

Exotic earthworm invasion increases soil carbon and nitrogen in an old-growth forest in southern Quebec

M. Wironen and T.R. Moore

Abstract: To test whether invasion of exotic earthworms affects soil carbon (C) and nitrogen (N), we sampled the litter and upper mineral soil (to 30 cm) at a series of sites varying in their earthworm populations in an old-growth beech–maple forest at Mont St. Hilaire, southern Quebec. We measured earthworm abundance and biomass using hand-sorting and chemical extraction (allyl isothiocyanate) methods. They gave similar results, though there was evidence of size and species-specific biases. Abundance and biomass of the earthworms ranged from <10 to >100 earthworms·m⁻² and from <10 to 125 g·m⁻², respectively, and were correlated with distance from a nearby lake (negatively) and soil pH (positively). The presence of earthworms was associated with a decrease in the mass and thickness and an increase in the C/N quotient of the litter layer. There were no significant changes in C and N mass of the mineral soil between 0 and 10 cm, but the underlying layers (10–20 and 20–30 cm) in sites with >10 earthworms·m⁻² showed significantly ($p < 0.05$) greater concentrations and masses of both C and N than did sites with <10 earthworms·m⁻². The overall profile (litter plus soil to 30 cm) average C was 13.7 and 10.1 kg·m⁻² with and without earthworms, respectively, and the equivalent figures for N were 1.01 and 0.68 kg·m⁻². These results demonstrate that invasion of earthworms into deciduous forests affects both the litter and mineral soil, and sampling to a depth of 30 cm suggests that earthworm invasion (from <10 to >10 earthworms·m⁻²) may increase overall C and N.

Résumé : Pour tester si l'invasion par des vers de terres exotiques affecte le carbone (C) et l'azote (N) dans le sol, les auteurs ont échantillonné la litière et la partie supérieure du sol minéral (jusqu'à 30 cm) dans une série de sites avec diverses populations de vers de terre, dans une vieille forêt de hêtre et d'érable au mont Saint-Hilaire dans le sud du Québec. Ils ont mesuré l'abondance et la biomasse des vers de terre en utilisant le triage à la main et l'extraction chimique (isothiocyanate d'allyle). Ces méthodes ont produit des résultats similaires malgré des indices de biais spécifiques à la dimension et à l'espèce. L'abondance et la biomasse des vers de terre variaient respectivement de <10 à >100 vers de terre·m⁻² et de <10 à 125 g·m⁻² et étaient corrélées avec la distance d'un lac situé à proximité (négativement) et le pH du sol (positivement). La présence des vers de terres était associée à une diminution de la masse et de l'épaisseur de la litière ainsi qu'à une augmentation du rapport C/N de la litière. Il n'y avait pas de changement significatif dans la masse de C et N du sol minéral entre 0 et 10 cm mais les horizons inférieurs (10–20 et 20–30 cm) dans les sites avec plus de 10 vers de terre·m⁻² avaient des concentrations et des masses de C et N significativement plus élevées ($p < 0,05$) que les sites avec moins de 10 vers de terre par m². L'ensemble du profil de sol (litière et sol minéral jusqu'à 30 cm de profondeur) avait en moyenne respectivement 13,7 et 10,1 kg C·m⁻² et 1,01 et 0,68 kg N·m⁻² avec et sans vers de terre. Ces résultats montrent que l'invasion des vers de terre dans les forêts feuillues affecte tant la litière que le sol minéral et l'échantillonnage jusqu'à une profondeur de 30 cm indique que l'invasion des vers de terre (de <10 à >10 vers de terre·m⁻²) peut entraîner une augmentation de la quantité globale de C et N.

[Traduit par la Rédaction]

Introduction

Most Canadian forests have developed on soils that were exposed by the retreat of ice sheets or water between 5000 and 12 000 years ago. At this time, soil development started without earthworms, which were restricted to nonglaciated areas, though many nonglaciated areas also were free of worms (James 1995). Rates of earthworm dispersal through soil are poorly known, perhaps 10 m·year⁻¹ (Marinissen and

van den Bosch 1992). To date, native species of earthworms have recolonized forest soils as far north as Massachusetts, so that most Canadian forest soils have developed in the absence of a large native earthworm population.

Exotic earthworms have been introduced into many North American forest soils, via various anthropogenic vectors (Langmaid 1964; Lee 1985; Edwards and Bohlen 1996; Dymond et al. 1997; Steinberg et al. 1997; Burtelow et al. 1998; Frelich and Holdsworth 2002; Gundale 2002; Hendrix

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and Bohlen 2002; Bohlen et al. 2004a). More than 45 species of earthworms have been introduced, primarily of a European origin (Lee 1985; Reynolds 1998). Recent research suggests that exotic earthworm invasion in northern forests is a widespread phenomenon (Alban and Berry 1994; Dymond et al. 1997; Frelich and Holdsworth 2002), with potential ecosystem-level implications, particularly in forests without native earthworm populations (Hendrix and Bohlen 2002; Bohlen et al. 2004a, 2004c).

Earthworms can have profound effects on soil properties, arising from the ingestion and excretion of soil and litter and the mixing of soil materials in the profile. Changes include the creation of soil aggregates (casts) and changes in porosity and other physical and hydraulic properties (Lee 1985; Edwards and Bohlen 1996). Earthworm consumption of the litter layer in forests may lead to changes in plant species composition and has raised concerns about the viability of rare endemic species, such as *Botrychium mormo*, in forests that have been invaded by exotic earthworms (e.g., Frelich and Holdsworth 2002; Gundale 2002).

Earthworms have also been shown to affect the amount and distribution of organic matter and to increase the rates of organic matter decomposition. Earthworms transport large quantities of carbon (C) from the surface of the soil to the lower horizons, effectively mixing the soil and increase humification rates and overall decomposition rates significantly (Langmaid 1964; Lee 1985; Alban and Berry 1994; Edwards and Bohlen 1996; Burtelow et al. 1998; Li et al. 2002; Bohlen et al. 2004b). The effects of earthworm activity on nitrogen (N) cycling are complex and far more uncertain than for C cycling (Bohlen et al. 2004b, 2004c). Earthworms increase labile stores of N and mix N stored in surface organic material into the lower mineral soil (Lee 1985; Edwards and Bohlen 1996; Burtelow et al. 1998; Lavelle et al. 1998; Bohlen et al. 2004b).

