

# Exotic Earthworm Invasion and Microbial Biomass in Temperate Forest Soils

Peter M. Groffman,<sup>1\*</sup> Patrick J. Bohlen,<sup>2</sup> Melany C. Fisk,<sup>3</sup> and Timothy J. Fahey<sup>3</sup>

<sup>1</sup>*Institute of Ecosystem Studies, Box AB, Millbrook, New York 12545, USA;* <sup>2</sup>*Archbold Biological Station, Lake Placid, Florida 33852, USA;* <sup>3</sup>*Department of Natural Resources, Cornell University, Ithaca, New York 14853, USA*

## ABSTRACT

Invasion of north temperate forest soils by exotic earthworms has the potential to alter microbial biomass and activity over large areas of North America. We measured the distribution and activity of microbial biomass in forest stands invaded by earthworms and in adjacent stands lacking earthworms in sugar maple-dominated forests in two locations in New York State, USA: one with a history of cultivation and thin organic surface soil horizons (forest floors) and the other with no history of cultivation and a thick (3–5 cm) forest floor. Earthworm invasion greatly reduced pools of microbial biomass in the forest floor and increased pools in the mineral soil. Enrichment of the mineral soil was much more marked at the site with thick forest floors. The increase in microbial biomass carbon (C) and nitro-

gen (N) in the mineral soil at this site was larger than the decrease in the forest floor, resulting in a net increase in total soil profile microbial biomass in the invaded plots. There was an increase in respiration in the mineral soil at both sites, which is consistent with a movement of organic matter and microbial biomass into the mineral soil. However, N-cycle processes (mineralization and nitrification) did not increase along with respiration. It is likely that the earthworm-induced input of C into the mineral soil created a microbial “sink” for N, preventing an increase in net mineralization and nitrification and conserving N in the soil profile.

**Key words:** nitrogen cycling; nitrogen mineralization; nitrification; respiration.

## INTRODUCTION

Soil microorganisms play a critical role in ecosystem nutrient cycling, facilitating the decomposition of organic matter, the release of nutrients contained therein and specific processes that influence the flow of these nutrients to plants and hydrological and gaseous losses to surrounding environments (Paul and Clark 1996; Bauhus and Khanna 1999; Groffman and Bohlen 1999). Soil microbial biomass and activity also strongly influence ecosystem retention of carbon (C) and (N) and soil fluxes of

trace gases (for example, methane and nitrous oxide) that influence the chemistry and physics of the atmosphere (Mooney and others 1987).

The biomass and activity of microorganisms in soil are regulated by complex interactions among plant production, which supplies organic matter to fuel microbial growth, climate, which constrains microbial decomposition activity, soil physical properties, which regulate habitat availability and “carrying capacity” for soil microbes, and trophic dynamics that can regulate microbes by predation (Zak and others 1994; Paul and Clark 1996; Bauhus and Khanna 1999; Bohlen and others 2001). Trophic control of soil microbial biomass and activity is not well understood due to difficulties in defining and studying soil food webs (Beare and others

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\*Corresponding author; e-mail: groffmanp@ecostudies.org

1992; Brussard and others 1997; Wall and Moore 1999; Anderson 2000). Interest in soil trophic dynamics has increased in recent years due to recognition that these dynamics influence the response of ecosystems to C additions from increased levels of atmospheric carbon dioxide (CO<sub>2</sub>) and to concerns about exotic species invasions of soil communities (Coleman and others 1992; Groffman and Jones 2000; Callahan and others 2001).

Invasion of north temperate forest soils by exotic earthworms has the potential to alter soil microbial biomass and activity, nutrient cycling, and environmental quality over large areas of North America. Native species of earthworms are rare in glaciated regions of North America, and many areas, especially forest ecosystems, have few or no earthworms (Gates 1976; James 1995). Invasion of temperate forests by European and Asian earthworm species has resulted in marked changes in the structure of soil profiles (for example, loss of the surface organic horizons or "forest floor") and patterns of nutrient cycling and loss (Alban and Berry 1994; Burtelow and others 1998; McLean and Parkinson 1997a, 1997b; Steinberg and others 1997; Scheu and Parkinson 1994a, 1994b). Alteration of the distribution and activity of soil microbial biomass by earthworm invasion is likely central to these changes (Brown and others 2000). Earthworm invasion effects on the amount, distribution, and activity of soil microbial biomass may thus be a critical regulator of forest ecosystem fertility, C storage, N retention, and trace gas flux functions in many forests over the next 100 years (Groffman and Bohlen 1999).

