1.346 mg N/L ± 0.448), although this trend for TN did not hold at plot 3. At Tompkins Farm, tension lysimeter DON concentrations decreased significantly with greater soil depth in the

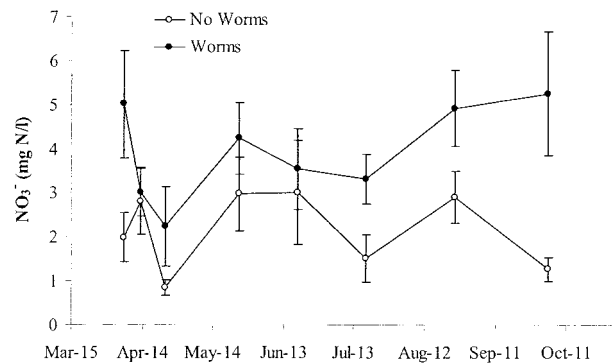


Figure 7. Average dissolved nitrate concentrations (mg NO₃⁻/L) in zero-tension lysimeters at the Tompkins Farm site. There were no significant differences in nitrate concentrations between the two depths. Therefore, a mean value from each plot was calculated (*n* = 4 lysimeters), and the overall means shown in the figure were calculated as the mean of all three plots. Error bars represent SE.

earthworm plots (*P* = 0.015) but not in the reference plots.

DOC. Average DOC concentrations in zero-tension lysimeters at Arnot were significantly lower (*P* = 0.046) in earthworm plots (6.35 mg C/L ± 1.11) than in reference plots (7.13 mg C/L ± 1.03). However, volume-weighted DOC concentrations in zero-tension lysimeters were actually significantly higher (*P* = 0.026) in the earthworm plots—the reverse of the results based on raw concentrations. Concentrations of DOC in tension lysimeters at Arnot were significantly lower (*P* = 0.009) in earthworm plots than in reference plots and decreased uniformly over depth in both treatments (*P* = 0.006), (Table 3). Concentrations of DOC in zero-tension lysimeters did not differ between the two depths.

Table 3. Average Dissolved Organic Carbon (DOC) Concentrations (mg C/L) in Tension and Zero-tension Lysimeters at Arnot Forest

Worm Plot and Soil Layer	Tension DOC (mg/L)	Zero-tension DOC (mg/L)
<i>Reference</i>		
Shallow (15 cm)	9.89 ± 2.29	6.63 ± 0.91
Deep (40 cm)	4.80 ± 1.11	8.23 ± 2.51
<i>Earthworm</i>		
Shallow (15 cm)	5.04 ± 1.65	7.08 ± 1.81
Deep (40 cm)	2.68 ± 0.70	5.49 ± 1.08

Values are means ± SE for 4 sample dates between March 2000 and May 2000 for tension lysimeters and 5 sample dates between March 2000 and October 2000 for zero-tension lysimeters.

DISCUSSION

Earthworm Effects on Pools of Soil C and N

Our results suggest that invasive earthworms have the potential to influence soil organic matter in northern forests to a degree comparable to or greater than other anthropogenic influences. Earthworms at the Arnot Forest site decreased soil surface organic matter storage by 28%. By comparison, logging has been reported either to cause increases of 22% to 61% (Hendrickson and others 1989; Mattson and Swank 1989; Johnson and others 1995) or decreases of 17% to 71% (Mattson and Smith 1993; Brais and others 1995; Johnson and others 1995) in forest floor C storage. In northern hardwood forests, recent analysis indicates that logging has small and inconsistent effects on forest floor mass (Yanai and others 2000). Nitrogen fertilization appears to increase the proportion of litter that becomes humus and to slow humus decay (Magill and Aber 1998), but these effects are slow and cumulative relative to the effects of earthworm invasion, which can be rapid and acute.

Earthworm introduction or invasion has been shown in several other studies to cause a decline in total soil C. For example, earthworm invasion of mixed forest in northern Minnesota decreased soil C to a depth of 50 cm at a rate of 0.6 Mg ha⁻¹ y⁻¹ over a period of 14 years (Alban and Berry 1994). Similarly, the introduction of earthworms into pastures in New Zealand increased C loss by an estimated 0.3–1.0 Mg ha⁻¹ y⁻¹ (O'Brien and Stout 1978). Earthworm activity in forests with long-established populations can contribute to the stabilization of soil organic matter by facilitating the formation of stable aggregates and mixing together

mineral and organic soil fractions (Scheu and Wolters 1991). However, earthworms have an important trophic role that is not replicated by other organisms in many ecosystems, and the introduction of earthworms into systems where they are lacking is likely to lead to a lower equilibrium level of soil C. We do not know when earthworms were introduced into our plots and so cannot estimate the rate of loss of soil C after their invasion at Arnot forest. However, the average magnitude of C loss at Arnot Forest (15.5 Mg C/ha) was much larger than the loss that occurred over 14 years after the invasion of a mixed forest in northern Minnesota (8.45 Mg C/ha). Investigation across a wider number of sites that differ in geographic location, soils, and forest type would improve our understanding of controls on the rate and magnitude of the changes in soil C and N that occur after earthworm invasion.