The effect of exotic earthworms on total soil C and N in North American forests has been addressed in several studies. Alban and Berry (1994) found that earthworm invasion of a Minnesota forest resulted in an annual loss (over the course of a 14-year study) of 60 g C·m⁻² from the upper 50 cm of the soil. Burtelow et al. (1998) reported a reduction in organic matter concentration from the litter layer with a minor increase in the A horizon total. Bohlen et al. (2004b) recorded a 28% decrease in upper-layer (0–12 cm depth) soil C storage at earthworm-invaded sites, but no change in total N storage. In Minnesota forests, at the leading edge of worm invasion, Hale et al. (2005a, 2005b) showed a correlation of increasing worm biomass with a decrease in the thickness of the O horizon, an increase in the thickness of the A horizon, and a resulting increase in the overall storage of organic matter in the top 12 cm. Thus, variable results have been observed on the effects of earthworm invasion on soil C and N storage.

In this paper, we examine soil characteristics in an undisturbed, old-growth deciduous forest in southeastern Canada, in soils that had initially developed with few earthworms. European introduction of exotic earthworms, probably from an adjacent lake and surrounding settlements in the past two centuries, has created soils with varying earthworm densities. At sites within the forest, we determine earthworm densities by number and mass, identify the major species, and develop a model of their distribution. We then examine the

distribution of C and N in the litter and soil as a function of earthworm density, controlling for soil and forest characteristics. We hypothesize that earthworm density is inversely related to distance from the lake edge and that the presence of earthworms reduces litter C and N mass, decreases overall soil profile C and N mass, and results in changes in C/N ratios.

Methods

Study area

This study was conducted at the Gault Nature Reserve on Mont St. Hilaire (MSH) (45°32'N, 73°80'W), 32 km east of Montréal, Quebec (Gault Nature Reserve 2004). MSH comprises eight peaks (maximum elevation of 415 m), covering an area of 5550 ha surrounding 32 ha Lac Hertel, and contains the largest remaining parcel of primeval forest in the St. Lawrence Lowlands (Gault Nature Reserve 2004). The climate is cool-continental, with a mean annual precipitation of 1058 mm, 200 mm of which falls as snow, and the mean annual temperature is 6.2 °C (Environment Canada 2004).

The mountain is composed of a series of igneous intrusions of Cretaceous age, ranging from gabbro to syenite. The mountain became free of ice by 12 500 years BP, with the flanks of the mountain engulfed by the Champlain Sea and the freshwater Lake Lampsilis. The mountain was initially covered by tundra vegetation, which was replaced by a mixed-deciduous forest cover and then a dominantly hardwood forest about 8000 years BP (Gault Nature Reserve 2004). The present forest canopy, which covers over 90% of MSH, is dominated by sugar maple (*Acer saccharum* Marsh.) and American beech (*Fagus grandifolia* Ehrh.).

After field surveys, six sites were chosen for study near streams or the lake, but at least 5 m from the source of water. They all had similar parent material, a mix of gabbro and nepheline syenite, and similar slope (<15°) and aspect (S to SE). The sites had soils that were well-drained sandy loam Brunisols, and there was no evidence of mottling in the profile to a depth of 30 cm, which corresponds to the start of the C horizon in the shallowest soil profiles. Sites 1 and 2 were located furthest from the lake and at the highest elevation (250 to 275 m a.s.l.), and the elevation for sites 3 to 6 ranged from 175 to 225 m.

Three sites (2, 4, and 5) showed evidence of extensive earthworm activity, such as surface casts, while the other three did not. At each site, a 100 m transect was established to cover the range of local habitat, containing three 100 m² sample plots, each spaced 50 m apart. Each had three sampling points 3 m from the centre in a triangular pattern. However, all results reported in this paper use data from 14 of the original 18 plots, as 4 of the sample plots were incompletely sampled and thus could not be included. The location of each plot was determined using a Garmin hand-held GPS unit. ArcView 3.3 and ArcGIS 9.0 were used to determine the linear distance from each plot to Lac Hertel. All trees in each plot with a diameter at breast height greater than 4 cm were counted and identified.

Sites 1 to 3 were dominated almost completely by *A. saccharum*, while sites 4 to 6 were not dominated by any single species, but rather a mix of hardwoods, primarily *A. saccharum*, *Fagus grandifolia*, *Fraxinus americana* L., *Quercus rubra* L., and *Tilia americana* L. *Acer saccharum*

Table 1. Earthworms found at Mont St. Hilaire from all plots (comprising 42 sample points).

Species	Ecological category ^a	Characteristics ^a	Total no. retrieved	Mean biomass per individual (g)
<i>Aporrectodea tuberculata</i>	Endoanecic	Mid-sized, widely distributed, bait worm, pH 4.8–7.5	165	0.36
<i>Dendrobaena octaedra</i>	Epigeic	Small, prefers undisturbed sites, pH 3.0–7.7	22	0.13
<i>Lumbricus terrestris</i>	Anecic	Large, widely distributed, bait worm, pH 4.0–8.0	23	4.54
<i>Lumbricus rubellus</i>	Epiendogeic	Mid-sized, widely distributed, bait worm, pH 3.8–8.0	26	1.89
<i>Eisenia rosea</i>	Epigeic	Small, widely distributed, prefers wetter sites, pH 4.9–8.0	28	0.19
Unknown juvenile	—	—	140	0.19
Juvenile <i>Lumbricus</i> spp.	—	—	46	1.36

^aEcological category and characteristics are derived from Reynolds (1977), Shakir and Dindal (1997), Hendrix and Bohlen (2002), Li et al. (2002), and Bohlen et al. (2004a).

and *Fagus grandifolia* were the dominant canopy species in the sample plots, constituting 52% and 15% of the total stand basal area, respectively. *Acer saccharum* was found in all plots, while *Fagus grandifolia* was found in 10 of the 14 plots. The remaining stand basal area comprised mixed hardwoods, including *Fraxinus americana* (10%), *Q. rubra* (6%), *T. americana* (6%), *Acer pennsylvanicum* L. (4%), *Betula alleghaniensis* Britt. (4%), *Ostrya virginiana* (P. Mill.) K. Koch. (3%), and *Betula papyrifera* Marsh. (<1%). None of these species was found in more than five of the plots.

Earthworm sampling

Earthworms at each site were sampled over a 5-day period in late May 2004, following about 15 mm of rain. Two methods were employed: hand sorting and chemical extraction. For hand sorting, a soil pit 42 cm × 42 cm × 30 cm deep was dug at the centre of each plot. All excavated soil was placed on a tarp and then hand sorted for earthworms (Reynolds 1977; Lee 1985; Lawrence and Bowers 2002). Earthworms were killed in a 70% ethanol solution, rinsed in the laboratory, and stored in a 5% formalin solution (Reynolds 1977).