Earthworm invasion effects on soil microbial biomass and activity are complex and difficult to predict. Direct predation by worms has been shown to reduce pool sizes and to increase the activity of soil microbial biomass (Scheu and Parkinson 1994b; Bohlen and Edwards 1995; Devliegher and Verstraete 1995). However, these predation effects need to be evaluated along with habitat change effects (for example, shifting of forest floor organic matter into the mineral soil), which will strongly influence the response of microbial biomass to climate (for instance, drought) and other environmental factors. Effects of earthworm invasion on the maintenance of soil microbial biomass should also vary with site factors (for example, forest floor thickness and soil texture) that influence organic matter supply and habitat conditions for biomass (Callahan and Blair 1999).

The research presented here is part of a larger project that is testing the global hypothesis that earthworm invasion of north temperate forests will have large consequences for nutrient retention and

uptake in these ecosystems. One approach that we are using in this project is to compare forest stands already invaded by earthworms with adjacent stands lacking earthworms, viewing these stands as end points along a gradient of earthworm invasion. We are conducting our experiments in sugar maple-dominated forests in two locations, one in eastern and the other in central New York. The site in eastern New York (Tompkins Farm) has a history of agricultural cultivation ending around 1925 and thin organic surface soil horizons. The site in western New York (Arnot Forest) has no history of cultivation and a thick (3–5 cm) forest floor. Our project is documenting the obvious and expected decline in forest floor depth and redistribution of surface organic matter in the soil profile following earthworm invasion. We are also conducting detailed studies on the distribution and activity of microbial biomass and fine roots and other nutrient-cycling parameters as described in the articles by Bohlen and others (2004a, 2004b), Suárez and others (2004), and Fisk and others (2004) in this issue of *Ecosystems*.

In this article, we present data on spatial and seasonal variation in microbial biomass and activity in invaded and reference stands. Our specific objectives were (a) to determine whether earthworm invasion alters the amount and distribution of microbial biomass in the soil profile, (b) to assess whether these effects vary with site land-use history, and (c) to examine changes in the nature of the soil microbial biomass by examining ratios between microbial biomass C and N and total soil C and N pools and rates of potential net N mineralization, nitrification, and respiration per unit of microbial biomass.

## METHODS

### Sites

*Arnot Forest.* The Arnot Forest, located in central New York, USA (42°16'N, 76°28'W), is situated on the northern Allegheny Plateau physiographic province. Annual rainfall is 100 cm, and 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 acidic Dystrachrepts with a well-developed forest floor (organic horizon: Oe and Oa) 3–5 cm thick overlying an acidic (pH 4.5–5.0) mineral horizon, are well drained, and exhibit pit and mound microtopography. Forest composition and dynamics at Arnot Forest have been described in detail (Fain and others 1994; Volk and Fahey 1994; Fahey 1998). The current forest,

which was logged in the late 19th century and has developed with minimal disturbance since then, is dominated by six species characteristic of Allegheny northern hardwood forests (sugar maple, red maple, beech, white ash, basswood, and hemlock).

**Tompkins Farm.** Tompkins Farm, a part of the Cary Arboretum, is located in eastern New York (41° 50' N, 73° 45' W) and 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, and has a mean summer temperature of 21.8°C and winter temperature of -2.4°C. Late Cambrian-early Ordovician shales and slates underlie the upland forests of the site. Soils are principally acidic (pH 4.2-5.0) Dystrichrepts and are silt loams derived from glacial till and outwash (Secor and others 1955). The area has a complex but well-documented history of land use and three major forest community types (Glitzenstein and others 1990).

After extensive field surveys, we located three replicate forest stands at both the Arnot and Tompkins Farm sites. In each stand, we established a 20 × 20-m plot that is invaded by earthworms (> 150 individuals/m<sup>2</sup>) and a 20 × 20-m noninvaded (< 2 individuals/m<sup>2</sup>) "reference" plot. The plots were carefully chosen to avoid differences in soils, vegetation, and topography among the plots in each pair. In most cases, plots were located at different distances from obvious worm refugia, for example, wet areas or streams in similar slope positions and with similar vegetation. Earthworm population data in the different sites are presented in Bohlen and others (2004a). The sites at Arnot were dominated by *Lumbricus rubellus*, *L. terrestris*, and *Octolasion tytaeum*. The Tompkins sites were dominated by *L. terrestris*. Detailed data on earthworm distributions at the Arnot Forest site support the idea that our sites are experiencing a systematic invasion from adjacent areas (Bohlen and others 2004a).