Earthworm invasion can significantly alter the distribution, retention, and quality of forest soil organic matter, but our results suggest that these effects may be strongly influenced by the land-use history of the invaded site. This interpretation is somewhat limited by the lack of replication of forests with different land-use histories in our study, but our observations are consistent with what one might expect for C-rich versus more C-depleted forest soils. The presence of earthworms was clearly associated with a reduction in the amount of organic matter in the forest floor and the redistribution of organic matter in the soil profile at Arnot Forest but not at Tompkins Farm. There were few observed differences in organic matter storage and distribution in plots with or without earthworms at Tompkins Farm, suggesting that the effects of previous agricultural practices at this site obscured any effects of earthworms on soil organic matter storage or dynamics.

The lower soil C:N ratios, greater N concentrations, and much greater nitrate concentrations in lysimeters at Tompkins Farm than at Arnot Forest are consistent with what would be expected for second-growth forest sites on previously cultivated land. In a comparison of forests in France that had been in pasture, cropland, forest, or other agricultural land use in the early 19th century, forests that had been in agricultural land use had lower soil C:N ratios and more P than continuously forested areas (Koerner and others 1997). In central New England, USA, nitrification rates in previously cultivated forest stands 100 years after reforestation were much greater than nitrification rates in stands that had been in pastures or woodlots (Compton and Boone 2000). Such changes in nutrient pools, organic matter quality, and nutrient transformation

rates due to land-use history are consistent with the differences in nutrient storage and N transformation rates that we observed at our two study locations (see also Groffman and others 2004) and may have consequences for the ability of these forests to respond to increases in N deposition and other environmental changes (Magill and others 1997; Fenn and others 1998).

The effects of earthworm invasion on organic matter distribution and quality at Arnot Forest were similar to the effects of cultivation, in that the worms incorporated the forest floor into the mineral soil and enhanced its mineralization, narrowing soil C:N ratios and decreasing overall C storage in surface soil (Davidson and Ackerman 1993). Such a narrowing of soil C:N in the upper mineral soil could only occur if earthworms had differential effects on the retention or loss of C and N (Table 1) whereby they increased the mineralization and loss of C while having little effect on the loss of N from the system.

The increase in the C:N ratio of forest floor material in plots with worms was likely due to the fact that the forest floor material in plots with worms was comprised of a greater portion of recalcitrant lignified material, such as twigs, stems, and leaf veins. These unpalatable materials have a wider C:N ratio than the decomposing leaves and partially decomposed plant remains that comprise the forest floor. Earthworms have been shown to selectively remove material with a lower C:N ratio and leave behind surface litter with a wider C:N ratio (Bohlen and others 1997). At Arnot, the $\delta^{13}\text{C}$ of forest floor material was significantly lower in plots with worms than in plots without worms, probably because the forest floor material in plots with worms consisted largely of stems and twigs, which are more depleted in ^{13}C than less lignified plant parts, such as leaves (Nadelhoffer and Fry 1988).

A general pattern of enrichment of soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with depth can be explained by the fact that litter inputs are depleted in ^{13}C and ^{15}N relative to soil and that discrimination against the heavy isotopes during decomposition increases $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with increasing soil depth (Nadelhoffer and Fry 1988; Garten and others 2000; Heil and others 2000). At Arnot, the finding of lower mineral soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in plots with earthworms relative to plots with no earthworms suggests that the worms incorporated forest floor material that was depleted of the heavy isotopes into the mineral soil (Figure 2,3). At the Tompkins Farm site, earthworms did not influence the stable isotopic signature of soil C or N, probably because there was less initial forest floor material to incor-

porate, and the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were already decreased due to past agricultural activity. There was no difference between earthworm and reference plots in litterfall amounts at either study site (Fisk and others 2004); thus, differences in isotopic signatures of soil between plots with and without earthworms are not likely due to differences in total inputs of litterfall, although they may have been due to differences in the isotopic signatures of litter, which we did not measure.