Chemical sampling was made using allyl isothiocyanate (AITC, mustard powder) at three points in each plot. This chemical expellant was chosen because of its nontoxic effects on understory vegetation and many soil organisms, compared with the effects of formalin (Lawrence and Bowers 2002; Zaborski 2003). A stock solution of 5 g of AITC (94%) in 1 L of isopropanol (100%) was prepared and diluted to 100 mg·L⁻¹ for field application (Zaborski 2003). Seven litres of the AITC solution was poured onto a 42 cm × 42 cm (0.18 m²) frame in two applications, 10 min apart (Zaborski 2003). The AITC irritates the epidermis of the earthworms, and they typically move towards the surface, where they were collected and preserved as for hand sorting (Lawrence and Bowers 2002; Zaborski 2003).

Individual worm biomass was determined by weighing the preserved samples. Speciation was performed using Reynolds's (1977) dichotomous key.

Soil sampling and analytical methods

At the centre of each plot, a 42 cm × 42 cm soil pit was excavated deep enough to expose part of the C horizon, or until an impenetrable layer was encountered (typically bedrock). Before digging, the thickness of the litter layer was measured and then collected. In the mineral soil a sample of each major horizon was collected. In the absence of clear horizonation, a sample was taken every 10 cm. Bulk density

cores were taken from depths of 0–10, 20, and 30 cm. Samples were refrigerated until they could be air dried.

Soil samples were air dried and weighed to determine moisture content. The litter samples were homogenized by hand, and a subsample was ground using a Wiley mill. The mineral soil samples were crushed and passed through a 2 mm sieve. All visible roots that passed through the 2 mm sieve were removed using tweezers before being sent for total C and N analysis. Each homogenized and sieved air-dry soil sample was then divided in two, and one subsample was oven-dried at 105 °C for 24 h to determine moisture content and bulk density (Carter 1993). Total C and N concentrations were determined on the oven-dry soil with a Carlo Erba NC-2500 elemental analyzer. The pH of the air-dry soil samples was determined in both distilled water and in 0.1 mol·L⁻¹ CaCl₂ solutions with an Orion Triode™ pH probe and model 290A meter (Carter 1993).

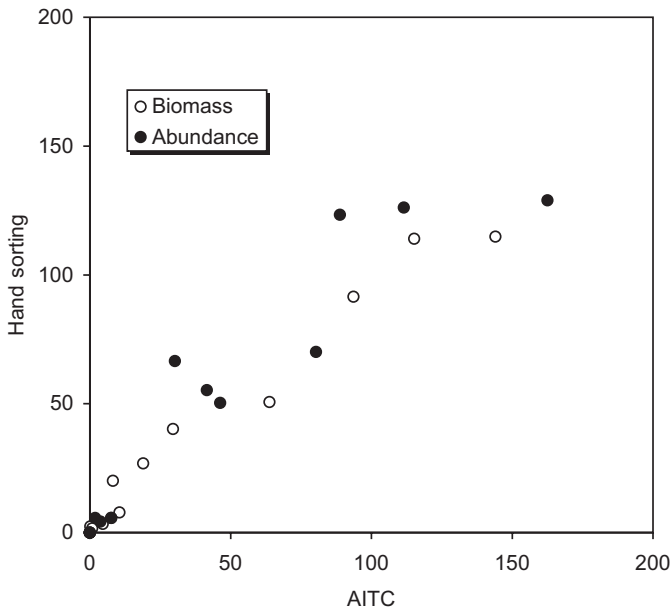
Statistical analyses were performed using SPSS 12.0 for Windows. A stepwise linear regression model was developed to explain earthworm abundance at the various sample plots. Two models were developed, the first using abundance and the second using biomass per square metre. Independent variables included were gravimetric soil moisture and pH (in CaCl₂) of the upper 10 cm of mineral soil as an expression of habitat limitation, total stand basal area as a surrogate for litter (food) production, and distance to Lac Hertel, the hypothesized introduction point. Data were log transformed where necessary. All model parameters were assessed for multicollinearity to ensure their utility. Student's *t* tests (which assume heteroscedasticity) and Wilcoxon signed-ranks tests were used to identify earthworm sampling biases. Both univariate linear regression and Student's *t* tests were used when analyzing soils data.

Results

Earthworms and their distribution

Individual sample points ranged in earthworm abundance from 0 to >200 earthworms·m⁻², and at one point near Lac Hertel over 114 g·m⁻² of worms was found, or more than 1 t earthworms·ha⁻¹. Five species of earthworm were found, all from the Lumbricidae; the most abundant species was *Aporrectodea tuberculata* (Eisen) and the large *Lumbricus terrestris* L. formed the greatest biomass (Table 1). Over 40% (29% of total earthworm biomass) of the recovered earthworms were juvenile, of which 25% (70% of total juvenile biomass) could be identified as members of the genus

Fig. 1. Relationship between biomass ($\text{g}\cdot\text{m}^{-2}$) and abundance (no. earthworms $\cdot\text{m}^{-2}$) of earthworms extracted by allyl isothiocyanate and hand sorting.



Lumbricus. Plots were grouped by earthworm population density — “few worms” for plots with a density <10 earthworms $\cdot\text{m}^{-2}$ and “heavily invaded” for plots with larger earthworm populations. It is noteworthy that no plots were found with earthworm densities >6 earthworms $\cdot\text{m}^{-2}$ and <50 earthworms $\cdot\text{m}^{-2}$.

There was no statistically significant difference ($p < 0.05$, Student's t test) between the number and biomass of earthworms collected by the two extraction methods (hand sorting and AITC) (Fig. 1, Table 2a). The results suggest, however, that AITC sampling resulted in fewer large worms and hand sorting resulted in a greater number of small worms, resulting in a potential species-level sampling bias. There was no significant difference in the proportion of juveniles sampled by the two methods. There were, however, significantly ($p < 0.05$) fewer *A. tuberculata* and more *L. terrestris* collected by the AITC method than by hand sorting, though there was no statistically significant difference for the other three species (Table 2b).

