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Indirect and direct effects of exotic earthworms on soil nutrient and carbon pools in North American temperate forests

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ABSTRACT

Earthworm invasions in North American temperate forests cause considerable changes to soil and litter horizons, which can lead to changes in soil biogeochemistry and plant communities. These ecosystem changes have complex causal relationships, and the cascades of indirect effects from earthworm burrowing and feeding may have larger net effects on soil biogeochemistry than direct effects. In this study we partitioned the effects of earthworms on particular soil nutrient and carbon pools into direct and indirect effects. We defined direct effects as consisting of the association between the soil nutrient or carbon pool and earthworm biomass, whilst indirect effects included the impacts earthworms have on these soil pools through changing litter layer depth and soil chemical and biotic factors. We quantified these direct and indirect effects using a path analysis approach applied to data collected from 24 plots across an earthworm gradient in a northern hardwood forest stand in Ontario, Canada. As potential predictors, we measured earthworm functional group biomass, litter depth, microbial biomass, soil pH, texture, and organic matter. We related these predictors to extractable nutrient and carbon concentrations, including nitrate/nitrite, ammonium, orthophosphate, and dissolved organic nitrogen, phosphorus, and carbon. We found few direct effects and primarily indirect effects of earthworms on the soil nutrient and carbon pools we measured. Endogeic earthworms had the strongest indirect effects via changes to pH, microbial biomass carbon, and proportion of soil organic matter. Anecic earthworms only indirectly affected soil and nutrient pools through changes in pH, and epigeic earthworms did not have either direct or indirect effects. Because endogeic earthworms had indirect effects through changing multiple soil factors, for some soil and nutrient pools these indirect effects augmented each other (primarily yielding negative effects), although in some cases indirect positive effects mitigated negative effects. Overall, the net effects of exotic earthworms on soil and nutrient pools were mostly negative. Of particular concern was the potential exacerbation by endogeic earthworms of phosphorus limitation in N-saturated forest systems, as well as carbon loss from mineral soils in addition to losses from the forest floor.

1. Introduction

Invasive ecosystem engineers, such as exotic earthworms, have strong impacts on nutrient, trophic, and physical resources in soils (Crooks, 2002). Non-native earthworms are recognized as an emerging threat to temperate North American forest ecosystems, and are considered one of the most globally important agents of change to biodiversity and associated ecological and evolutionary processes (Sutherland et al., 2011). Earthworms change forest floor systems by rapidly consuming litter and homogenizing the organic and mineral layers of soil, leading to a gradual elimination of the litter layer (Langmaid, 1964; Alban and Berry, 1994). Earthworm invasion in forests has also been associated with changes to soil chemistry (Edwards and Bohlen, 1996), altered microbial communities and processes (Burtelow et al., 1998; Li et al., 2002; Groffman et al., 2004; McLean et al., 2006), shifts in microarthropod communities (McLean and Parkinson, 2000; Migge-Kleian et al., 2006; Straube et al., 2009) and shifts in nutrient and carbon pools and dynamics (Bohlen et al., 2004a; Suárez et al., 2004; Hale et al., 2005; Wironen and Moore, 2006; Costello and Lamberti, 2009). These changes to nutrient availability and the soil environment may contribute to observed changes to the plant community that are correlated with earthworms, including seed banks

(Hopfensperger et al., 2011), community composition (Hale et al., 2006; Holdsworth et al., 2007) and growth (Larson et al., 2010).

The multiple ecosystem changes observed with earthworm invasion have complex causal relationships. In the conceptual model presented by Bohlen et al. (2004b) earthworms cause three inter-dependent categories of changes: physical effects (e.g., changed soil and litter structure), geochemical effects (e.g., homogenized soil horizons), and biological effects (e.g., altered nutrient cycling, microbial communities). These changes lead to ecological consequences which include short-term carbon loss and changes to soil N and P availability. In this study we explored a model pathway of the links between edaphic changes due to earthworms and shifts in soil nutrient and carbon pools. We suggest that earthworm burrowing and litter consumption may directly change nutrient and carbon concentrations, and also indirectly affect these pools by altering litter layer depth, and soil pH, organic matter, texture, and microbial biomass (Fig. 1). Although previous studies have quantified the direct relationships between earthworms and soil nutrient and carbon pools (Bohlen et al., 2004b; Addison, 2009), and earthworms and pH, organic matter, and microbial biomass (Haimi and Huhta, 1990; Schrader, 1994; Edwards and Bohlen, 1996), no studies, to our knowledge, have partitioned the effects of earthworms into direct and indirect components.

