

Effects of European Earthworm Invasion on Soil Characteristics in Northern Hardwood Forests of Minnesota, USA

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ABSTRACT

European earthworms are colonizing worm-free hardwood forests across North America. Leading edges of earthworm invasion in forests of northern Minnesota provide a rare opportunity to document changes in soil characteristics as earthworm invasions are occurring. Across leading edges of earthworm invasion in four northern hardwood stands, increasing total earthworm biomass was associated with rapid disappearance of the O horizon. Concurrently, the thickness, bulk density and total soil organic matter content of the A horizon increased, and its percent organic matter and fine root density decreased. Different earthworm species assemblages influenced the magnitude and type of change in these soil parameters. Soil N and P availability were lower in plots with high earthworm biomass compared to plots with low worm biomass. Decreases in soil nitrogen availability associated with high earthworm biomass were re-

flected in decreased foliar nitrogen content for *Carex pensylvanica*, *Acer saccharum* and *Asarum canadense* but increased foliar N for *Athyrium filix-femina*. Overall, high earthworm biomass resulted in increased foliar carbon to nitrogen ratios. The effects of earthworm species assemblages on forest soil properties are related to their feeding and burrowing habits in addition to effects related to total biomass. The potential for large ecosystem consequences following exotic earthworm invasion has only recently been recognized by forest ecologists. In the face of rapid change and multiple pressures on native forest ecosystems, the impacts of earthworm invasion on forest soil structure and function must be considered.

Key words: earthworm invasion; northern hardwood forests; forest soils; Lumbricidae; exotic species invasion; ecosystem change; *Acer saccharum*.

INTRODUCTION

European earthworms have been colonizing the North American continent since European settlement (Reynolds 1977; Gates 1982; Steinberg and others 1997). Although exotic earthworm species are invading much of North America, large areas of cold-temperate North America with no native

earthworms (Gates 1982; James 1998) may be most affected by exotic earthworm invasion. Recent research suggests that earthworm invasions in cold temperate, North American forests may result in widespread declines in diversity and abundance of native understory plants (Gundale 2002; Hale 2004) and large changes in soil structure and nutrient dynamics (Alban and Berry 1994; Scheu and Parkinson 1994; Bohlen and others 2004a). Despite this, the nature and extent of earthworm invasions and their impacts on these forest eco-

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systems remain largely unknown across the region (Hendrix and Bohlen 2002).

Earthworms affect ecosystem structure and function directly by ingesting, altering and mixing organic matter and mineral soil (Lavelle and others 1998), thereby changing soil structure, chemistry and biology (Devliegher and Verstraete 1997). Most research on earthworm effects on ecosystem function has been conducted in agricultural systems, where earthworms have been nearly universally credited with improving soil structure and increasing rates of nutrient cycling (Lee 1995; Lavelle and others 1998; Parmelee and others 1998). However, much of the work done on earthworm effects on soil structure, organic matter dynamics and nutrient cycling processes has been done at the cast or microsite level over short time frames (Scheu 1987; Blair and others 1997; Subler and others 1997; Chapuis-Lardy and others 1998) and the net long term and large scale ecosystem effects are largely unknown (James 1991; Shakir and Dindal 1997). Earthworm effects on plant roots are poorly understood. In crop plants, changes in root biomass in response to earthworm activity are plant specific (Syers and Springett 1984; Alpehi and others 1996; Derouard and others 1997) and the impacts of earthworm activity on the roots of native plants is largely unknown (James and Seastedt 1986). Earthworms may affect fine roots through direct consumption (Cortez and Bouché 1992; James and Cunningham 1989) or indirectly through alteration of the chemical and physical characteristics of the rhizosphere (Blair and others 1995; Edwards and others 1995).

To a first approximation, the effects of earthworms are expected to be proportional to their biomass (Edwards 1967; Daniel and others 1996; Bouché and Aladdan 1997; Zaller and Arnone 1999) and in fact a range of soil properties have been shown to change proportionally to earthworm biomass (McLean and Parkinson 1997a, b, 2000). Therefore, previously worm free ecosystems that have the ability to support large earthworm populations may be expected to experience the largest impacts when earthworms invade.

But because different species of earthworms often have different feeding and burrowing behaviors, their effects on soil structure and processes could also differ independently of effects due to biomass alone (Springett 1983; Shaw and Pawluk 1986; Hughes and others 1994; Scheu and Parkinson 1994). Consequently, the effects of different species assemblages may not always be simply the sum of their individual species effects and the assemblage effects may be out of proportion to

worm biomass alone (Nielsen and Hole 1964; Shaw and Pawluk 1986; Tomlin and others 1995; Lavelle 1997).

Previously worm free, cold-temperate hardwood forests of North America have the potential to support large and diverse exotic earthworm populations and consequently, large effects may be expected following earthworm invasion (Nielsen and Hole 1964; Reynolds 1972; Ponge and Delhay 1995; Hale and others 2005). Exotic earthworm invasions have been associated with decreasing forest floor thickness and the development of thick A horizons (Langmaid 1964; Alban and Berry 1994; Shakir and Dindal 1997; Bohlen and others 2004b). In these forests, as is the case in agricultural soils, nutrient content, mineralization and nitrification in forest soils appears to increase in fresh earthworm cast material (Spiers and others 1986; Steinberg and others 1997; Bohlen and others 2004b). However, increased leaching and immobilization of nitrogen and phosphorous may ultimately lead to lower nutrient availability (Scheu and Parkinson 1994; Bohlen and others 2004b; Suárez and others 2004). In the one published study that examined root dynamics following earthworm invasion in forest soils, earthworms were associated with decreased fine root biomass and affected the distribution and function of fine roots (Fisk and others 2004).

Published studies comparing site specific conditions before and after earthworm introductions, with few exceptions (Yeates 1981; Stockdill 1982; Hoogerkamp and others 1983), were nearly all conducted in areas where worms were chemically or physically removed, as opposed to documenting changes following invasion in areas previously free of earthworms (Edwards and Lofty 1977; Springett and others 1992; Pashanasi and others 1996). Therefore, the value of these results in predicting impacts to previously worm-free hardwood forests is uncertain. Studies comparing worm free and worm invaded sites provide valuable insights into the potential impacts of earthworm invasion (Bohlen and others 2004a). However, land-use history and other site-specific factors may confound studies comparing worm free to worm invaded sites (Callaham and Blair 1999; Jordon and others 1999; Cortez and others 2000; Bohlen and others 2004a). Studies of site-specific soil conditions before and after initial invasion by earthworms may be the most insightful (Alban and Berry 1994; McLean and Parkinson 2000).

Leading edges of earthworm invasion in forests of northern Minnesota (Hale and others 2005) provide an opportunity to document the gradient

of changes in soil characteristics associated with earthworm invasion. At the leading edges, there are steep gradients of earthworm population densities, species assemblages and their effects (Hale and others 2005; Holdsworth and others unpublished data — University of Minnesota, Dept. of Forest Resources). The different assemblages of epigeic (strict litter dwellers), epi-endogeic (litter and upper soil dwellers), endogeic (soil dwellers) and anecic (deep burrowing surface feeders) species associated with leading edges in different sites provide a rare opportunity to examine the effects of different earthworm species assemblages within a given stand as the invasion occurs rather than simply comparing worm-free sites with heavily invaded sites.

The objective of this study was to describe the changes in soil properties associated with gradients of earthworm biomass and species assemblages across advancing leading edges of earthworm invasion. We measured bulk density, soil organic matter, horizon thickness, root biomass, soil nutrient availability and foliar nutrient concentrations. We hypothesized that (1) increasing earthworm biomass would be associated with decreased O horizon thickness, increased A horizon thickness and bulk density, shifts in the distribution of soil organic matter in the upper soil horizons, decreased fine root biomass and lower nitrogen and phosphorous availability. We further hypothesized that species-specific effects would be present, such that (2) the strictly epigeic (that is, litter dwelling) species *Dendrobaena octaedra* would have no effect on O horizon thickness, whereas *Lumbricus rubellus*, a species that consumes fresh and old forest floor litter, would have a greater effect than that suggested by biomass alone; (3) species assemblages dominated by either endogeic or anecic species, both soil dwelling groups, will create similar physical soil characteristics despite differences in total biomass; and (4) the most diverse species assemblage group, with the greatest range of feeding and burrowing behaviors, will result in the largest effect on root biomass and nutrient availability.