### Sampling

Soil samples were taken for microbial biomass and activity analysis in fall 1998 and in spring, summer, and fall of 1999 and 2000, for a total of seven sample dates. Samples were not taken at Tompkins Farm in summer 1999 due to extremely dry soil conditions. Three samples were taken at random from each of the six plots at each site. Forest floor and mineral soil (0-12 cm) were analyzed separately. Soils from the Arnot site were shipped to the Institute of Ecosystem Studies in Millbrook for analyses. All soils were stored at field moisture content at 4°C between time of sampling and analysis (ap-

proximately 1 week). Because samplings for earthworm populations and microbial biomass were done independently, we can only make comparison between no-worm and worm treatments and cannot explore relationships between earthworm density and response variables.

### Laboratory Analyses

Samples were thoroughly mixed by hand, and large (> 2 mm) roots and stones were removed. A 10-g subsample was oven-dried at 60°C to determine gravimetric water content. Field moist soil was used in the assays for microbial biomass, respiration, and potential net N mineralization and nitrification. Soil inorganic N was extracted with 2 M KCl and analyzed colorimetrically using a Perstorp 3000 Series autoanalyzer (Perstorp Analytical).

Soil microbial biomass was measured using the chloroform fumigation-incubation method (Jenkinson and Powlson 1976; Voroney and Paul 1984). A 20-g subsample of soil was fumigated for 24 h with CHCl<sub>3</sub> in a humid vacuum desiccator. After fumigation, the samples were inoculated with 0.5 g of fresh soil, placed in 1-L airtight glass jars, and incubated for 10 days at room temperature. Controls consisting of 20.5-g subsamples of soil were placed in separate jars at the time the fumigated soils were reinoculated, and were incubated alongside the fumigated samples. Accumulation of CO<sub>2</sub> in the headspace of the jars was determined by gas chromatography (Tracor 540 gas chromatograph, thermal conductivity detector). Nitrogen accumulation was determined by extracting incubated samples as already described.

Microbial biomass C was calculated as  $B_C = (F_C / K_C)$ , where  $B_C$  is biomass C,  $F_C$  is the flush of C from fumigated samples, and  $K_C = 0.41$ . Microbial biomass N was calculated just as  $F_{Nv}$ , or the flush of N from the fumigated samples. Accumulation of CO<sub>2</sub> in the unfumigated controls was used as a measure of soil respiration. Accumulation of inorganic N was used as a measure of potential net N mineralization, and accumulation of NO<sub>3</sub><sup>-</sup> was used as a measure of potential net nitrification.

### Statistical Analyses

Results from laboratory analyses were combined with soil horizon mass data from Bohlen and others (2004b) so that results could be expressed on an areal (g m<sup>-2</sup>) basis. Data were analyzed by analysis of variance (ANOVA) using the general linear models routine of the Statistical Analysis System (SAS, 1988) with *site*, *treatment*, and *date* as main effects. There were numerous site-treatment-date interac-

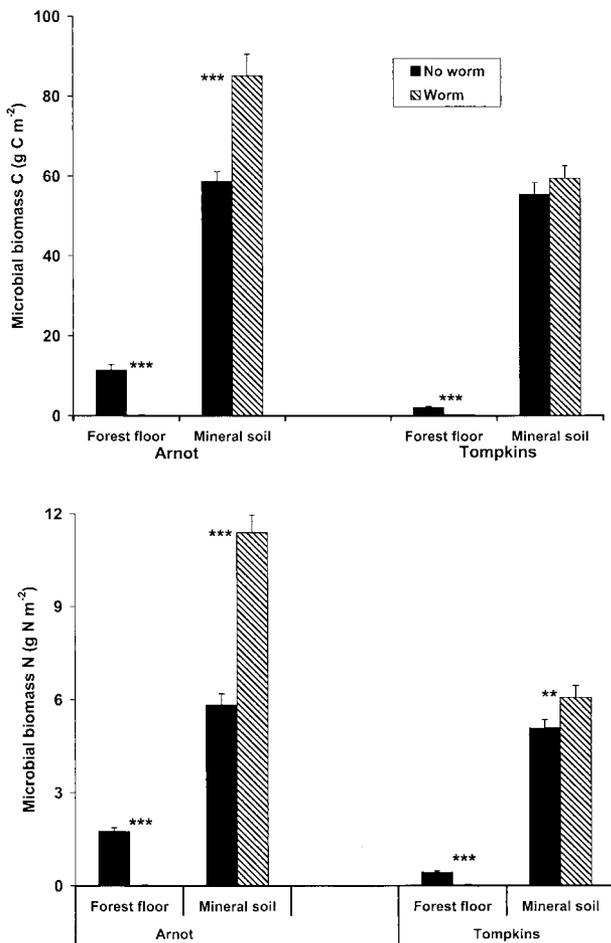


Figure 1. Microbial biomass carbon (C) (top) and nitrogen (N) (bottom) content in the forest floor and mineral soil (0–12 cm) of earthworm-invaded and reference plots in western (Arnot) and eastern (Tompkins) New York State, USA. Values are mean (standard error) of three invaded and reference plots at each site, sampled six or seven times over a 2-year period from November 1998 to November 2000. \*\*\*, \*\*Statistically significant differences between treatments at  $P < 0.01$  and  $0.05$ , respectively.