Earthworm distribution was positively related to soil pH (CaCl_2) in the upper 10 cm and negatively to distance (m) from the adjacent Lac Hertel (Fig. 2a). Abundance (earthworms $\cdot\text{m}^{-2}$) and biomass ($\text{g}\cdot\text{m}^{-2}$) of earthworms could be correlated with these two variables, which explained 70% and 88% of the variance, respectively:

$$\text{Abundance} = 27.06 (\text{pH}) - 22.07 \ln (\text{distance}) + 65.25$$

$$r^2 = 0.80, n = 14, \text{SEE} = 25.68$$

$$\text{Biomass} = 18.01 (\text{pH}) - 22.39 \ln (\text{distance}) + 90.22$$

$$r^2 = 0.88, n = 14, \text{SEE} = 16.04$$

Soil moisture and total stand basal area did not significantly explain any variance in earthworm abundance or biomass and thus were excluded from the model.

Using data from Melillo et al. (1982) and Finzi et al. (1998), the canopy species were grouped into high (*Fagus*

and *Quercus* spp.) and low (remaining tree species) C/N classes. This was done to further assess the role of litter (food) quality on earthworm abundance, and stand basal area was used as a surrogate for litter production. The net stand basal areas for the two C/N classes were regressed as independent variables against the unstandardized residuals from the original distribution model, to control for canopy species – pH interactions. Both variables were insignificant (low C/N, $p < 0.75$; high C/N, $p < 0.53$) and explained only 7% of the variance in the residuals.

Lumbricus terrestris and *Eisenia rosea* (Savigny) are narrowly confined to the immediate environs of Lac Hertel (Fig. 2b). *Dendrobaena octaedra* (Savigny) was found only at locations considerably removed from Lac Hertel. *Lumbricus rubellus* (Hoffmeister) occurs over a broader range, and *A. tuberculata* is the most widely dispersed species occurring at both the nearest and furthest site from the lake.

Soil properties

Soils with small to nonexistent earthworm populations were generally classified as Orthic Dystric Brunisols or Orthic Sombric Brunisols, following the Canadian Soil Information System (Agriculture and Agri-Food Canada 2005). Dystric and Sombric Brunisols are acidic, with a pH (CaCl_2) below 5.5, and Sombric Brunisols have an Ah horizon thicker than 10 cm. The underlying Bm horizons occasionally showed evidence of podzolization, with inconsistent, trace Ae horizons. Soils with large populations of earthworms showed an almost complete lack of horizonation, with the upper 30 cm or more of the pedon showing signs of heavy bioturbation often with a crumb-like structure. Soil pH of the top 10 cm measured in $0.01 \text{ mol}\cdot\text{L}^{-1} \text{ CaCl}_2$ ranged from 3.0 to 5.2 with a mean of 4.3 and a standard deviation of 0.5. At the time of earthworm sampling, gravimetric soil moisture content in the top 10 cm averaged 31% (range 26%–42%) and from 10 to 20 cm averaged 29% (range 22%–37%). There is no adequate classification for these soils under the Canadian Soil Information System (D. Kroetsch, soil specialist, personal communication, 2005), and they were classified as Orthic Vermic Brunisols.

There was a significant ($p < 0.01$) exponential decrease in both the thickness (from 5 to 1 cm) and the mass (from 1000 to 300 $\text{g}\cdot\text{m}^{-2}$) of the litter layer at the sites as a function of earthworm biomass (Fig. 3a, 3b). There was also a significant increase in litter C/N quotient (from 23 to 40) with increasing earthworm biomass (Fig. 3c). Concentrations of C and N within profiles generally decreased with depth, but those profiles with a large number of earthworms (>10 earthworms $\cdot\text{m}^{-2}$) showed smaller C and N concentrations in the upper soil layer (0–10 cm) and a larger concentration in the lower soil layer (deeper than 20 cm) than those at the sites with a small number of earthworms (<10 earthworms $\cdot\text{m}^{-2}$) (Fig. 4). For the litter layer and soils sampled at depths of 10 and 20 cm, the presence of earthworms was associated with significant differences in C and N concentrations and C/N quotient, the effect varying with depth (Table 3). Earthworms significantly ($p < 0.05$) decreased C and N concentrations and increased C/N quotient in the litter and significantly increased C and N concentrations at 10 and 20 cm, with no major difference in C/N quotient at these depths.

C and N mass can be calculated by merging the concentration and bulk density results. The decline in litter C and N

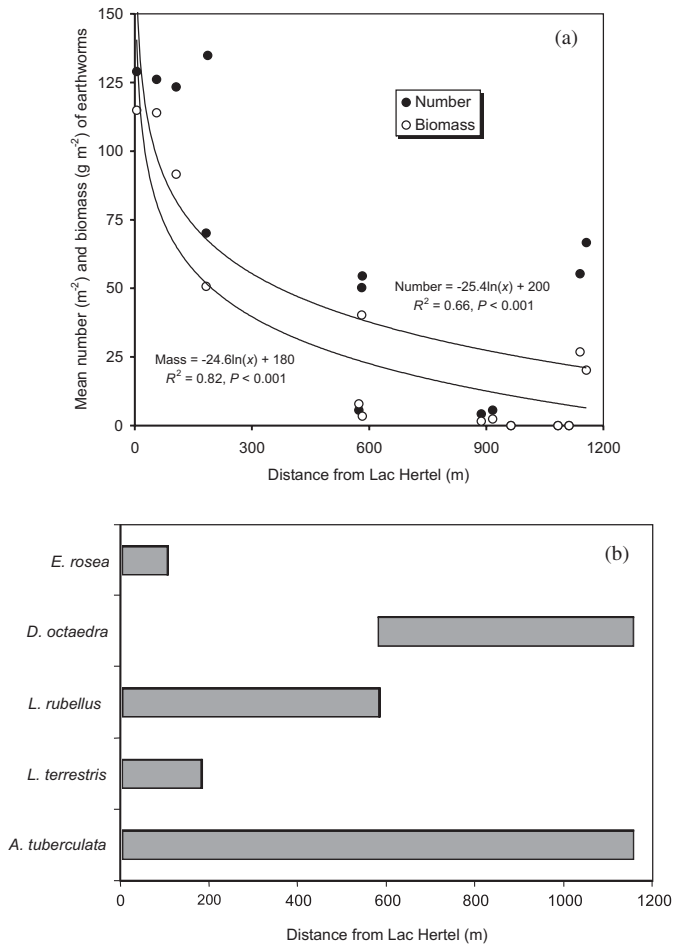
Table 2. The effects of the allyl isothiocyanate (AITC) and hand-sorting extraction methods, using the pooled data for (a) mean abundance, biomass, and proportion of juveniles and (b) mean abundance per species.