METHODS

Study Sites and Sampling Design

The study was conducted over 4 years (1998–2001) in four mature northern hardwood stands that contain leading edges of earthworm invasion located on the Chippewa National Forest of north-central Minnesota (Hale and others 2005, Fig-

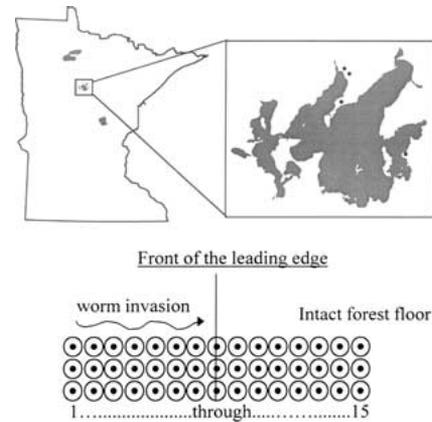


Figure 1. The locations of four study sites in the Chippewa National Forest near Leech Lake in north central Minnesota. An illustration of the sample grid (30 × 150 m) of 45 points laid perpendicular to the leading edge of earthworm invasion in each study site. Sample points are 10 m apart.

ure 1). The study sites had similar overstory composition, soils and stand history. Sugar maple (*Acer saccharum*, Marshall) was the dominant tree species making up 50–90% of basal area among stands with yellow birch (*Betula alleghaniensis*, Britton), paper birch (*Betula papyrifera*, Marshall) and basswood (*Tilia americana*, Linnaeus) as secondary species. Soils are a deep, well-drained and light-colored silty clay loam Eutroboralf (Warba series) associated with the Guthrie Till Plain (USDA 1997). Under worm-free conditions these soils have a thick forest floor composed of O_i, O_e and O_a layers. The climate is humid, continental, and cold temperate. Mean annual precipitation is 65 cm and the median growing season is 134 days with snow cover from late November to early April. Normal mean temperatures in January and July are –15 and 20°C, respectively (Minnesota State Climatology Office 2003).

A leading edge was defined as a discrete area in the forest where a transition from a thick forest floor to thin forest floor with large patches of bare mineral soil took place within 75–100 m (Hale and others 2005). At each site, a 30 × 150 m sample grid was established, consisting of 45 sample points 10 m apart in three parallel transects with 15 points each (Figure 1). Transects were placed perpendicular to the identified leading edge with the midpoint, sample point 8, located where forest floor thickness first dropped to zero. From sample point 8, the transects extended 75 m into the zone of well-developed forest floor in front of the leading edge with seven sample points, 10 m apart up to sample point 15. Similarly, the transects extended

Table 1. Grouping of Earthworm Species used in Analysis

Taxonomic group	Ecological group	Species included
<i>Dendrobaena</i>	epigeic	<i>Dendrobaena octaedra</i> , <i>Dendrodrilus rubidus</i> (rare)
<i>L. rubellus</i>	epi-endogetic	<i>Lumbricus rubellus</i> adults
<i>L. juveniles</i>	epi-endogetic / anecic	<i>Lumbricus juveniles</i>
<i>Aporrectodea</i>	endogetic	<i>A. caliginosa</i> , <i>A. tuberculata</i> , <i>A. trapezoides</i> , <i>A. rosea</i>
<i>Octolasion</i>	endogetic	<i>Octolasion tyrtaeum</i>
<i>L. terrestris</i>	anecic	<i>Lumbricus terrestris</i> adults

behind the leading edge for seven more sample points, 10 m apart to sample point 1.

Earthworm Sampling. In 1999 and 2000, earthworm populations were censused using mustard liquid extraction (40 g powdered mustard /41 water) in 0.12 m² (35 × 35 cm) subplots located 2.0 m from each sample point at a randomly selected cardinal direction (Lawrence and Bowers 2002; Hale and others 2005). In previous comparisons, earthworm biomass measures from hand sampling and liquid sampling were well correlated and for all earthworm species groups, except *Aporrectodea*, where liquid extraction sampling often resulted in higher biomass values than did hand sampling (Zaborski 2003; Hale and others 2005). For each subplot, total ash-free dry (AFD) earthworm biomass was determined for each species (Hale and others 2004). For the purposes of analyses, closely related earthworm species with similar habitat and feeding preferences were combined into taxonomic groups (Table 1) including a separate group for juvenile *Lumbricus* specimens where species identification was impossible.

Earthworm biomass measures can vary greatly due to interannual or seasonal variation in soil moisture conditions (Hale and others 200). In 1999 and 2000, there were no or very minimal seasonal soil moisture deficits in the study area (Hale and others 2005) ensuring that the earthworms present were not aestivating and would be responsive to the liquid extraction sampling method used. Further, earthworm populations were sampled during September and October of each year because at that time soil moisture had recovered from the normal mid-summer dry period. At this time, the earthworm population also has a relatively higher proportion of sexually mature individuals that are easier to identify to species than juveniles.

Forest Floor and Upper Soil Horizons. In 1999, soil cores 6 cm in diameter and 15 cm deep were collected adjacent to all odd numbered sample points and point 8 in each site. In each soil core, the thickness (cm) of the O and A horizons was measured ($n = 96$). Percent organic matter was deter-

mined by loss on ignition for each horizon present ($n = 48$ and $n = 93$ for O and A horizons, respectively).

In 2000, soil cores were collected adjacent to all sample points in each site. In each core, the thickness of the O and A horizons was measured ($n = 179$). In cores from all odd numbered sample points and point 8, bulk density (dry g cm⁻³) was determined for each horizon present ($n = 22$ and $n = 105$ for O and A horizons, respectively).

In 2001, total fine root density was measured in two soil cores collected adjacent to sample points 1, 8 and 15 in each site. Each core was separated into O, A and upper E horizon and the thickness of each horizon collected was recorded. Cores were returned to the lab and washed to collect all fine roots (≤ 1.0 mm), which were dried at 60°C for 24 h and then weighed. Mean fine root density (mg cm⁻³) was calculated for each sample point by averaging the two replicate samples. Total fine root biomass to 10 cm depth (g m⁻²) was calculated for each core.

Nutrient Availability. To assess soil nitrogen and phosphorus availability, six ion exchange resin bags, each containing 5 g of Rexyn I-300 (Fischer Scientific, Fairlawn, New Jersey, USA) mixed-bed, cation-anion exchange resin (Binkley 1984), were buried (8–10 cm deep) in the mineral soil at randomly selected locations 2 m from sample points 1 and 15 at opposite ends of each transect at each site ($n = 144$). Bags were buried on June 28–29, 2001 and removed September 21, 2001. Ammonium, nitrate and phosphate were extracted from 3 g subsamples of resin with 100 mL of 1 mol L⁻¹ KCl; the resins were shaken for 15 min in 25 mL KCl, decanted, shaken again in another 25 mL aliquot, poured into small Buchner funnels (5.5 cm diameter) equipped with pre-washed #1 Whatman filters, and washed with additional KCl before the filtrate was brought to 100 mL volume. The extracts were analyzed by standard methods for μg of N as ammonium (NH₄-N) or as nitrate (NO₃-N) and P as phosphate (PO₄-P) on a Lachat autoanalyzer (Lachat Instruments, Milwaukee, Wisconsin, USA).