Our results are consistent with the idea that differences in isotopic signatures are easier to detect for N than C. Specifically, N detection is easier because isotopic fractionation during nutrient transformation and loss is three times greater for N than for C (Nadelhoffer and Fry 1988). Recent evidence from an experiment examining forest soil respiration following the addition of various C_3 -, C_4 -, and ^{13}C -labeled sugars confirms that discrimination against ^{13}C during respiration in forest soils is of minor importance (Ekblad and others 2002). Consequently, small changes in the isotopic enrichment of different soil layers are easier to detect for N than for C. For example, in plots without earthworms at Arnot Forest, the difference in ^{15}N enrichment between the forest floor and the 25-cm soil depth was 7.83‰, whereas the difference in ^{13}C enrichment between those two soil layers was only 2.16‰ (Figures 2,3).

Another explanation for the greater shift in the isotopic signature of N than of C in the mineral soil of invaded plots at Arnot is that the earthworms had a differential effect on the loss or retention of these two elements, as suggested by data on total soil C and N and soil C:N ratios. If earthworms had increased C loss by increasing the C mineralization of forest floor material, they would have decreased soil $\delta^{13}\text{C}$ less than would have been expected if all the forest floor material were retained in the mineral soil. If they had increased N loss, by increasing gaseous or leaching N flux, they would have to increase the ^{15}N concentrations in the remaining material because discrimination against the heavy isotope occurs during transformations that lead to loss (nitrification, denitrification). This fractionation of N during mineralization/loss would to some degree offset the decreases in heavy isotope enrichment of the mineral soil caused by incorporation of isotopically depleted surface layers into the mineral soil by earthworms.

Alternatively, the relationship between earthworms and the distribution of stable isotopes can be examined by using a simple mixing model. The model predicts the shift in the isotopic signature of mineral soil that would have occurred if earth-

worms mixed all of the C and N from the forest floor into the mineral soil with no transformation or loss. The model assumes that the isotopic signature of mineral soil prior to invasion was equivalent to current values in plots without earthworms. Using $\delta^{13}\text{C}$ as an example, the model would be:

$$\text{Predicted } \delta^{13}\text{C}_{\text{Emin}} = [(\delta^{13}\text{C}_{\text{Rff}} \times \text{Rff}) + (\delta^{13}\text{C}_{\text{Rmin}} \times \text{Rmin})] / \text{Emin}$$

where $\delta^{13}\text{C}_{\text{Emin}}$ is the $\delta^{13}\text{C}$ signature of the upper 12 cm of soil in earthworm plots; $\delta^{13}\text{C}_{\text{Rff}}$ and $\delta^{13}\text{C}_{\text{Rmin}}$ are, respectively, the $\delta^{13}\text{C}$ signature of the forest floor and the upper 12 cm of mineral soil in the reference plots; and Rff, Rmin, and Emin are, respectively, the mass of the forest floor, the mass of the upper 12 cm of mineral soil in the reference plots, and the mass of the upper 12 cm of mineral soil in the earthworm plots. For these calculations, all values for the mineral soil were adjusted by summing values from the four different depth increments sampled. At Arnot Forest, the $\delta^{13}\text{C}$ value that would have been observed in mineral soil if earthworms had simply mixed the forest floor into the mineral soil was -26.563 , reflecting incorporation of forest floor material depleted in the heavy isotope. However, the actual $\delta^{13}\text{C}$ of mineral soil in plots with worms was -26.234 , indicating that the mineral soil was more enriched with ^{13}C than predicted probably because much of the ^{13}C -depleted forest floor C was mineralized after the earthworm invasion. The average $\delta^{15}\text{N}$ value of mineral soil in plots without worms at Arnot was 4.932 . The predicted and actual values for mineral soil in plots with earthworms were, respectively, 3.909 and 3.675 , meaning that the mineral soil in plots with worms was more depleted in ^{15}N than would be predicted by simple mixing, indicating that most of the forest floor N retained in the mineral soil following invasion. The difference in the behavior of C and N could not be attributed to differences in discrimination against the heavy isotopes during metabolic transformations, because, such fractionation is greater for ^{15}N than for ^{13}C and would have contributed to a pattern opposite of that observed here. Thus, the differential effect of earthworms on stable isotopic signature of C and N provides additional evidence that there was greater retention of forest floor N than C in mineral soil after earthworm invasion at Arnot Forest.