(a) Pooled data.										
	AITC		Hand							
Abundance (earthworms·m ⁻²)	52.9		74.7							
<i>t</i> (<i>p</i> value)			-0.75 (0.23)							
Biomass (g·m ⁻²)	44.5		38.8							
<i>t</i> (<i>p</i> value)			0.29 (0.39)							
Proportion of juveniles	0.35		0.29							
<i>t</i> (<i>p</i> value)			0.68 (0.25)							

(b) Species data.										
	<i>L. terrestris</i>		<i>D. octaedra</i>		<i>L. rubellus</i>		<i>A. tuberculata</i>		<i>E. rosea</i>	
	AITC	Hand	AITC	Hand	AITC	Hand	AITC	Hand	AITC	Hand
Abundance (earthworms·m ⁻²)	10.21	2.84	5.67	6.80	3.97	12.47	20.98	41.39	11.91	26.65
<i>t</i> (<i>p</i> value)	2.11 (0.04)		-0.26 (0.40)		-1.16 (0.15)		-1.81 (0.05)		-0.94 (0.21)	
Wilcoxon <i>Z</i>	-1.47	-0.41	-1.21	-2.19	-0.54					

Note: These data exclude all plots without earthworms. All reported *p* values are one-tailed.

Fig. 2. Relationship between distance from Lac Hertel and (a) earthworm abundance and biomass and (b) earthworm species presence.



the 10–20 and 20–30 cm depths (Table 4). When the litter plus upper 30 cm of soil were compared, those with earthworms contained an average of 13.7 kg C·m⁻² compared to 10.1 kg C·m⁻² in those without earthworms (significantly different at *p* < 0.05). There was a similar, significant difference in N mass of 1.01 and 0.68 kg N·m⁻², in soils with and without earthworms, respectively.

Discussion

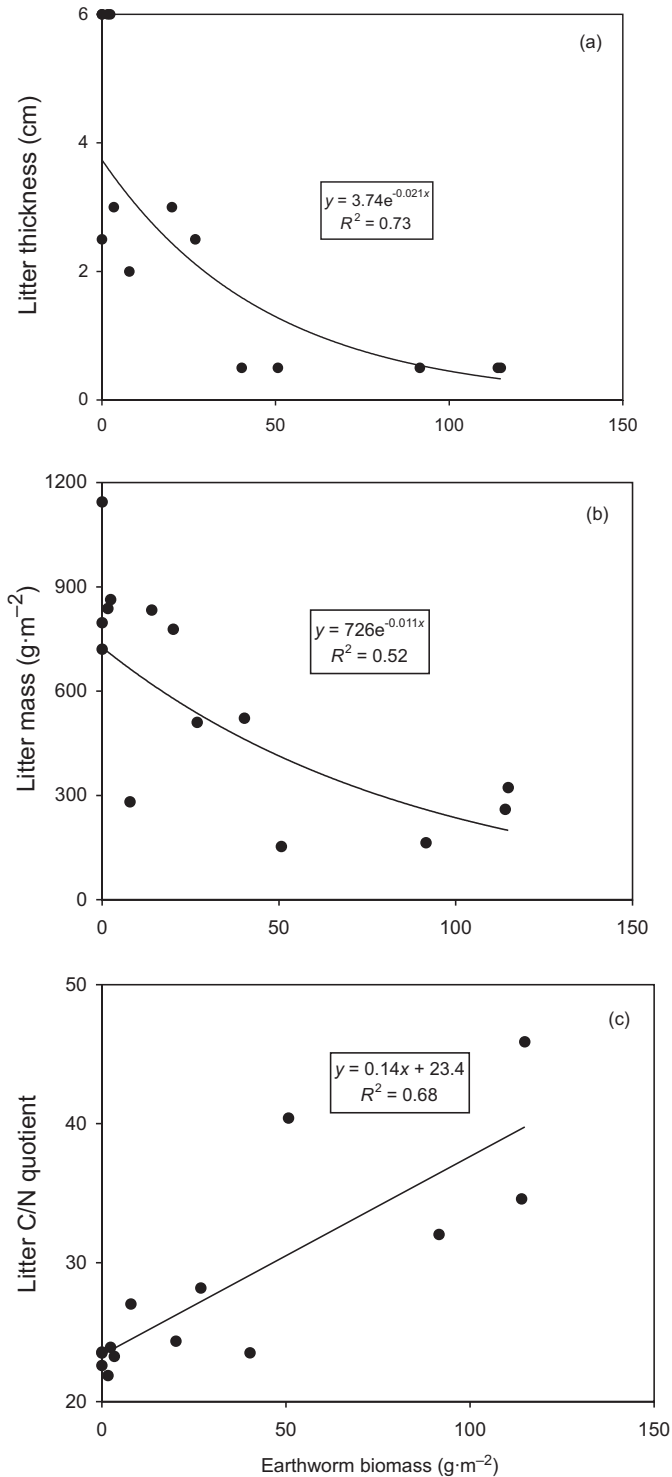
Earthworm population and distribution

The results of the earthworm sampling suggest that exotic earthworm invasion has occurred at a diversity of sites throughout MSH. The most abundant species, *A. tuberculata*, was found over 1 km from Lac Hertel, its suspected introduction point. The species richness (five) found at MSH is typical of temperate earthworm populations and represents almost 20% of the earthworm diversity found in Quebec (Reynolds 1998). The high proportion of juveniles (40% by number, 29% by biomass) is not surprising given the May sampling date, as earthworm activity and abundance is at a peak in late spring, when there is a large influx of recently hatched juveniles (Reynolds 1977; Shakir and Dindal 1997).

The strong correlation between hand sorting and AITC extraction for earthworm biomass and abundance is similar to that reported by Hale et al. (2005a) in Minnesota forests. The statistical analyses of sampling results between AITC extraction and hand sorting suggest that some species-specific ecological bias occurs, which was also reported by Hale et al. (2005a). Although no significant bias was found for the aggregated (all species) data, AITC sampling yielded significantly greater numbers of *L. terrestris* and fewer *A. tuberculata*. *Lumbricus terrestris* is a deep-dwelling anecic that creates semipermanent vertical burrows, so a chemical expellant would rapidly flow down these macropores, returning larger numbers of these worms compared to that returned by hand sorting (Reynolds 1977; Chan and Munro 2001; Zaborski 2003). *Aporrectodea tuberculata*, an endoanecic species, was retrieved in significantly smaller numbers by AITC extraction. Zaborski (2003) reported similar findings when sampling