Foliage samples of the current year's growth were collected in early September, 2001. Whole leaves were collected from up to eight understory plant species within 5 m of sample points 1 and 15 at opposite ends of the sample grid at each site. Samples were dried at 60°C for 24 h, weighed and then ground into a fine powder for analysis. Foliar nitrogen and carbon content were determined using the LECO CHN-800 elemental analyzer.

Statistical Methods

For all analyses, the 2-year mean (1999 and 2000) of earthworm biomass per sample point was used as the best estimate of mean earthworm biomass for each sample point (Hale and others 2005). Additionally, because some soil parameters were collected in different years, the 2-year mean of earthworm biomass provides consistency across all analyses. When we had multiple years of soil parameter data (such as horizon thickness) concurrent with multiple years of earthworm biomass, the results of analyses using the 2 year mean of earthworm biomass were comparable to results using individual year data of earthworm biomass.

For purposes of statistical analyses, earthworm biomass data were log transformed using a technique described in McCune and Grace (2002), which preserves both zero values and differences in magnitude that exist in the data set (Hale and others 2005). The transformed value (b) equals...

$$b = \log_{10}(x + d) - c \quad (1)$$

where c = the integer of $(\log_{10}(\min(x)))$, $\min(x)$ = the lowest non-zero value of x and d = inverse $\log_{10}(c)$.

For percent organic matter, fine root density, total soil organic matter and nutrient availability $\ln(x+1)$ transformations were used. No transformations were required for horizon thickness, bulk density or foliar nutrient content.

A combination of multiple regression analysis and a non-parametric Mantel test (McCune and Grace 2002; Hale and others 2005) were used to assess the spatial relationships of earthworm biomass to sample point position across the sample grid. To test the relationship of total earthworm biomass to changes in nutrient availability, fine root density and O and A horizon thickness, density and percent organic matter a combination of multiple and simple linear regression analysis was used (Montgomery and Peck 1992; SAS 2001).

The relationships of different earthworm species assemblages to changes in soil parameters were assessed using a combination of cluster analysis and

indicator species analysis (McCune and Mefford 1999) to sort sample points across all sites into earthworm species assemblage groups (Hale and others 2005) and then ANOVA and paired t -tests were used to test for differences in the means of soil parameters between assemblage groups (Montgomery and Peck 1992; SAS 2001).

Using mean earthworm species specific biomass, all sample points were sorted into species assemblage groups by the group average linkage method (a hierarchical, polythetic, agglomerative clustering method) as described by McCune and Grace (2002), using Sorensen distance measures. Six potential earthworm species assemblage groups were initially identified in the cluster analysis process. Then indicator species analysis (McCune and Mefford 1999) was used to identify a subset of groups that had unique species assemblages by identifying which subset of groups had both the lowest average P -values and the most significant indicator species (Dufrene and Legendre 1997). Monte Carlo randomization tests (1,000 runs) were used to assess the significance of indicator values for each species in each potential subset of earthworm assemblage groups.

RESULTS

Total Earthworm Biomass in Relation to Sample Point Position

Across all sites, total earthworm biomass decreased across the leading edge of invasion from sample point 1 through 15 ($r^2 = 0.59$, $P < 0.0001$). However, the spatial pattern of total earthworm biomass in relation to sample point position was nonlinear and varied with site. In three of the four sites, total earthworm biomass decreased (Figure 2) and the Mantel test (McCune and Grace 2002) indicated that the spatial structure of earthworm biomass varied significantly across the sample grid. The fourth site, Two Points, follows a similar pattern but with greater variability.

Soil Properties in Relation to Total Earthworm Biomass

Bulk Density in Relation to Total Earthworm Biomass. Among all sample points and sites, A horizon density generally increased with total earthworm biomass ($P < 0.0001$, Table 2) and ranged between 0.15 and 1.1 g cm⁻³. However, the pattern depended on site (Table 2). Examining each site individually shows significant positive correlations between A horizon density and earthworm biomass in two sites and no relationship

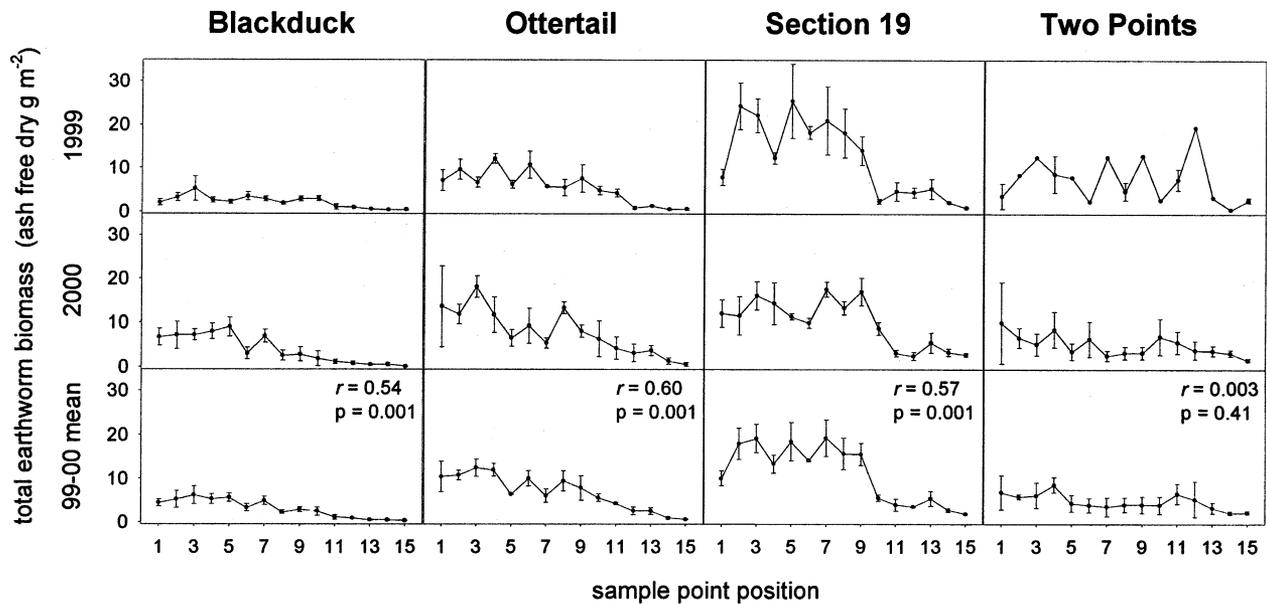


Figure 2. Mean and standard error scatter plots of total earthworm biomass in 1999, 2000 and the 2 year mean (1999 and 2000) in relation to sample point position across the sample grid in each study site. The standardized Mantel statistic (r) indicates the strength of the relationship between sample point position and earthworm biomass and the associated P -value indicates the significance of that relationship ($n = 45$ per site).

in the other two (Figure 3). O horizon density had no relationship with total earthworm biomass or site ($P > 0.50$). E horizon density varied between sites ($P = 0.002$) but had no relationship with total earthworm biomass ($P = 0.75$).

Percent Organic Matter in Relation to Total Earthworm Biomass. Across all sample points and sites, A horizon percent organic matter decreased with increasing total earthworm biomass ($P = 0.01$, Table 2) and ranged between 11 and 54%. O horizon percent organic matter also decreased with increasing total earthworm biomass ($P = 0.05$) and ranged between 37 and 83%. However, there are no data points for the O horizon above 11 ash free dry g m^{-2} of earthworm biomass because the O horizon had been eliminated in those sample points. Percent organic matter in the E horizon did not change in relation to either total earthworm biomass or site.

Horizon Thickness in Relation to Total Earthworm Biomass. In both 1999 and 2000, A horizon thickness increased with total earthworm biomass ($P < 0.0001$) across all sample points and sites (Table 2). Although the relationships were generally similar at all sites, they were statistically different (Table 2, Figure 4).

Many soil cores lacked an O horizon (48% of cores in 1999 and 75% in 2000). The lack of an O horizon is an important measurable impact of earthworms.