At Tompkins Farm, the increase in the heavy isotopic signature with increasing depth was not as great as at Arnot Forest, presumably because Tompkins Farm has a legacy of agricultural land use, which contributes to weak increases in ^{15}N with soil

depth (Riga and others 1971; Rennie and others 1976). However, at Tompkins Farm 25- and 35-cm depth, $\delta^{15}\text{N}$ was significantly lower in plots with the worm than in plots without worms. It is possible that this difference was due to the incorporation of isotopically depleted surface organic matter deeper into the soil profile by *L. terrestris*, a deep-burrowing species that dominated the earthworm community at the Tompkins site and is capable of incorporating organic matter deep into the soil profile (Edwards and Bohlen 1996).

Pathways of Nutrient Loss

We hypothesized that the ability of earthworms to increase organic matter mineralization rates and to create preferential flowpaths in soil would result in higher soil solution concentrations of inorganic N and increased N leaching. However, this hypothesis was not supported by our observations at Arnot Forest and was only partially supported at Tompkins Farm, suggesting that the effects of earthworms on soil solution chemistry and nutrient leaching involve more complicated mechanisms than simple increases in net mineralization and preferential flowpaths. The lack of response of N leaching to earthworm invasion at Arnot is consistent with our conclusion that N was strongly retained in the soil following invasion. This retention was likely driven by earthworms incorporating forest floor organic matter into the mineral soil, creating an immobilization sink for N in the mineral soil and reducing the potential for leaching loss of N (Groffman and others 2001).

As earthworms ingest and mix the forest floor with mineral soil, their casting and burrowing activity can influence both N availability and the movement of dissolved N through the soil profile. Mineral N concentrations in earthworm casts are greater than in the surrounding soil, and labile C compounds (for example, carbohydrates) are also secreted with the casts initially resulting in much higher microbial activity and biomass in the casts (Lee 1985; Blair and others 1995). Earthworms also form macropores capable of conducting water through the soil and, as detailed elsewhere in this issue by Suarez and others 2004, preferential flow was particularly prominent in the earthworm-invaded plots at the Arnot Forest site. Earthworms can increase leaching losses of N in some agricultural settings (see for example, Beven and Germann 1982; Shipitalo and others 1994; Bouché and Al-Addan 1997), mainly by increasing soil water flux rather than by increasing N concentration in leaching water (Subler and others 1997; Dominguez and others Forthcoming).

This apparent effect of earthworms on water percolation through soil in the Arnot Forest plots calls into question comparisons based only on concentration data. Volume-weighting of the concentration data eliminated differences between earthworm and reference plots for NO_3^- and TN. Hence, there is still considerable uncertainty in our conclusions about the effects of earthworms on the leaching of N and DOC from the Arnot site, but there is little evidence that earthworms significantly increased current N leaching losses at this site.

The difference in land-use history at the two sites likely explains the significantly lower soil organic matter pools and higher nitrate concentrations at Tompkins Farm. Mineral N concentrations in soil solutions at Arnot Forest fell within the same range as other northeastern forests without an agricultural legacy (Currie and others 1996; Fernandez and others 1995; Friedland and others 1991), whereas Tompkins Farm nitrate concentrations were three to four times greater, probably due to the persistent effects of previous agricultural land use (Compton and Boone 2000).

There is evidence that earthworms may have had a greater effect on N leaching losses at Tompkins Farm than at Arnot Forest. Unlike Arnot, at Tompkins Farm, both NO_3^- concentrations and the ratio of $\text{NO}_3^-:\text{NH}_4^+$ in zero-tension lysimeters were significantly higher in earthworm plots than in reference ones ($P = 0.023$ and $P = 0.040$, respectively). The high nitrification rates at Tompkins Farm (Groffman and others 2004) suggest that nitrifiers have greater access to recently mineralized NH_4^+ as a result of the lower potential for immobilization in the N-rich but C-poor soils at that site. Earthworm-induced increases in preferential flowpaths may therefore lead to greater N leaching losses at Tompkins Farm than would occur in forest soils with a stronger potential for immobilizing N, such as those at Arnot Forest.

We have little evidence that earthworms significantly altered other pathways of N loss from our study plots. Although there is some evidence that earthworms may enhance denitrification (Burtelow and others 1998; Knight and others 1992), data collected from our site showed that denitrification rates in the plots were very low and that there was no clear influence of earthworms on denitrification (P. M. Groffman unpublished). Overall, our data suggest that earthworms did not greatly increase pathways of N loss in these forests, except perhaps to slightly increase nitrate leaching at Tompkins Farm. These findings are consistent with our conclusion that earthworm invasion did not lead to significant loss of N from the soil profile.