tion effects, so analyses were run separately for each site and date. Statistically significant results were verified with nonparametric tests (Wilcoxon and Kruskal–Wallis) when there were concerns about nonnormally distributed data and nonhomogeneity of variance.

## RESULTS

Earthworm invasion greatly reduced the mass of forest floor and its pools of microbial biomass C and N (Figure 1). At the Arnot site, which has thick forest floors in noninvaded plots, invasion resulted in an increase ( $P < 0.01$ ) in biomass C and N in the

mineral soil (Figure 1). At the Tompkins site, which has a history of agricultural cultivation and very thin forest floors in noninvaded plots, invasion caused a much less marked increase in biomass C and N in the mineral soil (Figure 1). Although forest floor material has high amounts of microbial biomass C and N on a “per gram of soil” basis (data not presented), the low density and thickness of this material results in relatively small pools of biomass in the forest floor relative to the mineral soil in all treatments (Figure 1).

The earthworm-induced increase in biomass C and N in the mineral soil at the Arnot site was larger than the decrease in biomass in the forest floor. As a result, total soil profile (forest floor plus 0–12 cm) biomass C and N were higher ( $P < 0.01$ ,  $0.05$ , and  $0.10$ ) in worm-invaded plots than in reference sites at most dates, at the Arnot site (Figure 2 and Table 1). The Tompkins site had lower ( $P < 0.01$ ) levels of biomass than Arnot, and there was very little stimulation of total soil profile microbial biomass by earthworm invasion at this site (Figure 2 and Table 1).

The earthworm invasion-induced increase in microbial biomass in the mineral soil did not lead to an increase in rates of potential net N mineralization and nitrification at either site (Figure 3 and Table 1). Rates of these processes were greatly reduced ( $P < 0.01$ ) in the forest floor of invaded plots, but there was no increase in the mineral soil or the entire soil profile as there was for microbial biomass, or for soil respiration (Figure 3 and Table 1).

There were marked differences in rates of N-cycle processes between the two sites. Levels of inorganic N, especially  $\text{NO}_3^-$ , and potential net nitrification were almost universally higher ( $P < 0.01$  and  $0.05$ ) at the Tompkins site (Figure 4 and Table 1). Given that the Tompkins site had lower levels of microbial biomass than the Arnot site, rates of potential net N mineralization and nitrification per unit of microbial biomass were higher ( $P < 0.01$ ,  $0.05$ , and  $0.10$ ) at this site (Table 2).

There were very few effects of earthworm invasion on rates of microbial activity per unit of microbial biomass (Table 2). There were a few earthworm-induced differences in rates of activity per unit of biomass in the forest floor. However, these are not very meaningful because of the extremely low amounts of forest floor in the invaded plots.

## DISCUSSION

As described in other reports in this special feature of *Ecosystems*, the most dramatic effect of earthworm invasion was the loss of the forest floor, which