However, in regression analyses a large number of zeros in the data matrix might unduly influence the results and for this reason zeros are often removed (Montgomery and Peck 1992). Therefore, analyses using the full data set and a data set with zero values removed were conducted and compared (Table 2, Figure 4). In 1999 and 2000, O horizon thickness decreased with increasing total earthworm biomass across all sample points and sites when using the full data set ($P < 0.0001$, Table 2) and in 1999 for the data set when all zero values of O horizon thickness were deleted from the analysis ($P = 0.03$). These relationships varied somewhat among sites (Table 2, Figure 4).

Total Soil Organic Matter Content in Relation to Total Earthworm Biomass. A horizon total soil organic matter (SOM) ranged from 0.8 to 14.5 Mg m^{-2} . The combined effect of changes in bulk density, percent organic matter and thickness for each soil horizon caused A horizon SOM to increase with total earthworm biomass ($P = 0.007$, Table 2) across all sample points and sites. O horizon SOM ranged from 0.0 to 7.6 Mg m^{-2} . Across all sample points and sites, O horizon SOM decreased in relation to increasing total earthworm biomass ($P = 0.001$), with significant variability among sites (Table 2). However, when all points with no measurable O horizon were deleted from the analysis, O horizon SOM had no relationship to earthworm

Table 2. Summary Statistics: Multiple Regression Results of Soil Parameters in Relation to Site and Total Earthworm Biomass

Soil parameters	Full model ^a	Regression parameters		
		Worm	Site	Site * Worm
Bulk Density				
A horizon	$R^2 = 0.45$	+	Yes	Yes
O horizon	NS	NS	NS	NS
E horizon	$R^2 = 0.18$	NS	Yes	NS
Percent Organic Matter				
A horizon	$R^2 = 0.23$	-	Yes	NS
O horizon	NS	-	NS	NS
E horizon	NS	NS	NS	NS
Horizon Thickness (1999)				
A horizon	$R^2 = 0.53$	+	Yes	Yes
O horizon (all data)	$R^2 = 0.47$	-	Yes	Yes
O horizon (zeros removed)	$R^2 = 0.34$	-	Yes	NS
Horizon Thickness (2000)				
A horizon	$R^2 = 0.53$	+	Yes	Yes
O horizon (all data)	$R^2 = 0.59$	-	Yes	Yes
O horizon (zeros removed)	$R^2 = 0.33$	NS	Yes	NS
Soil Organic Matter Content				
Total to 12 cm depth	$R^2 = 0.42$	+	Yes	NS
A horizon	$R^2 = 0.40$	+	Yes	NS
O horizon (all data)	$R^2 = 0.66$	-	Yes	Yes
O horizon (zeros removed)	NS	NS	NS	NS
E horizon	NS	NS	Yes	NS
Fine Root Density				
A horizon	$R^2 = 0.40$	-	NS	not included ^b
O horizon	NS	NS	NS	NS
E horizon	NS	-	NS	not included ^b

Symbols - or + indicate the direction of the relationship of each soil parameter to increasing total earthworm biomass ($P \leq 0.05$). Yes or NS in subsequent columns indicate significant or non-significant site and site*worm interaction effects, respectively ($P \leq 0.05$).

^aAll models $P \leq 0.0001$.

^bWhere the interaction term was not significant in the full model it was removed and model run again with only main effects.

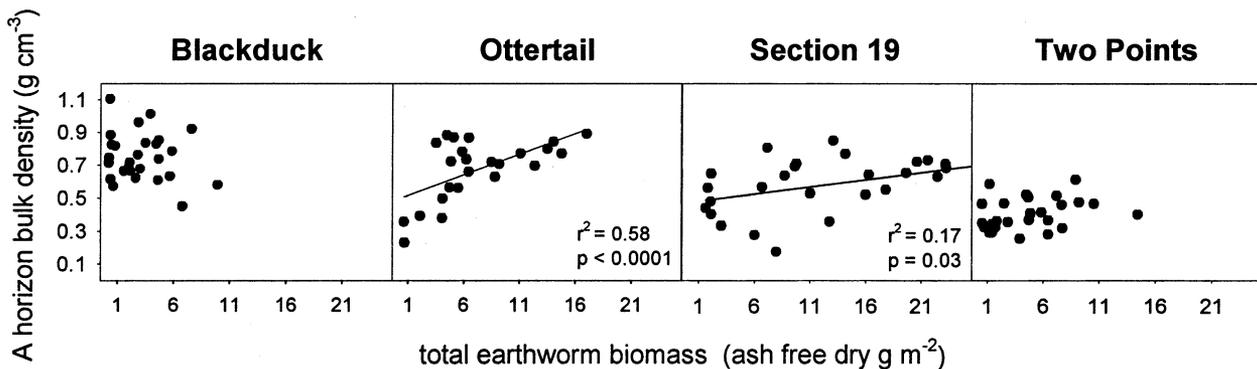


Figure 3. Scatter plots of A horizon bulk density in relation to mean total earthworm biomass in each study site. $n = 27$ in each site except Ottertail where $n = 24$.

biomass. E horizon SOM ranged from 1.1 to 7.2 Mg m^{-2} and had no relationship to total earthworm biomass. Combined total soil organic

matter to a depth of 12 cm ranged from 5.5 to 16.5 Mg m^{-2} and increased somewhat ($P = 0.06$) with total earthworm biomass.

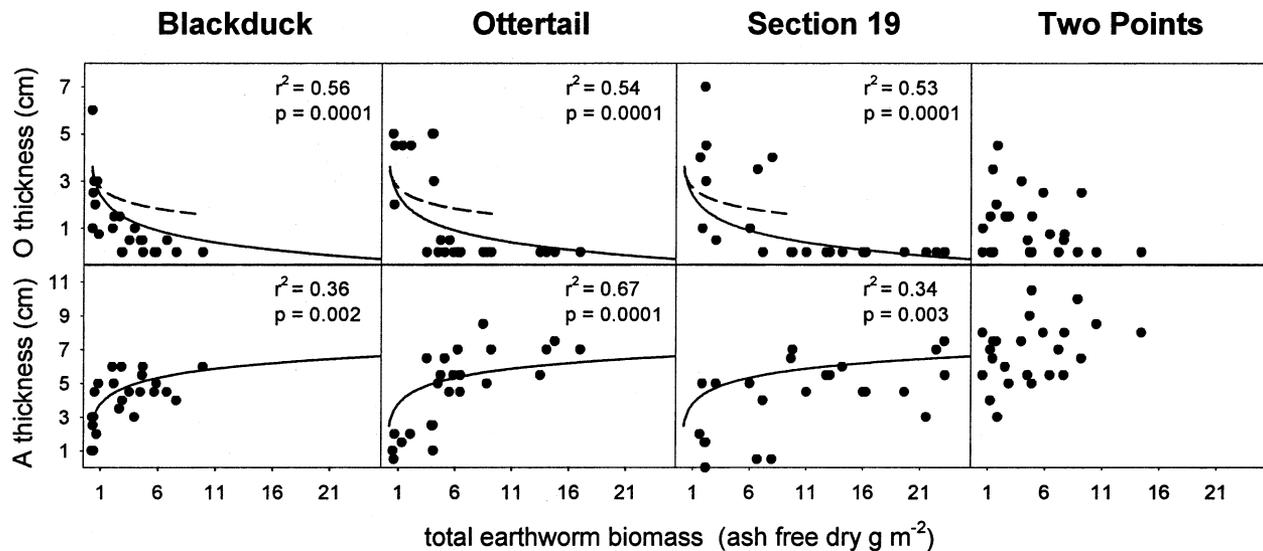


Figure 4. Scatter plots of 1999 O and A horizon thickness in relation to mean total earthworm biomass in each study site. Fitted relationships with solid lines include all data points, $n = 24$ in each site. For comparison, O horizon fitted relationships with all zero values deleted are provided (*dashed lines*), however, only for the Blackduck site is this relationship significant ($r^2 = 0.45$, $P = 0.004$).