Because the forest floor is a significant source of the DOC in soil solutions in northern forest soils (McDowell and Likens 1988; Kaiser and Zech 1998; Kalbitz and others 2000), we expected that without a forest floor, earthworm plots would have lower DOC concentrations than reference plots. At Arnot Forest, DOC and DON concentrations in tension lysimeters were, in fact, lower in all plots with earthworms than in plots without earthworms (Tables 23). On average, DOC concentrations in the tension lysimeters were 50% lower in earthworm plots than in reference plots, while DON concentrations were on average 35% lower in earthworm plots. However, DOC concentrations were only slightly lower in zero-tension lysimeters in earthworm plots than in reference plots, and volume-weighted amounts of DOC were actually greater in earthworm than reference plots due to the greater volume of leachates produced in earthworm plots. It is possible that earthworm activity caused differences in organic matter properties, altered the movement of DOC through the profile, or altered other biological processes known to influence DOC concentrations in tension lysimeters, such as root exudation of soluble organic matter or microbial utilization of DOC (Kalbitz and others 2000).

The average C:N ratio of dissolved organic matter from the earthworm plots (12.1 ± 3.2) was approximately half that of the reference plots (22.4 ± 2.6), which is consistent with the lower C:N ratio of soil organic matter in plots with earthworms (Figure 1). The C:N ratio of soil solution DOC from the reference plots was slightly lower than the overall mean ratio (25.7 ± 3.1 ; $n = 22$) reported in a recent review of temperate forest soil solutions (Michalzik and others 2001). The ratio from earthworm plots was much lower than this reported value, but it was consistent with the C:N ratios of whole soil that were observed in the plots (Figure 1).

CONCLUSIONS

Earthworm invasion of northern hardwood forests clearly influenced soil organic matter distribution and storage at a relatively undisturbed forest site, but it had little influence at a previously cultivated forest site, where the legacy of agricultural land use may have obscured any effects of earthworms. By eliminating the forest floor layers, invading earthworms reduced soil C storage in the undisturbed forest site. Although it is somewhat surprising that the accompanying increase in heterotrophic soil respiration did not result in significant differences in total soil respiration (TSR) between earthworm and reference plots (Fisk and others 2004; Groff-

man and others 2004), the magnitude of the increased C loss may be too small to detect against the high background and large temporal and spatial heterogeneity of TSR. For example, if the observed C loss at Arnot Forest were distributed across 20 years (a conservative estimate), then the increased respiration would be less than 10% of TSR at this site (Fisk and others 2004). Moreover, if soil C pools in invaded plots have reached a new lower equilibrium, then differences in C efflux would not be expected at the present time.

Earthworms had different effects on the pools of soil C and N. In particular, earthworm invasion of an undisturbed forest site caused a significant decline in storage of C but not of N in surface soil. Analysis of data from zero-tension and tension lysimeters pointed to substantial differences between study sites in concentrations of nitrate and DON, which were likely related to land-use differences, but it did not indicate that earthworms were currently contributing to increases in leaching of N from the soil. Increases in nitrate concentrations in lysimeters in plots with earthworms relative to plots without worms at Tompkins farm suggested that earthworms might have increased N leaching, possibly due to a lower potential for N immobilization at that relatively C-poor site. The results show that earthworm invasion can significantly alter the distribution and retention of soil C and N in north temperate forests, and should therefore, be considered along with the suite of other factors leading to ecological changes in these forests.

ACKNOWLEDGEMENTS

This research was supported by a grant from the National Science Foundation (DEB-9726869).

We thank Ann Gorham, Isabella Fiorentino, Emilee Stander, Alan Loreface, and Abraham Parker for help with fieldwork and laboratory and data analysis; and Melany Fisk and Ruth Sherman for help with installing zero-tension lysimeters at Arnot Forest.