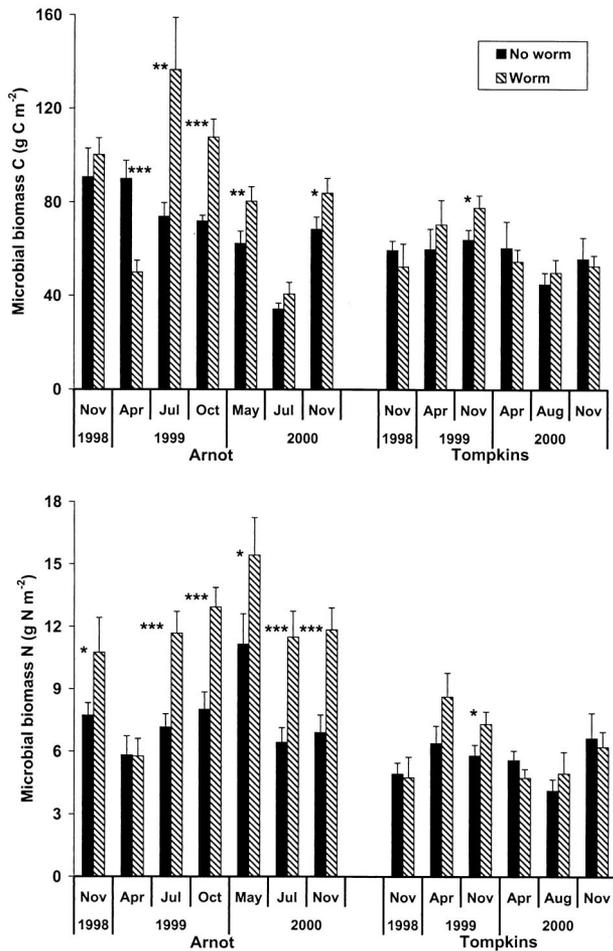


Figure 2. Microbial biomass carbon (C) (top) and nitrogen (N) (bottom) content in the soil profile (forest floor plus 0–12 cm mineral soil) of earthworm-invaded and reference plots in western (Arnot) and eastern (Tompkins) New York State, USA, from November 1998 to November 2000. Values are mean (standard error) of three invaded and reference plots at each site. \*\*\*, \*\*, \*Statistically significant differences between treatments and sites at  $P < 0.01$ ,  $0.05$ , and  $0.10$ , respectively.

dramatically altered the location and nature of nutrient-cycling activity in the soil profile. Alteration of the forest floor changed total C and P pools, C:N ratios, the loss and distribution of different soil P fractions, and the distribution and function of roots and microbes. Variation in the response to invasion was related to site characteristics and differences in earthworm species. Loss of the forest floor was much less marked at the Tompkins Farm site, which has a history of cultivation and inherently thin forest floors, compared to the Arnot Forest site, which has no history of cultivation and inherently thick forest floors. Differences in the burrowing activities of the dominant earthworm species in

different plots were another source of variation, especially for the distribution and loss of different fractions of soil P.

As expected, earthworm invasion greatly reduced pools of microbial biomass in the forest floor and increased pools in the mineral soil. Earthworms are known to “mix” soil profiles, eliminating the forest floor and enriching the mineral soil (Edwards and Bohlen 1996). However, our data suggest that site history strongly influences the nature and extent of earthworm-invasion effects on microbial biomass and activity. Enrichment of the mineral soil was much more marked at the Arnot site, which does not have a history of agricultural cultivation and has thick forest floors in areas that are not colonized by earthworms. At the Tompkins site, which has a very thin forest floor, very little organic matter was available before invasion to enrich the mineral soil. At this site, earthworm invasion removed the forest floor and its microbial biomass and produced very little enrichment of the mineral soil.

Examination of ratios between microbial biomass and total soil C and N, and between microbial activity and microbial biomass, suggests that earthworms did not alter the composition or metabolic state of the soil microbial community. It is important to note that these approaches may not be specific enough to evaluate such changes. For example, Li and others (2003) used more specific methods and found an increase in respiration per unit of microbial biomass in the mineral soil at the Arnot site during summer 2000. Several other studies have also found earthworm-induced increases in respiration per unit of biomass (Haimi and Huhta 1990; Scheu and Parkinson 1994b; Zhang and others 2000). In contrast, McLean and Parkinson (1997b) observed a decrease in respiration per unit of biomass following earthworm invasion of lodgepole pine forest by *Dendrobaena octaedra*. Our data also do not enable us to evaluate earthworm effects on microbial community composition. For example, McLean and Parkinson (2000) used specific cultivation techniques and found that invasion caused significant changes in fungal community composition and dynamics. Zhang and others (2000) and Lachniet and Hendrix (2001) observed little or no earthworm effect on overall bacterial–fungal ratios.

The increase in microbial biomass C and N in the mineral soil at the Arnot site was larger than the decrease in the forest floor, resulting in a net increase in total soil profile microbial biomass in the invaded plots. These results suggest that the mineral soil has a higher “carrying” or “preservation” capacity for microbial biomass than the forest floor (Paul

**Table 1.** Microbial Biomass Carbon (C) and Nitrogen (N), Potential Net N Mineralization and Nitrification, and Soil Respiration in the Soil Profile (Forest Floor plus 0–12 cm Mineral Soil) of Earthworm-invaded and Reference Plots in Western (Arnot) and Eastern (Tompkins) New York State, USA