Total Fine Root Density and Biomass in Relation to Total Earthworm Biomass. Across all sample points and sites, fine root density decreased with increasing total earthworm biomass in the A and E horizon ($P = 0.04$, Table 2, Figure 5), but not in the O horizon. Total fine root biomass to 10 cm depth ranged from 127 to 876 g m^{-2} and had no relationship to either site or total earthworm biomass.

Nutrient Availability in Relation to Total Earthworm Biomass. Soil nutrient availability and foliar nutrient content were compared at the extreme ends of each sample grid (sample points 1 and 15). Ammonium, nitrate and phosphate availabilities were lower in sample points with high total earthworm biomass compared with sample points of low total earthworm biomass (Figure 6). Of the eight understory plant species sampled, foliar nitrogen content was significantly lower in sample points with high total earthworm biomass compared with sample points of low total earthworm biomass for *Carex pensylvanica*, *Acer saccharum* and *Asarum canadense* but the inverse was true for *Athyrium felix-femina* (Figure 7). Foliage carbon content was significantly lower in plots with high earthworm biomass for *Carex pensylvanica* only (Figure 7). Carbon to nitrogen ratios increased significantly with increasing earthworm biomass for *Carex pensylvanica* and *Acer saccharum* but decreased with increasing earthworm biomass for *Athyrium felix-femina* (Figure 7).

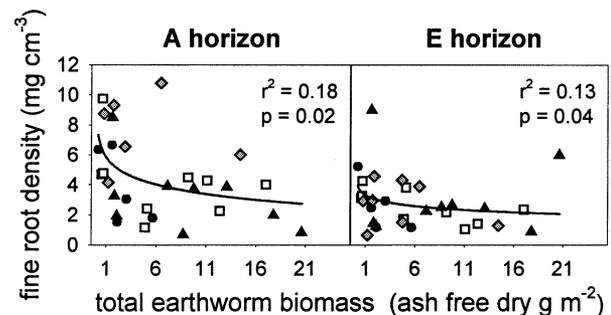


Figure 5. Scatter plots of A and E horizon fine root density in relation to mean total earthworm biomass in all study sites. Fitted relationships include all data points, $n = 29$ and 31 in A and E horizons, respectively; *closed circles*, Blackduck; *open squares*, Ottertail; *closed triangles*, Section 19; *gray diamonds*, Two Points.

Soil Properties in Relation to Earthworm Species Assemblages

Earthworm Species-specific Biomass. Earthworm species composition differed between sites. The biomass of individual species of earthworms changed significantly across the leading edge (Figure 8). However, the spatial patterns of change differed among species (Figure 8). *Dendrobaena* was found in all four sites and its biomass increased significantly across the leading edge from bare soil (sample point 1) to intact forest floor (sample point 15) in three sites (Ottertail, Section 19 and Two Points). *Aporrectodea* species was found in all four

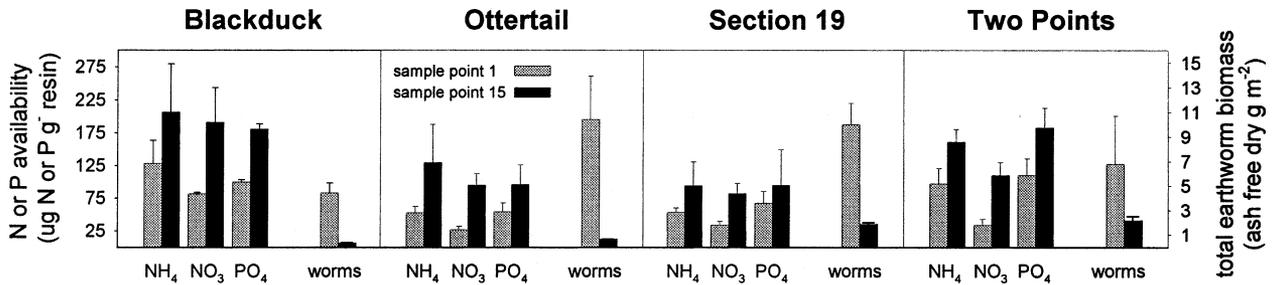


Figure 6. Paired comparisons of mean N (as ammonium and nitrate) and P (as phosphate) availability and total earthworm biomass at opposite ends of the sample grid in each site, $n = 6$ in each site. All comparisons are significant at $P \leq 0.02$.

sites and its biomass decreased significantly across the leading edge in three sites (Blackduck, Ottertail and Section 19). *L. rubellus* adults, *L. terrestris* adults and *L.* (juveniles) were found in two or three sites, and their biomass generally decreased across the leading edge. *Octolasion* was found in only one site (Section 19) and its biomass decreased significantly across the leading edge.

Earthworm Species Assemblage Groups. The species assemblage groups were associated with sites due to differences in overall species composition seen between the study sites (Figure 8). There was substantial overlap in the relative positions of different species assemblage groups across the leading edges among sites, thereby limiting the degree to which the effects of the different assemblage groups might be confounded with time since invasion. Assemblage group 1 contained sample points with *Dendrobaena* only and had a total of 20 sample points from three sites (Blackduck, Ottertail and Two Points). Assemblage group 2 contained sample points with only *Lumbricus rubellus* adults, *Lumbricus* juveniles and *Dendrobaena* and had a total of 13 sample points from two sites (Ottertail and Section 19). *Aporrectodea* species and *Dendrobaena* were the dominant taxonomic groups in assemblage group 3, which contained a total of 74 sample points from two sites (Blackduck and Two Points). Assemblage group 4 was identified by the presence of *Lumbricus terrestris* adults and *Octolasion tyrtaeum* but contained all six taxonomic groups. It contained a total of 70 sample points primarily from two sites (Ottertail and Section 19). Total earthworm biomass was lowest in group 1, increased to an intermediate value in groups 2 and 3 and was highest in group 4 (Figure 9).

Soil Attributes in Relation to Earthworm Species Assemblages. A horizon bulk density was lowest in the *L. rubellus* dominated group (assemblage group 2) relative to all other groups (Figure 9). A horizon percent organic matter declined modestly from the

Dendrobaena group (assemblage group 2) through the *L. terrestris* dominated group (assemblage group 4) (Figure 9). In 1999, the *Dendrobaena* and *L. rubellus* dominated groups had similar thickness of O horizons and A horizons, whereas the *Aporrectodea* dominated group (assemblage group 3) and *L. terrestris* dominated group had very thick A horizons and thin O horizons (Figure 9). In 2000, these patterns were similar, except the *L. rubellus* dominated group had switched to a thin O horizon and thick A horizon comparable to the *Aporrectodea* and *L. terrestris* dominated groups.

Changes in bulk density, percent organic matter and horizon thickness compensated each other, so total soil organic matter content to 12 cm depth did not change in relation to earthworm species assemblage groups. However, comparisons with the *L. rubellus* dominated group were impossible due to its small sample size. Soil organic matter content of the O horizon was higher in the *Dendrobaena* group relative to the *Aporrectodea* and *L. terrestris* dominated groups and A horizon soil organic matter content was higher in the *Aporrectodea* dominated group compared with the *Dendrobaena* and *L. terrestris* dominated groups (Figure 9).

Fine Root Density and Nutrient Availability in Relation to Earthworm Assemblages. A horizon fine root density (g cm^{-3}) was lower in the *L. terrestris* dominated group compared with all other groups (Figure 9). Mean total fine root biomass (g m^{-2}) to a depth of 10 cm was lowest in the *L. terrestris* dominated group compared with all other groups (Figure 9). Nitrogen and phosphorus availability, in the forms of ammonium nitrate, and phosphate, were lower in the *L. terrestris* dominated group than in all other groups (Figure 9).