REFERENCES

- Alban DH, Berry E. 1994. Effects of earthworm invasion on morphology, carbon, and nitrogen of a forest soil. *Appl Soil Ecol* 1:246–9.
- Beven K, Germann P. 1982. Macropores and water flow in soils. *Water Resources Res* 18:1311–25.
- Blair JM, Parmelee RW, Lavelle P. 1995. Influences of earthworms on biogeochemistry In: Hendrix PF, Eds. *Earthworm ecology and biogeography in North America*. Boca Raton (FL): Lewis. p 127–58.
- Bohlen PJ, Groffman PM, Fahey TJ, Fisk MC, Suarez ER, Pelletier DM, Fahey RT. 2004. Ecosystem consequences of exotic earthworm invasion of north temperate forests. *Ecosystems* 7:1–13.
- Bohlen PJ, Parmelee RW, McCartney DA, Edwards CA. 1997. Earthworm effects on carbon and nitrogen dynamics of surface litter in corn agroecosystems. *Ecol Appl* 7:1341–9.
- Bouché MB, Al-Addan F. 1997. Earthworms, water infiltration and soil stability: some new assessments. *Soil Biol Biochem* 29:441–52.
- Brais S, Camirre C, Paré D. 1995. Impacts of whole tree harvesting and winter windrowing on soil pH and base status of clayey sites of northwestern Quebec. *Can J For Res* 25:997–1007.
- Burtelow AE, Bohlen PJ, Groffman PM. 1998. Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. *Appl Soil Ecol* 9:197–202.
- Compton JE, Boone RD. 2000. Long-term impacts of agriculture on soil carbon and nitrogen in New England forests. *Ecology* 81:2314–30.
- Currie WS, Aber JD, McDowell WM, Boone RD, Magill AH. 1996. Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. *Biogeochemistry* 35:471–505.
- Davidson EA. 1995. Linkages between carbon and nitrogen cycling and their implications for storage of carbon in terrestrial ecosystems In: Woodwell GM, Mackenzie FT, Eds. *Biotic feedbacks in the global climatic system: will the warming feed the warming?* New York: Oxford University Press. p 219–32.
- Davidson EA, Ackerman I. 1993. Changes in soil carbon inventories following cultivation of previously untilled soils. *Biogeochemistry* 20:161–93.
- D'Elia CF, Steudler PA, Corwin N. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnol Oceanogr* 22:760–4.
- Dixon RK, Brown S, Houghton RA, Solomon AM, Trexler MC, Wisniewski J. 1994. Carbon pools and flux of global forest ecosystems. *Science* 263:185–90.
- Dominguez J, Bohlen PJ, Parmelee RW "Earthworms increase nitrogen leaching losses from agroecosystems." *Ecosystems*. Forthcoming
- Dymond P, Scheu S, Parkinson D. 1997. Density and distribution of *Dendrobaena octaedra* (Lumbricidae) in aspen and pine forests in the Canadian Rocky Mountains (Alberta). *Soil Biol Biochem* 29:265–73.
- Edwards CA, Bohlen PJ. 1996. *Biology and ecology of earthworms*. New York: Chapman and Hall.
- Ekblad A, Nyberg G, Hogberg P. 2002. C-13-discrimination during microbial respiration of added C-3-, C-4- and C-13-labelled sugars to a C-3-forest soil. *Oecologia* 131:245–9.
- Fahey TJ. 1998. Recent changes in an upland forest in south-central New York. *J Torrey Bot Soc* 121:51–7.
- Fain JJ, Volk TA, Fahey TJ. 1994. Fifty years of change in an upland forest in south-central New York: general patterns. *Bull Torrey Bot Club* 121:130–9.
- Fenn ME, Poth MA, Aber JD, Baron JS, Bormann BT, Johnson DW, Lemly AD, McNulty SG, Ryan DF, Stottlemeyer R. 1998. Nitrogen excess in North American ecosystems: predisposing factors, ecosystem responses, and management strategies. *Ecol Appl* 8:706–33.
- Fernandez IJ, Lawrence GB, Son Y. 1995. Soil-solution chemistry in a low-elevation spruce–fir ecosystem, Howland, Maine. *Water Air Soil Pollut* 84:129–45.