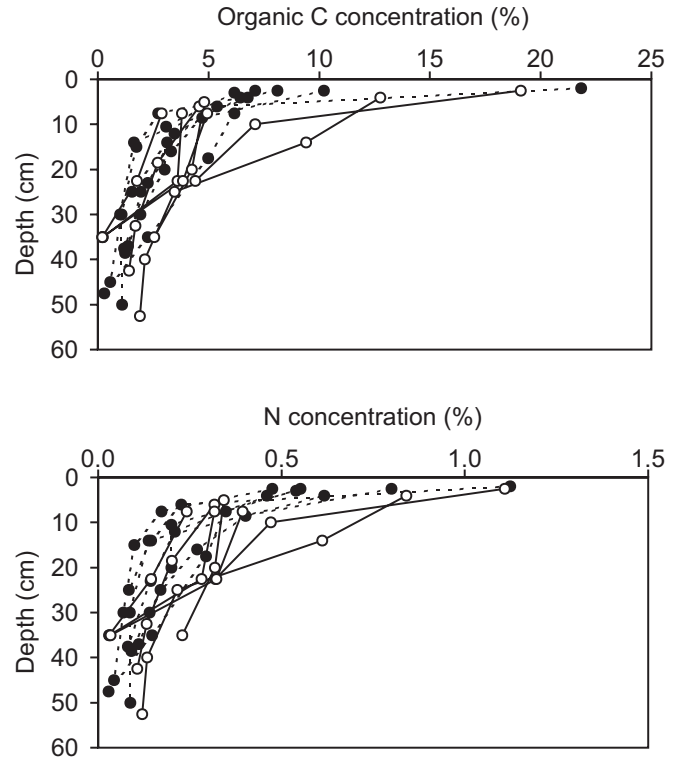
mass was followed by no significant differences at 0–10 cm depth and by a significant (*p* < 0.05) increase in soil C and N mass in earthworm compared to non-earthworm soils at

Fig. 3. Relationship between earthworm biomass and (a) litter thickness, (b) litter mass, and (c) litter carbon/nitrogen (C/N) quotient.



A. tuberculata with AITC, and Hale et al. (2005a) noted this for *Aporrectodea* species. This trend was also observed by Chan and Munro (2001) and Zaborski (2003) for a similar species, *Aporrectodea trapezoides*. The trend is likely due to the horizontal burrowing patterns of *Aporrectodea* species, which result in a horizontal movement away from the sam-

Fig. 4. Soil profile concentrations of carbon (C) and nitrogen (N), differentiated by earthworm population (<10 earthworms·m⁻² (filled circles); >10 earthworms·m⁻² (open circles)).



ple point when contacted by the expellant. This species may be a habitat generalist, since it is listed as endoanecic, endogeic, and anecic in different papers in the literature (see Table 1) (Shakir and Dindal 1997; Chan and Munro 2001; Li et al. 2002; Zaborski 2003; Bohlen et al. 2004a). The hand-sorting bias suggests that it may reside nearer to the surface at MSH. The significance of the species-specific analyses may have been constrained by the low sample size for rarer species. It is possible that there was a within-method sampling bias for AITC extraction, where sites with wetter soils had slower and shallower penetration of the expellant compared to those at or below field capacity. This was not tested, but represents a limitation of all chemical expellants that is particularly prominent when working in heterogeneous soil environments such as forests (Reynolds 1977).

The statistical model developed to explain the distribution and abundance of earthworms at MSH showed that soil pH and distance to the lake are the only significant factors. The insignificance of soil moisture is surprising, given that many authors have noted that this variable can control earthworm distribution (Lee 1985; Edwards and Bohlen 1996), but this may be due to the relative homogeneity in moisture conditions across sites at the time of sampling. The soil moisture content fell within the optimal range of 20%–35%, as suggested by Shakir and Dindal (1997), but as soil moisture content was measured only once, these data are limited in their explanatory power. While the sites were chosen in an effort to reduce variance in edaphic conditions, a more comprehensive assessment of seasonal soil moisture conditions would have provided more power in explaining earthworm

Table 3. Mean organic carbon (C) and nitrogen (N) concentrations and C/N quotient in the litter and soil at 10 and 20 cm depths, separated into those with (>10 earthworms·m⁻²) and without (<10 earthworms·m⁻²) earthworms.

Depth	C concentration (%)		N concentration (%)		C/N quotient	
	With	Without	With	Without	With	Without
Litter	34.4 (7.5)	44.5 (0.8)	1.10 (0.35)	1.89 (0.14)	31.3 (8.3)	23.5 (1.6)
<i>t</i> (<i>p</i> value)	-3.57 (0.006)		-5.54 (<0.001)		2.83 (0.015)	
10 cm	5.32 (2.21)	3.40 (1.36)	0.38 (0.11)	0.22 (0.12)	14.0 (1.4)	15.5 (4.4)
<i>t</i> (<i>p</i> value)	1.95 (0.040)		2.71 (0.010)		-1.81 (0.057)	
20 cm	3.85 (1.45)	2.56 (1.23)	0.29 (0.09)	0.18 (0.10)	13.3 (1.1)	14.2 (4.3)
<i>t</i> (<i>p</i> value)	1.78 (0.050)		2.06 (0.032)		-1.02 (0.171)	

Note: Statistical comparisons between earthworm categories are made within each layer and property.

Table 4. Litter and soil carbon (C) and nitrogen (N) mass (mean and standard deviation) in the litter and soil to 30 cm at sites with (>10 earthworms·m⁻²) and without (<10 earthworms·m⁻²) earthworms.

Depth	C mass (kg·m ⁻²)		N mass (kg·m ⁻²)	
	With	Without	With	Without
Litter	0.14 (0.10)	0.35 (0.11)	0.005 (0.004)	0.015 (0.005)
<i>t</i> (<i>p</i> value)	-3.71 (<0.001)		-4.03 (<0.001)	
0–10 cm	4.48 (1.84)	4.47 (0.49)	0.32 (0.11)	0.32 (0.07)
<i>t</i> (<i>p</i> value)	0.01 (0.496)		0.14 (0.448)	
10–20 cm	4.70 (1.60)	2.91 (1.19)	0.35 (0.10)	0.19 (0.08)
<i>t</i> (<i>p</i> value)	2.38 (0.019)		3.42 (0.004)	
20–30 cm	4.36 (1.29)	2.41 (0.98)	0.33 (0.12)	0.17 (0.06)
<i>t</i> (<i>p</i> value)	3.18 (0.005)		3.36 (0.005)	
Litter plus 0–30 cm	13.67 (3.62)	10.14 (2.42)	1.01 (0.23)	0.68 (0.18)
<i>t</i> (<i>p</i> value)	2.15 (0.030)		2.90 (0.008)	