DISCUSSION AND CONCLUSIONS

The gradients of earthworm biomass and species assemblages associated with leading edges of

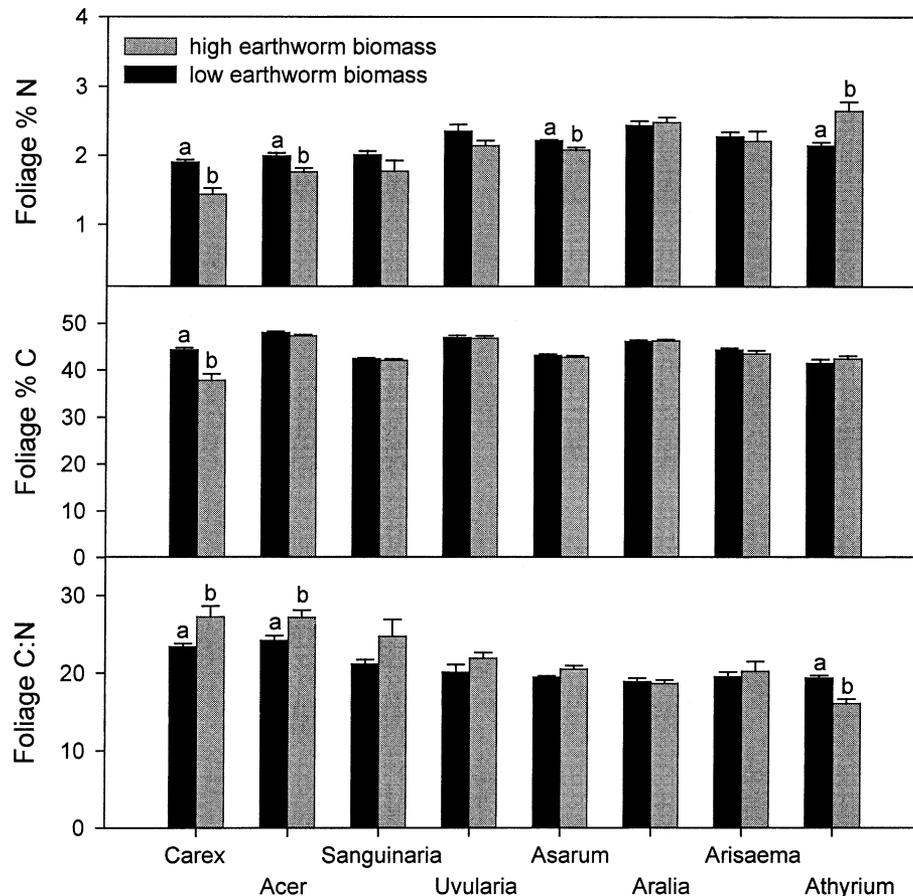


Figure 7. Paired comparisons of foliage mean percent nitrogen, percent carbon and carbon to nitrogen ratios in eight understory plant species collected at opposite ends of the sample grid under high or low earthworm biomass conditions (Figure 6). Plant species included *Carex pensylvanica* ($n = 13$), *Acer saccharum* ($n = 13$), *Sanguinaria canadensis* ($n = 6$), *Uvularia grandiflora* ($n = 6$), *Asarum canadense* ($n = 3$), *Aralia racemosa* ($n = 3$) and *Athyrium felix-femina* ($n = 3$). All comparisons indicated by different letters are significant at $P \leq 0.02$.

earthworm invasion provided an opportunity to examine changes in soil characteristics and processes related to exotic earthworm invasion without the confounding factors often associated with comparative field studies. Overall, increasing earthworm biomass homogenized the upper soil horizons. The trajectories of change in bulk density, percent organic matter, fine root density and nutrient availability in relation to increasing earthworm biomass are, in part, the result of decreased variability (Figures 3, 4 and 5). Under worm-free conditions, numerous factors may determine conditions at any given point and the system is highly heterogeneous as indicated by high variability in measured soil parameters when earthworm biomass is low (Webb 1972). After earthworms invade, they come to have a dominant effect on soil structure and function resulting in more homogeneous and less variable soil conditions (Edwards and others 1995).

As we hypothesized, there was a net relocation of organic matter from the forest floor down into the upper mineral soil horizon in response to increasing earthworm biomass (Figure 4, Table 2). O horizon thickness declined rapidly to zero (Figure 4), which may have precluded the detection of any decrease in its density in relation to earthworm biomass. Increases in A horizon thickness and bulk density were commensurate with the loss of the O horizon. A horizon percent organic matter by weight decreased with increasing total earthworm biomass as a result of increased bulk density with only a small increase in total soil organic matter content in the A horizon. Similarly, total soil organic matter to 12 cm depth increased slightly with increasing earthworm biomass. Our results support the conclusions of other studies, that earthworm invasion into previously worm free forests generally results in the translocation of surface litter in a newly developed A horizon (Al-