Variable	Arnot		Tompkins		Site Effects	
	No Worm	Worm	No Worm	Worm	No Worm	Worm
Biomass C (g C m <sup>-2</sup> )	70 (3)	*** 86 (5)	57 (3)	60 (3)	***	***
Biomass N (g N m <sup>-2</sup> )	7.6 (0.4)	*** 12 (0.6)	5.5 (0.4)	6.1 (0.3)	***	***
Mineralization (mg N m <sup>-2</sup> d <sup>-1</sup> )	87 (11)	64 (15)	105 (11)	84 (24)		
Nitrification (mg N m <sup>-2</sup> d <sup>1</sup> )	56 (11)	44 (9)	103 (7)	89 (7)	***	***
Respiration (g C m <sup>-2</sup> d <sup>-1</sup> )	1.9 (0.1)	** 2.3 (0.2)	1.2 (0.1)	** 1.6 (0.1)	***	***

Values are mean (standard error) of three invaded and reference plots at each site, sampled six or seven times over a 2-year period from November 1998 to November 2000. \*\*\*, \*\*Statistically significant differences between treatments and sites at  $P < 0.01$  and  $0.05$ , respectively.

and Clark 1996). This enhanced capacity is likely a product of the physical protection and water-holding capacity provided by mineral soil particles. Increases in soil aggregation by earthworms (Edwards and Bohlen 1996) may also have increased the preservation capacity of the mineral soil for microbial biomass. In the forest floor, microbial biomass is likely more susceptible to predation and desiccation than in the mineral soil. As a result, earthworm invasion results in a net increase in total microbial biomass in the soil profile, at least in sites with inherently thick forest floors.

The net effect of earthworms on the size of the soil microbial biomass has been a topic of some controversy in the literature. Several studies have shown that earthworms reduce microbial biomass, primarily by consumption, as soil passes through the earthworm gut (Wolters and Joergenson 1992; Bohlen and Edwards 1995; Devliegher and Verstraete 1995; Zhang and Hendrix 1995; Gorres and others 1997; Callahan and Hendrix 1998; Saetre 1998; Zhang and others 2000; Lachnicht and Hendrix 2001). In contrast, other studies have found earthworm-induced increases in microbial biomass (Parle 1963; Shaw and Pawluk 1986; Daniel and Anderson 1992; Scholle and others 1992; Tiwari and Mishra 1993; Burtelow and others 1998; Bohlen and others 1999). Devliegher and Verstraete (1995) suggest that the net effect of earthworms on microbial biomass is a product of reductions in biomass during gut passage and stimulation due to mixing of organic matter into the soil profile. Brown and others (2000) emphasize the importance of temporal and spatial scale when evaluating the effects of earthworms on the soil profile, suggesting that fresh earthworm casts behave differently than aged casts and that earthworm effects are often restricted to specific areas in soil (the drilo-

sphere). The variation in results from different studies likely arises from several sources, including the species of earthworm involved, characteristics of the site (soil texture and forest floor depth), and the time course of invasion (McLean and Parkinson 1997b).

The earthworm-induced redistribution of microbial biomass that we observed had complex effects on microbial activity in the soil profiles. We observed an earthworm-induced increase in total soil profile respiration at both sites, which is consistent with a movement of labile organic matter and microbial biomass into the mineral soil. However, N-cycle processes (mineralization and nitrification) did not increase along with respiration. It is likely that the earthworm-induced input of C into the mineral soil created a microbial "sink" for N, preventing an increase in net mineralization and nitrification. These results help to explain the high retention of N in the face of earthworm invasion at our sites reported by Bohlen and others (2004b). They reported that while earthworm invasion reduced total soil profile C by 25%, total soil profile N was not reduced. We suggest that C-driven microbial retention of N accounts for this difference.

Differential redistribution of biomass between the two sites helps explain results reported by Bohlen and others (2004b) showing that earthworms decreased nitrate concentrations in lysimeters at Arnot and increased nitrate loss at Tompkins. At Arnot, movement of forest floor-derived C into the mineral soil created a conservation sink for N that reduced leaching loss relative to the noninvaded plots. At Tompkins, there was too little movement of forest floor-derived C into the mineral soil, and earthworm effects on the movement of water likely led to increased leaching losses at this site.