Note: Statistical comparisons are made within each layer and property between earthworm category.

population distributions. Earthworms are known to prefer soils with a higher pH, but at the same time can raise the pH of the soils that they occupy (Reynolds 1977; Lee 1985; Edwards and Bohlen 1996; Burtelow et al. 1998). The insignificance of other soil properties in explaining earthworm abundance and biomass was not unprecedented. There has been considerable debate about whether earthworm population aggregations are correlated with certain soil properties, with conflicting conclusions (Lee 1985; Edwards and Bohlen 1996; Shakir and Dindal 1997; Rossi 2003; Whalen and Costa 2003). Soil properties may constrain earthworm populations only at the extremes of each variable (Edwards and Bohlen 1996), and the factors that determine earthworm population aggregations may operate at different scales from those that determine soil physical–chemical properties (Whalen and Costa 2003). Rossi (2003) suggests that the scale and distribution of earthworm population aggregates may limit the utility of any general soil property indicators of earthworm distribution.

Additionally, total, net low C/N, and net high C/N stand basal area were not significant factors in explaining the distribution of earthworms at MSH. This is likely due to the low variance between plots: all plots were comprised of relatively mature stands of mixed hardwoods. As mentioned in the site descriptions, there was a qualitative difference in canopy composition with distance from the lake, in that transects at greater elevation tended to be dominated primarily by *A. saccharum*, a species with a low C/N ratio. How-

ever, earthworm populations were found at some of these sites, but not extensively, and were found in greater abundance at sites dominated by species with litter of a higher C/N ratio. This may be due to distributional effects as well as edaphic factors, confounding any site-specific assessment of the role of litter quality on worm distribution.

Earthworm abundance and biomass decrease exponentially with increasing distance from the lake, supporting the argument that the lake is the introduction area. Lac Hertel is the most heavily visited part of the park, and earthworms may have been introduced as cocoons in the boots of hikers and the treads of all-terrain vehicles (Langmaid 1964; Dymond et al. 1997). Additionally, they may have been introduced via bait release due to illegal fishing or prior to the ban. The exponential decline in abundance and biomass with distance from the lake is likely due to the overwhelming influence of continuing high propagule pressure at the heavily visited lake, rather than solely neighbourhood diffusion from a single introduction episode, which would result in a normal distribution around the introduction point. Earthworm species richness was also negatively correlated with distance from the lake, resulting from several factors. Firstly, propagule pressure may be greatest surrounding the lake, resulting in a higher probability of successful colonization for any introduced species (as seen by Frelich and Holdsworth (2002)). Secondly, there may be a distance partitioning effect caused by species-specific dispersal rates. Finally, the habitat surrounding the lake may be more suitable for certain species

and thus these species do not spread from this region, although this was controlled for in this study.

The species-specific results show that earthworms are partitioned in certain areas of MSH. For example, *L. terrestris* and *E. rosea* occur only in a narrow band surrounding Lac Hertel, suggesting that their present range is limited by dispersal and (or) habitat. *Lumbricus rubellus* is more broadly distributed, while *A. tuberculata* occurs over the entire range of distances. Interestingly, *D. octaedra* is confined to sites 500 m or further from the lake and is known to occupy mostly undisturbed, forested sites (Reynolds 1977). The areas surrounding Lac Hertel are subject to heavy human traffic and disruption. Furthermore, *D. octaedra* is a true epigeic species, which means that it inhabits solely the litter layer. As species like *L. terrestris* and *L. rubellus* invade, they consume the litter layer, essentially eating the habitat of *D. octaedra* out from under it. This habitat limitation could explain why one does not find *D. octaedra* in areas populated by these species (Fig. 2b) and why it is not found in the heavily invaded areas surrounding Lac Hertel.

Earthworms and soil C and N

This study indicates that exotic earthworms are a large and influential force in shaping the soils of MSH, with earthworms eliminating visible horizonation. Earthworm activity was strongly correlated with a decrease in the mass and depth and an increase in the C/N ratio of the leaf litter layer. The change in ratio is tied to earthworm feeding preference for material with a low C/N ratio (Lee 1985; Edwards and Bohlen 1996; Shakir and Dindal 1997; Lavelle et al. 1998; Bohlen et al. 2004b). The soil surface following earthworm invasion is often strewn with small woody debris and few leaves, suggesting that the “higher quality” leaf litter has already been consumed (Frelich and Holdsworth 2002; Bohlen et al. 2004b). At MSH, earthworm activity has changed the forest floor from a mor type to a duff mull.

In earthworm-occupied plots, the loss (or removal) of the surface litter layer was accompanied by an increase in the C and N concentrations of the mineral soil. The statistically significantly greater C and N concentrations at 10 and 20 cm depths suggest that earthworm invasion results in a mixing and homogenization of the litter layer and A and B horizons of the soil profile. This faunal activity results in a more moderate, less stratified gradation of C and N concentrations from the surface of the mineral profile to the lower horizons and may have implications for forest soil and plant communities. Our study indicates that earthworm invasion may result in an overall increased storage of soil C and N. The increase in C and N concentration at lower depths in the soil profile was accompanied by an increase in the total mass of C and N stored at 10–20 and 20–30 cm depths. No significant change in mass of C and N occurred in the upper 10 cm of the mineral soil, and this may be explained by the greater bulk density observed in the 0–10 cm horizon of the earthworm-invaded plots. A reduction in the magnitude of difference between earthworm-invaded and worm-free plots of 20% did not substantially alter the results. Yanai et al. (2003) suggested that changes of soil C greater than 20% in magnitude were necessary for any level of confidence in interpretation, owing to the high levels of spatial variability in soil C and N stocks. This sensitivity analysis shows that changes in stor-

age in the upper 20 cm of the soil profile are most sensitive to measurement error, but are still robust.