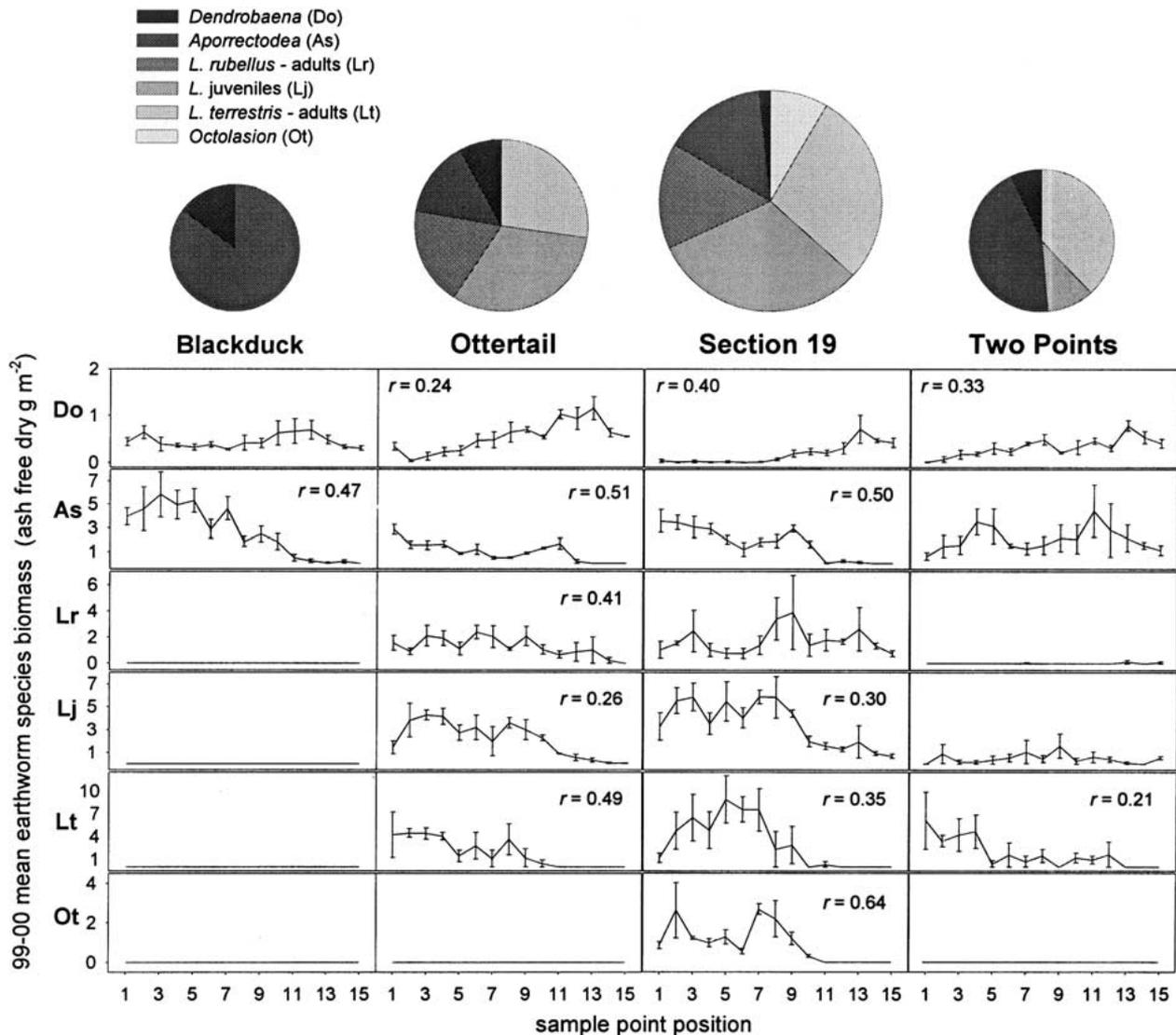


Figure 8. Pie charts of 1999–2000 mean earthworm biomass (ash-free dry g m^{-2}) for each taxonomic group present in each site. The size of the pie chart is proportional to mean total earthworm biomass for each site where the minimum (Blackduck) and maximum (Section 19) were 3.0 and 11.1 ash-free dry g m^{-2} , respectively. Mean and standard error scatter plots of 99–00 mean earthworm biomass for each taxonomic group in relation to sample point position are shown. The standardized Mantel statistic (r), indicates the strength of the relationship between sample point position and earthworm species biomass, when (r) is provided $P \leq 0.01$.

ban and Berry 1994; Bohlen and others 2004b). The fact that we saw no decline in total soil organic matter, while both of these previous studies saw moderate declines, may be a short term condition seen during initial invasion. In the many subsequent years or decades following earthworm invasion we could expect gradual loss of carbon as was documented by Alban and Berry (1994), resulting in long term decreases in total soil carbon such as those documented by Bohlen and other (2004b).

The predicted increase in A horizon bulk density with total earthworm biomass occurred, despite the

incorporation of surface litter, as a result of processes mediated by the earthworm gut which modify, compact and mix organic materials with mucus and mineral soil in the formation of earthworm cast material (Devliegher and Verstraete 1995). In this study, A horizon bulk density nearly doubled with increasing earthworm biomass, to values comparable to those reported by Alban and Berry (1994) following earthworm invasion in another Minnesota forest type.

This increase in bulk density is the opposite of that generally reported for agricultural soils after earthworm introduction. In studies of agricultural

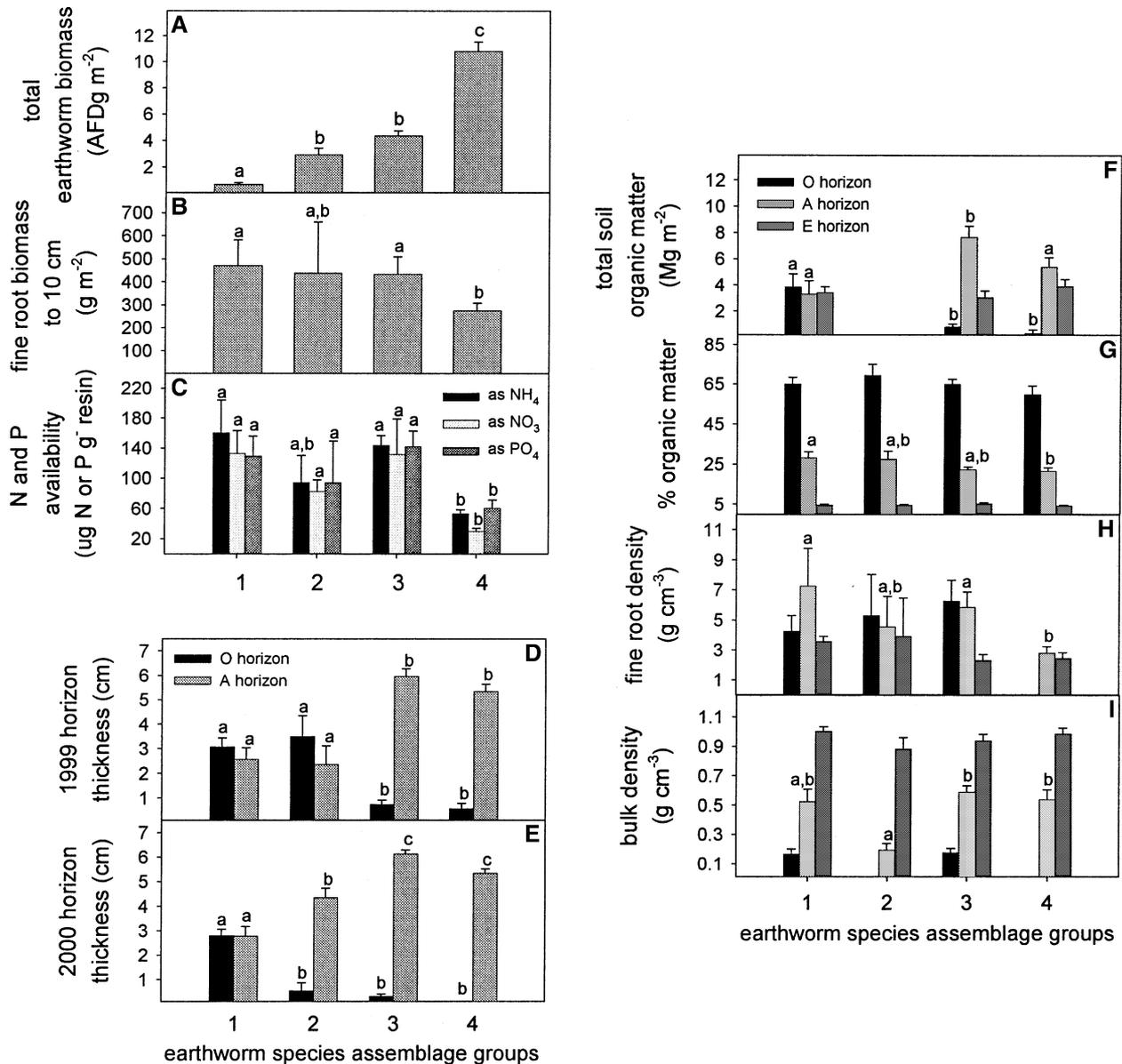


Figure 9. Comparisons of total earthworm biomass (ash-free dry g m⁻²) and soil parameters in relation to earthworm species assemblage groups. Where differences existed between species assemblage groups, bars with different letters indicate a significant difference ($P \leq 0.05$). Assemblage group 1 contains sample points with the *Dendrobaena* taxonomic group only ($n = 20$); Assemblage group 2 contains sample points with *Lumbricus rubellus*, *Lumbricus juveniles* and *Dendrobaena* taxonomic groups ($n = 13$); Assemblage group 3 contains sample points dominated by the *Aporrectodea* and *Dendrobaena* taxonomic groups ($n = 74$); Assemblage group 4 contains sample points with all taxonomic groups but were identified by the presence of the *L. terrestris* and *Octolasion* taxonomic groups ($n = 70$). Sample sizes differed in each earthworm species assemblage group depending on the soil parameter, for groups 1 through 4 respectively: graph A ($n = 20, 13, 74$ and 70); graph B ($n = 3, 3, 7$ and 12); graph C ($n = 5, 3, 6$ and 6); graph D ($n = 13, 7, 36$ and 38); graph E ($n = 20, 13, 74$ and 70); graph F ($n = 6, 2, 11$ and 12); graph G ($n = 12, 6, 36$ and 37); graph H ($n = 2, 3, 10$ and 12); graph I ($n = 11, 7, 42$ and 43).

soils, bulk densities before the addition of earthworms are very high as a result of compaction by farm equipment. In agricultural soils, earthworms reverse the compaction and hence decrease bulk density (Lavelle and others 1998). If earthworm

populations dominated by endogeic and anecic species lead to moderate bulk densities, then whether they loosen or compact the soil depends on the starting bulk density before earthworms invade. Although water infiltration rates in arable

lands often increase with earthworm activity, the inverse may be the rule in previously worm-free forests with low native bulk densities where earthworm invasion results in increased soil bulk density. The combination of the loss of surface litter and increased soil bulk density is likely to lead to increased surface run off and erosion in earthworm invaded forests (Willoughby and others 1997; Shuster and others 2000).