Carbon control of N-cycle processes also likely

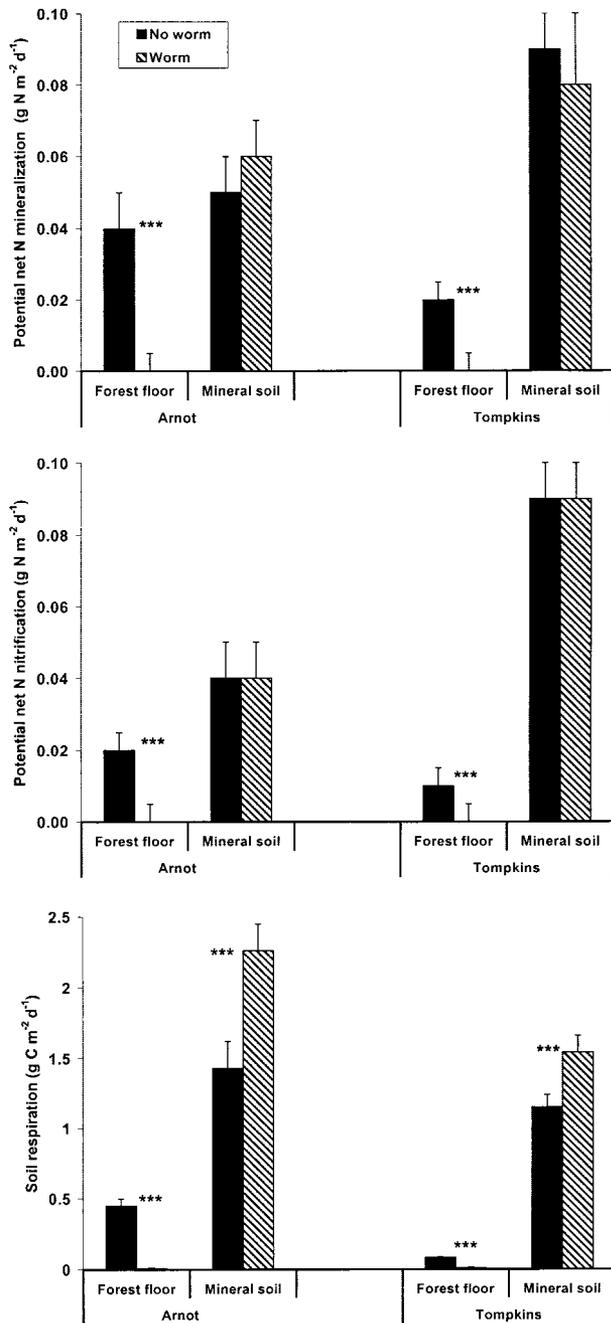


Figure 3. Potential net nitrogen (N) mineralization (top), potential net nitrification (middle), and soil respiration (bottom) in the forest floor and mineral soil (0–12 cm) of earthworm-invaded and reference plots in western (Arnot) and eastern (Tompkins) New York State, USA. Values are mean (standard error) of three invaded and reference plots at each site, sampled six or seven times over a 2-year period from November 1998 to November 2000. \*\*\*Statistically significant differences between treatments at  $P < 0.01$ .