Whether exotic earthworm invasion leads to a decrease or an increase in total soil C and N has been raised many times (Alban and Berry 1994; Burtelow et al. 1998; Yanai et al. 2003; Bohlen et al. 2004b, 2004c). Alban and Berry (1994) found that earthworm invasion of a Minnesota forest over 14 years resulted in a 0.6 kg·m⁻², or 9%, decrease in C from the upper 50 cm of the soil, resulting from a decrease in the mass of the forest floor and an increase in C storage at the 0–10 cm depth (Table 5). Burtelow et al. (1998) reported a 36% decrease in organic matter concentration in the O horizon and a 9% increase in the A horizon of maple sites in New York State, but the absence of bulk density data precludes a conversion to changes in C mass. For two maple sites affected by earthworms, Bohlen et al. (2004b) noted a 90% and 33% decrease in forest-floor C, and an increase of 5% in 0–12 cm mineral soil, resulting in overall decreases of 0.6 and 0.0 kg·m⁻², or 28% and 0%, respectively (Table 5). Similar changes in forest floor, mineral soil, and overall soil were reported for N (Table 5). All these results show convincing evidence that earthworm invasion results in a significant loss of soil C from the soil and that N storage is not substantially affected by invasion. The Burtelow et al. (1998) and Bohlen et al. (2004b) studies are limited by the depth of sampling: 10 and 12 cm in the mineral soil, respectively. Our study shows no significant change in A horizon (0–10 cm) C storage, but an increase below this layer depth to 30 cm, the limit of visible evidence of earthworm activity (Table 4). Bohlen et al. (2004b) found no increase in total soil respiration at earthworm-invaded sites, despite a 28% decrease in litter and A horizon C storage. It is possible that much of the reported loss of soil C was actually redistributed into the mineral soil below 12 cm.

Large (48%–89%) declines in litter and forest-floor N mass with earthworm invasion are reported, with increases (7%–167%) in the upper mineral soil and profile changes from a loss of 7%, or 0.2 kg·m⁻², to an increase of 49%, or 0.33 kg·m⁻² (Table 5). In general, N loss is smaller than that of C at earthworm-affected sites. The preferential use by earthworms of litter with low C/N ratios and its transfer to lower layers may account for the increase in N mass at depth, relative to C (Table 5). While worms speed up decomposition, resulting in a loss of C from the upper layers of soil, the formation of stable organic matter – mineral complexes in the subsoil may override this effect, resulting in an increase in total soil C in the profile. It is likely that the initial effects of earthworm invasion (increased decomposition rates, consumption of the litter layer) cause a decrease in total soil C, while in the long term total soil C increases as stable organic matter – mineral complexes accumulate (Bohlen et al. 2004c). The uncertainty as to which of the two processes dominates is reflected throughout much of the literature and may also explain the unresolved question about whether earthworms increase or decrease soil respiration rates (Yanai et al. 2003; Bohlen et al. 2004b, 2004c).

While we chose sites in an effort to maximize similarity and minimize differences, earthworms are particularly adept at detecting small variations in soil conditions. As our study is comparative in nature and does not document soil changes before and after earthworm invasion, it is possible that there

Table 5. Collation of literature values on the effects of earthworm invasion (with or without earthworms) on litter and soil carbon (C) and (N) mass.

Property	Alban and Berry (1994)		Bohlen et al. (2004b) ^a				Present study	
	60-year-old birch–maple– aspen		Undisturbed maple ^a		Regrowth maple ^a		Undisturbed beech– maple	
	Without	With	Without	With	Without	With	Without	With
Litter and forest-floor C (kg·m ⁻²)	1.5	0.3	1.95	0.20	0.45	0.30	0.35	0.14
Litter and forest-floor N (kg·m ⁻²)	0.04	0.01	0.09	0.01	0.21	0.11	0.015	0.005
Soil C (kg·m ⁻²)	2.3 ^b	2.7 ^b	3.66 ^c	3.86 ^c	3.09 ^c	3.24 ^c	9.78 ^d	13.5 ^d
Soil N (kg·m ⁻²)	0.13 ^b	0.15 ^b	0.28 ^c	0.26 ^c	0.06 ^c	0.16 ^c	0.67 ^d	1.01 ^d
Total C (kg·m ⁻²)	6.5 ^e	5.9 ^e	5.61 ^c	4.06 ^c	3.54 ^c	3.54 ^c	10.1 ^d	13.7 ^d
Total N (kg·m ⁻²)	0.37 ^e	0.38 ^e	0.29 ^c	0.27 ^c	0.27 ^c	0.27 ^c	0.68 ^d	1.01 ^d

^aArnot Forest (undisturbed maple), Tompkins Farm (regrowth maple), and Bohlen et al. (2004b) found no difference in C and N concentrations at 25 and 35 cm, but did not include bulk density measurements, and thus mass differences could not be calculated.

^bMineral soil was sampled to 10 cm depth.

^cMineral soil was sampled to 12 cm depth.

^dMineral soil was sampled to 30 cm depth.

^eMineral soil was sampled to 50 cm depth.

were preexisting differences in soil conditions before earthworm invasion. The most likely confounding factor is soil moisture: wetter sites are likely to have greater amounts of soil C. Furthermore, the correlation between earthworm density and soil pH may also lead to naturally elevated levels of soil C, since calcium can control C stabilization (Paul et al. 2003). However, the sites all had similar acidic parent material, and pH ranged from 3.0 to 5.2, so that calcium levels should not be substantially different. Furthermore, these concerns do not compromise the general validity of this study, but rather highlight one of the difficulties in all earthworm studies in natural ecosystems — the high spatial variability of soil properties and processes may be a key factor in contributing to variability in results (Yanai et al. 2003). Future studies could reduce this problem through controlled introduction of earthworms into previously sampled plots or through sampling the leading edge of earthworm invasions (e.g., Hale et al. 2005a, 2005b); however, this will only provide a short-term picture of the effects of exotic earthworm invasion on soil C and N. Long-term monitoring, as well as comparative studies, will be helpful in illuminating the long-term and large-scale effects of exotic earthworm invasion into earthworm-free forests.

Conclusion

Our study has documented the occurrence of earthworms in an old-growth deciduous forest in southern Quebec, showing that earthworm distribution is primarily related to distance from a nearby lake, the presumed entry point. Soil and vegetation characteristics were less important in explaining the distribution. Our results confirm the dramatic decrease in the thickness and mass of the leaf litter at sites with large earthworm numbers or biomass, but the overall change in soil C and N storage associated with earthworms may be dependent on the depth to which the soil is sampled. We found significantly (3 and 0.3 kg·m⁻² of C and N, respectively) larger storage to 30 cm in earthworm-affected soils, whereas most previous estimates were restricted to the forest floor and the upper 10 cm of the mineral soil. The increased rates of litter decomposition and nutrient mobilization and redis-

tribution associated with earthworm invasion may influence forest ecosystem functioning (Hendrix and Bohlen 2002; Bohlen et al. 2004a, 2004c). Furthermore, the loss of the protective leaf litter may expose the soil to harsher microclimates and affect the viability of understory plants and fungal networks.

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