Despite the fact that fine root density declined with increasing total earthworm biomass in the A and E horizons, total fine root biomass to 10 cm depth did not change in relation to total earthworm biomass. Under worm free conditions, the forest floor contains a large proportion of total fine root biomass (Fisk and others 2004). It is likely that the loss of fine roots associated with loss of the forest floor was compensated by the increase in A horizon thickness resulting in no net change in fine root biomass.

Soil nutrient availability was consistently lower in areas with high earthworm biomass (Figure 6) as reflected in the lower foliar nitrogen content and increased C:N when earthworm biomass was high (Figure 7). In previously worm-free hardwood forests of North America, nutrient availability declines following earthworm invasion (Scheu and Parkinson 1994; Bohlen and others 2004b; Suárez and others 2004). This decline may be caused by increased immobilization of nutrients by the microbial community as fresh litter is continuously incorporated into the mineral soil and by increased leaching losses. In this study, ammonium, nitrate and phosphate had very similar patterns despite large differences in potential leaching rates, suggesting that increased immobilization may be the primary cause of lowered availability. If increased leaching were the primary effect of earthworms on nutrient availability then nitrate, which is more mobile, would be expected to decrease more than ammonium and phosphate (Jeffrey 1987), but that was not the case here (Figures 6 and 8).

In these sites, different earthworm species assemblages had different effects on forest soil properties independent of biomass and time since invasion. The feeding and burrowing habits of different earthworm species also determine their impacts on soil structure and function, in addition to their biomass (Shipitalo and others 1988; James and Cunningham 1989; Hendriksen 1990; Scheu and Parkinson 1994). However, increasing species diversity is often associated with increasing earthworm biomass and time since invasion, making it difficult to determine species-specific effects under field conditions. The four sites in-

cluded in this study were on the same landform and soil unit, had the same overstory composition and age structure, had the same land use and disturbance history and were in close proximity so that they experienced the same local climate conditions. Therefore, the site effects seen in this study are likely due to the differences in earthworm populations seen between the sites and across each leading edge of earthworm invasion (Hale and others 2005; Bohlen and others 2004a). These population differences are represented by the species assemblage groups. Affects of the potentially confounding factor of time since invasion were minimized because the locations of different species assemblage groups across the sample grids overlapped among sites (Hale and others 2005).

As hypothesized, the strictly epigeic *Dendrobaena* group alone did not remove the forest floor or enhance the development of the A horizon, nor did it change other soil parameters. However, the assemblage group dominated by the epi-endogeic species *L. rubellus* had a much larger effect on O horizon removal and formation for the A horizon than would be expected from biomass alone (Figure 9) (Gundale 2002). Although, soil bulk density may decrease when the *L. rubellus* group is found in isolation, when found in species assemblages containing endogeic and anecic species (groups 3 and 4) soil bulk density increased (Figure 9).

The addition of the *Aporrectodea* taxonomic group to species assemblages resulted in the maximum A horizon thickness and bulk density measures (Figure 9). This was the case in both assemblage groups 3 and 4 even though total earthworm biomass in group 3 was nearly 1/3rd that of group 4. This illustrates the importance of the burrowing and feeding behaviors of endogeic species in the homogenization of the upper soil horizon and formation of the A horizon (Figure 9).

The *L. terrestris* dominated assemblage group contained the highest total biomass and the most species, including at least one earthworm species from each taxonomic group (Table 1). Therefore, this assemblage group represents the net affect of the feeding and burrowing behaviors of all ecological groups (epigeic, epi-endogeic, endogeic and anecic) and any synergistic effects that may exist due to the range of feeding and burrowing behaviors present (Shaw and Pawluk 1986). This full species assemblage resulted in the complete elimination of surface litter and the lowest percent organic matter in the A horizon. Fine root density and total fine root biomass were lower in this group compared with all other groups. The decline in fine

root biomass in response to the *L. terrestris* dominated group is comparable to that seen by Fisk and others (2004) in an uncultivated mature hardwood forest with a similar earthworm species composition (Bohlen and others 2004a). Because fine root biomass was unrelated to total earthworm biomass in this study, the higher earthworm biomass of this group alone does not explain this result, nor does time since invasion because both the *L. rubellus* and *Aporrectodea* dominated assemblage groups (2 and 3) were present at similar locations across the sample grid. It is likely that the combined affects of multiple earthworm species with a range of grazing and burrowing behaviors contributes to this result. Changes in fine root biomass may be the result of persistent disturbance by epi-endogeic and endogeic species in the rhizosphere (Blair and others 1995). Changes in soil temperature, depth of freezing and other factors associated with the loss of a previously thick forest floor layer following earthworm invasion may also be important contributors to changes in fine root distribution, biomass and function (Waisel and others 1996; Tierney and others 2003).

Nutrient availability was substantially lower in the *L. terrestris* dominated assemblage group compared to all other groups. The complete incorporation of all annual litter inputs by this assemblage group each year may lead to increased immobilization of nutrients relative to the other groups. Increased nitrification and leaching losses may also be occurring as a result of synergistic effects of the different earthworm species. However, the specific mechanism of the nutrient declines is beyond the scope of this study (Bohlen and others 2004b; Suárez and others 2004).

The four earthworm species assemblage groups in this study represent a typical successional sequence of earthworm assemblages across a leading edge of earthworm invasion (Hale and others 2005). Incipient invasions often begin with *Dendrobaena octaedra* (assemblage group 1) in advance of all other species, followed in succession by the addition of *L. rubellus* (assemblage group 2), *Aporrectodea* species (assemblage group 3) and finally *L. terrestris* (assemblage group 4), with total earthworm biomass generally increasing with diversity.

The *L. terrestris* dominated assemblage group represents a typical stable reproductive earthworm population in stands that have been invaded by European earthworms for multiple decades (C.M. Hale, personal observation; Holdsworth and others, unpublished data — University of Minnesota, Dept. of Forest Resources) and may therefore represent the long term effects that earthworm invasion may

have in hardwood forests of the western Great Lakes region. However, the rapid change in soil characters associated with the appearance of *L. rubellus* illustrates the importance of this species in ecosystem change during initial invasion.

The large effect of *L. rubellus* as compared to *Dendrobaena octaedra* also illustrates the need to consider specific feeding and burrowing behavior, and not simply location in the soil profile, when assessing potential impacts (Gundale 2002). The widely used categories of three ecological groups (epigeic, endogeic and anecic) are too general to adequately describe the diversity in function of different species (Lavelle and others 1998). In particular, *Dendrobaena octaedra* and *L. rubellus* are both categorized as epigeic species but they have very different effects due to the propensity of *L. rubellus* to burrow and cast in the surface mineral soil layer (Edwards and others 1995; Rozen 1988).

In contrast to results of earthworm activity in agricultural ecosystems, changes in fine root dynamics (Fisk and others 2004), declines in nutrient availability (Bohlen and others 2004b), the loss of the forest floor as habitat and seed bed (Baskin and Baskin 1998; Parkinson and McLean 1998) and changes in mycorrhizal fungi composition and colonization rates (Lawrence and others 2003) seen in north temperate hardwood forests represent important changes in ecosystem function that may ultimately lead to changes in forest composition and structure (Gundale 2002; Hale 2004). Although earthworm invasions have been occurring for many decades, earthworms have not been recognized as exotic species by forest ecologists in the region (Wagner and others 1977; Coderre and others 1995). In the face of rapid change and multiple pressures on native forest ecosystems, the potential impacts of earthworm invasion should be considered.

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