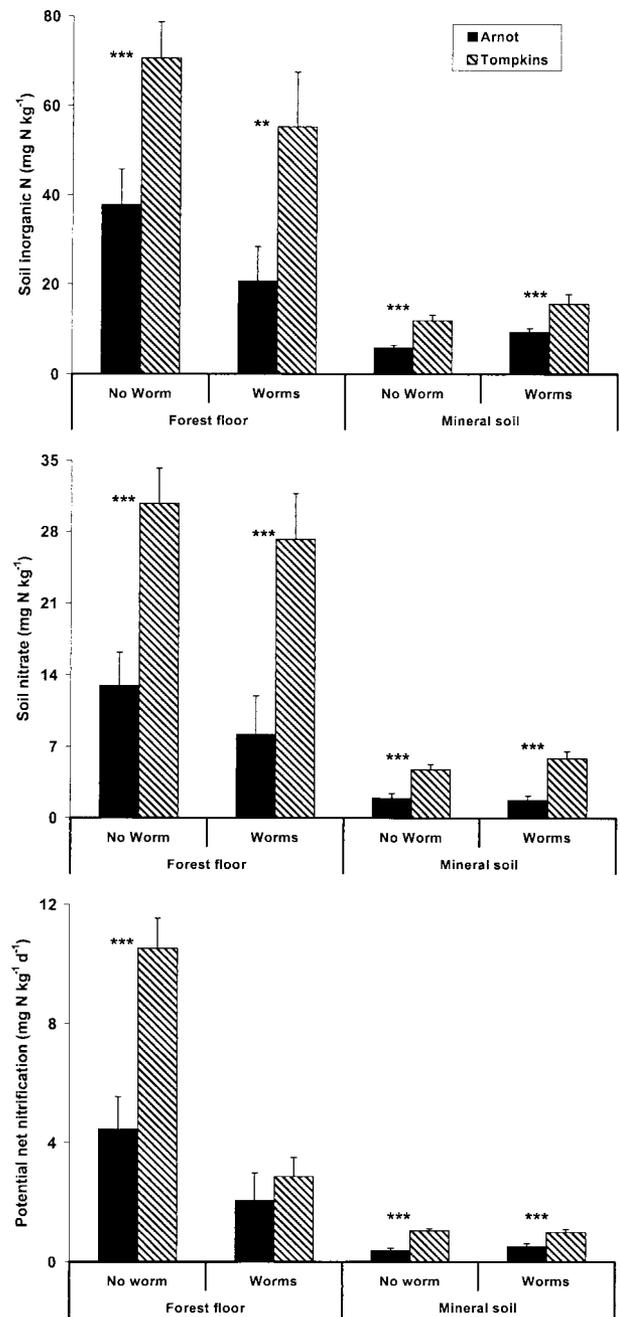


Figure 4. Total soil inorganic nitrogen (N) (top), soil nitrate (middle), and potential net nitrification (bottom) in the forest floor and mineral soil (0–12 cm) of earthworm-invaded and reference plots in western (Arnot) and eastern (Tompkins) New York State, USA. Values are mean (standard error) of three invaded and reference plots at each site, sampled six or seven times over a 2-year period from November 1998 to November 2000. \*\*\*, \*\*Statistically significant differences between sites at  $P < 0.01$  and  $0.05$ , respectively.

**Table 2.** Microbial Biomass per Unit of Total Soil Carbon (C) and Microbial Activity per Unit of Microbial Biomass in the Forest Floor and Mineral Soil (0–12 cm) of Earthworm-invaded and Reference Plots in Western (Arnot) and Eastern (Tompkins) New York State, USA

Site	Horizon	Treatment	Biomass C/ Total C	(N Mineralization/ Biomass C) * 1000	(Nitrification/ Biomass C) * 1000	(Respiration/ Biomass C) * 1000
Arnot	Forest floor	No worm	5.99 (0.9)**		3.16 (0.7)	41.8 (2.5)
		Worm	1.53 (0.5)		1.55 (0.8)	33.1 (2.7)
	Mineral soil	No worm	0.98 (0.2)		0.82 (0.2)	24.9 (1.5)
		Worm	0.92 (0.2)		0.67 (0.2)	26.7 (1.3)
Tompkins	Forest floor	No worm	8.28 (0.9)***		6.61 (0.7)	39.7 (2.1)**
		Worm	1.76 (0.4)		1.78 (0.3)	52.8 (7.4)
	Mineral soil	No worm	1.70 (0.2)		1.83 (0.2)	21.3 (1.5)**
		Worm	1.62 (0.1)		1.92 (0.3)	36.3 (7.2)
Site effects	Forest floor	No worm	*		***	NS
		Worm	NS		NS	**
	Mineral soil	No worm	***		***	*
		Worm	NS		***	NS

Values are mean (standard error) of three invaded and reference plots at each site, sampled six or seven times over a 2-year period from November 1998 to November 2000. \*\*\*, \*\*, \*Statistically significant differences between treatments or sites at  $P < 0.01$ ,  $0.05$ , and  $0.10$ , respectively. NS, not significant at  $P < 0.10$ .

explains the marked differences in N cycling and availability between sites that we observed. Rates of potential net mineralization and nitrification and levels of inorganic N, especially  $\text{NO}_3^-$ , were all higher at the Tompkins site than at the Arnot site. These results are consistent with the smaller pools of total C (Bohlen and others 2004b) and microbial biomass that we observed at the Tompkins site. Higher pools of C stimulate microbial growth and demand for N, reducing mineralization, nitrification, and inorganic N levels at the Arnot site.

The contrast in response to earthworm invasion between the two sites highlights the complexity of evaluating ecosystem response to environmental change. Earthworm invasion caused profound changes in the distribution and activity of microbial biomass in these forests, but the effects likely have different implications at the different sites. At the Arnot site, which had a well-developed forest floor prior to invasion, earthworm invasion increased total soil profile microbial biomass, with potentially positive effects on nutrient availability to plants and retention that may balance out the negative effects associated with the loss of the forest floor (increased potential for erosion, and reduction in habitat quality for roots). At the Tompkins site, earthworm invasion removed the small forest floor that was present at this site, with little enrichment of the mineral soil. Ultimately, evaluating earthworm effects on forest ecosystem fertility, C storage, N retention, and trace gas flux over the next 100 years will depend on developing an understanding of

how earthworm activities interact with multiple factors (earthworm and plant species, climate variation, and site history) in time and space.

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