- Fisk MC, Fahey TJ, Groffman PM, Bohlen PJ. 2004. Earthworm invasion, fine-root distributions, and soil respiration in north temperate forests. *Ecosystems* 7:55–62.
- Friedland AJ, Miller EK, Battles JJ, Thorne JF. 1991. Nitrogen deposition, distribution and cycling in a subalpine spruce–fir forest in the Adirondacks, New York, USA. *Biogeochemistry* 14:31–55.
- Garten CTJ, Cooper LW, Post WMI, Hanson PJ. 2000. Climate controls on forest soil C isotope ratios in the southern Appalachian Mountains. *Ecology* 81:1108–19.
- Glitzenstein JS, Canham CD, McDonnell MJ, Strong DR. 1990. Effects of environment and land-use history on upland forests of the Cary Arboretum. *Bull Torrey Bot Club* 117:106–22.
- Green RN, Trowbridge RL, Klinka K. 1993. Towards a taxonomic classification of humus forms. Bethesda (MD): Society of American Foresters.
- Groffman PM, Bohlen PJ. 1999. Soil and sediment biodiversity: cross-system comparisons and large-scale effects. *BioScience* 49:139–48.
- Groffman PM, Bohlen PJ, Fisk MC, Fahey TJ. 2004. Exotic earthworm invasion and microbial biomass in temperate forest soils. *Ecosystems* 7:45–54.
- Haimi J, Huhta V. 1990. Effects of earthworms on decomposition processes in raw humus forest soil: a microcosm study. *Biol Fertil Soils* 10:178–83.
- Haines BL, Waide JB, Todd RL. 1982. Soil solution nutrient concentrations sampled with tension and zero-tension lysimeters: report and discrepancies. *Soil Sci Soc Am J* 46:658–61.
- Heil B, Ludwig B, Flessa H, Beese F. 2000. C-13 and N-15 distributions in three spodic dystric cambisols under beech and spruce. *Isotopes Environ Health Studies* 36:35–47.
- Hendrickson OQ, Chatarpaul L, Burgess D. 1989. Nutrient cycling following whole tree and conventional harvest in northern mixed forest. *Can J For Res* 19:725–35.
- Huffman EWD. 1977. Performance of a new automatic CO₂ coulometer. *Microchem J* 22:567–73.
- James SW. 1995. Systematics, biogeography, and ecology of Nearctic earthworms from eastern, central, southern, and southwestern United States In: Hendrix PF, Eds. *Earthworm ecology and biogeography in North America*. Boca Raton (FL): Lewis. p 29–52.
- Johnson CE, Driscoll CT, Fahey TJ, Siccama TG, Hughes JW. 1995. Carbon dynamics following clear-cutting of a northern hardwood forest ecosystem. In: McFee WW, Kelly JM, Eds. *Carbon forms and functions in forest soils*. Madison, WI: American Society of Agronomy. p 463–488.
- Johnson DW, Curtis PS. 2001. Effects of forest management on soil C and N storage: meta analysis. *For Eco Manag* 140:227–38.
- Kaiser K, Zech W. 1998. Rates of dissolved organic matter release and sorption in forest soils. *Soil Sci* 163:714–25.
- Kalbitz K, Solinger S, Park JH, Michalzik B, Matzner E. 2000. Controls on the dynamics of dissolved organic matter in soils: a review. *Soil Sci* 165:277–304.
- Knight D, Elliot PW, Anderson JM, Scholefield D. 1992. The role of earthworms in permanent managed pastures in Devon, England. *Soil Biol Biochem* 24:1511–7.
- Kocner W, Dupouey JL, Dambrine E, Benoit M. 1997. Influence of past land use on the vegetation and soils of present day forest in the Vosges mountains, France. *J Ecol* 85:351–8.
- Landon MK, Delin GN, Komor SC, Regan CP. 1999. Comparison of the stable-isotopic composition of soil water collected from suction lysimeters, wick samplers, and cores in a sandy unsaturated zone. *J Hydrol* 224:45–54.
- Langmaid KK. 1964. Some effects of earthworm invasion in virgin podzols. *Can J Soil Sci* 44:34–7.
- Lee KE. 1985. *Earthworms: their ecology and relationships with soils and land use*. Sydney: Academic Press.
- Magid J, Christensen N, Nielsen H. 1992. Measuring P fluxes through the root zone of a layered sandy soil: comparisons between lysimeter and suction cell solution. *J Soil Sci* 43:739–47.
- Magid J, Christensen N. 1993. Soil solution sampled with and without tension in arable and heathland soils. *Soil Sci Soc Am J* 57:1463–9.
- Magill AH, Aber JD. 1998. Longterm effects of experimental nitrogen additions on foliar litter decay and humus formation in forest ecosystems. *Plant Soil* 203:301–11.
- Magill A, Aber J, Hendricks J, Bowden R, Melillo J, Steudler P. 1997. Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. *Ecol Appl* 7:402–15.
- Marques R, Ranger J, Gelhaye D, Pollier B, Ponette Q, Goedert O. 1996. Comparison of chemical composition of soil solutions collected by zero-tension plate lysimeters with those from ceramic cup lysimeters in a forest soil. *Eur J Soil Sci* 47:407–17.
- Mattson KG, Smith HC. 1993. Detrital organic matter and soil CO₂ efflux in forests regenerating from cutting in West Virginia. *Soil Biol Biochem* 25:1241–8.
- Mattson KG, Swank WT. 1989. Soil and detrital carbon dynamics following forest cutting in the southern Appalachians. *Biol Fertil Soils* 7:247–53.
- McDowell WH, Likens GE. 1988. Origin, composition, and flux of dissolved organic carbon in the Hubbard Brook Valley. *Ecol Monogr* 58:177–95.
- Michalzik B, Kalbitz K, Park J-H, Solinger S, Matzner E. 2001. Fluxes and concentrations of dissolved organic carbon and nitrogen—a synthesis for temperate forests. *Biogeochemistry* 52:173–205.
- Müller PE. 1884. *Studier over skovjord, som bidrag til skovdyrkningens teori II. Om muld og mor i esgekove og paa heder*. *Tidsskrift for Skovbrug* 7:1–232.
- Muys B, Granval P. 1997. Earthworms as bio-indicators of forest site quality. *Soil Biol Biochem* 29:323–8.
- Nadelhoffer KJ, Fry B. 1988. Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. *Soil Sci Soc Am J* 52:1633–40.
- Neilson GE, Hole FE. 1964. Earthworms and the development of coprogenous Al horizons in forest soils of Wisconsin. *Soil Sci Soc Am Proc* 28:426–30.
- O'Brien BJ, Stout JD. 1978. Movement and turnover of soil organic matter as indicated by carbon isotope measurements. *Soil Biol Biochem* 10:309–17.
- Prescott CE, Maynard DG, Laiho R. 2000. Humus in northern forests: friend or foe? *For Ecol Manage* 133:23–36.
- Rennie DA, Paul EA, Johns IE. 1976. Natural nitrogen-15 abundance of soil and plant samples. *Can J Soil Sci* 56:43–50.
- Riga A, Van Praag HJ, Brigode N. 1971. Rapport isotopique naturel de l'azote dans quelques sols forestiers et agricoles de Belgique soumis a divers traitements culturaux. *Geoderma* 6:213–22.
- SAS. 1988. *SAS/STAT user's guide*. Release 6.03. Cary, (NC): SAS Institute.

- Scheu S. 1987. The influence of earthworms Lumbricidae on the nitrogen dynamics in the soil litter system of a deciduous forest. *Oecologia* 72:197–201.
- Scheu S, Parkinson D. 1994a. Effects of earthworms on nutrient dynamics, carbon turnover and microorganisms in soils from cool temperate forests of the Canadian Rocky Mountains—laboratory studies. *Appl Soil Ecol* 1:113–25.
- Scheu S, Parkinson D. 1994b. Effects of invasion of an aspen forest (Canada) by *Dendrobaena octaedra* (Lumbricidae) on plant growth. *Ecology* 75:2348–61.
- Scheu S, Wolters V. 1991. Influence of fragmentation and bioturbation on the decomposition of carbon-14-labelled beech leaf litter. *Soil Biol Biochem* 23:1029–34.
- Schulze E-D. 2000. The carbon and nitrogen cycle of forest ecosystems In: Schulze E-D, Eds. Carbon and nitrogen cycling in European forest ecosystems. Berlin: Springer-Verlag. p 3–13.
- Shaffer KA, Fritton DD, Baker DE. 1979. Drainage water sampling in a wet, dual-pore soil system. *J Environ Qual* 8:241–6.
- Shipitalo MJ, Edwards WM, Redmond CE. 1994. Comparison of water movement and quality in earthworm burrows and pan lysimeters. *J Environ Qual* 23:1345–51.
- Steinberg DA, Pouyat RV, Parmelee RW, Groffman PM. 1997. Earthworm abundance and nitrogen mineralization rates along an urban–rural land use gradient. *Soil Biol Biochem* 29:427–30.
- Suarez E, Pelletier DM, Fahey TJ, Groffman PM, Bohlen PJ, Fisk MC. 2004. Effects of exotic earthworms on soil phosphorus cycling in two broadleaf temperate forests. *Ecosystems* 7:28–44.
- Subler S, Baranski CM, Edwards CA. 1997. Earthworm additions increased short-term nitrogen availability and leaching in two grain-crop agroecosystems. *Soil Biol Biochem* 29:413–21.
- Volk TA, Fahey TJ. 1994. Fifty-three years of change in an upland forest in south-central New York: growth, mortality and recruitment. *Bull Torrey Bot Soc* 121:140–7.
- Wilson SM, Pyatt DG, Malcolm DC, Connolly T. 2001. The use of ground vegetation and humus type as indicators of soil nutrient regime for an ecological site classification of British forests. *For Ecol Manage* 140:101–16.
- Yanai RD, Arthur MA, Siccama TG, Federer CA. 2000. Challenges of measuring forest floor organic matter dynamics: repeated measures from a chronosequence. *For Ecol Manage* 138:273–83.