The specification and testing of models that include direct and indirect effects is particularly valuable for understanding ecosystem consequences of invasive species (Gandhi and Herms, 2010; Weidenhamer and Callaway, 2010). Cascades of indirect effects from invasive species potentially cause larger and more persistent ecosystem changes than direct effects (e.g., Peltzer et al., 2010). Studies that quantify only direct effects (e.g., direct relationship between available nutrients and earthworm biomass) may underestimate the total earthworm effects on a response variable, which may be also affected indirectly via changes to other soil factors. Depending on the magnitude and direction of indirect effects they can either augment or mitigate other direct and indirect impacts of the invasive (Eubanks, 2001; Atwater et al., 2011).

One strategy that can be used to explore indirect and direct effects is path analysis, and this technique has been used to quantify the relative importance of the indirect and direct effects of predictor variables on response variables in many ecosystems (Mitchell, 1992; Wootton, 1994; Bakker et al., 2003; Vogel et al.,

Fig. 1. Direct and indirect pathways by which earthworm functional groups may alter soil nutrient and carbon pools. Black solid arrows indicate direct effects, while gray dashed arrows indicate indirect effects via changes to soil or litter factors.

2010), including the impacts from invasive species (Eubanks, 2001; Atwater et al., 2011). Path analysis is similar to multiple regression, as it is based on the analysis of correlations, but unlike multiple regression, it allows the partitioning the effects of predictor variables on a response variable into direct and indirect components (Fig. 1). The relative magnitude of these components can be calculated, and the total effect of a predictor on a response variable consists of the sum of direct and indirect effects (Quinn and Keough, 2002). Because path analysis uses correlations to calculate path coefficients, this method can only test how the data fit the proposed causal pathways, and not prove causality.

In this study we focus on the earthworm invasion of a northern temperate hardwood forest with acidic and nutrient-poor soils and quantify the direct and indirect (through changes in litter layer depth, pH, soil organic matter, soil texture, and microbial biomass) effects of earthworms in different functional groups (Edwards and Bohlen, 1996) on soil nutrient and carbon pools.

2. Materials and methods

2.1. Study site and sampling design

The study was conducted in 2010 at Haliburton Forest and Wild Life Reserve (HF), a mixed-use forest in eastern Ontario, Canada (45.29 N, −78.64 W) with no native earthworm species. Haliburton Forest is dominated by sugar maple (*Acer saccharum* Marshall), red maple (*Acer rubrum L.*), eastern hemlock (*Tsuga canadensis (L.) Carrière*), yellow birch (*Betula alleghaniensis* Britton), and American beech (*Fagus grandifolia* Ehrh.). This area receives ∼1100 mm of precipitation per year, and has a mean annual temperature of 4.9 °C. Soils, generally in the Dystric Brunisols great group (Soil Classification Working Group, 1998), have low pH (range: 4.2–5.1 (Gradowski and Thomas, 2006)) and base saturation and a sandy to sandy-loam texture overlaying granite bedrock. Although we do not know the exact dates of earthworm introduction at this site, earthworms were widespread across this rural county in the 1970s (Reynolds, 1972), and our site is within the main settlement area of Haliburton Forest which has been in constant use since its initial settlement as a farm in the 1870s, with subsequent use as a base for logging and recreation. We therefore suspect that earthworms have been invading this forest stand for decades. The study was conducted in a forest stand adjacent to two horse pastures near to the original settlement. The stand was primarily maple (80% by basal area) interspersed with hemlock and beech (each 9% basal area), and isolated black cherry (*Prunus serotina* Ehrh.) and yellow and white birch (*Betula papyrifera* Marsh.). This site was selected because there was a visible gradient of earthworm invasion in the forest from the sides closest to the pastures and continuing into the forest, providing us with an earthworm abundance gradient from high to low (zero) densities. In the forest, beginning 100 m and 15 m from the pastures (a distance beyond the transitional zone between pasture and forest), we set up 7 transects, 50 m apart, which ran perpendicular to the nearest pasture. Along each transect we sampled every 50 m until earthworms were not present at 2 consecutive sampling points, at which point we stopped extending the transect. To delineate the spread front more accurately we added an additional sample point on the transect 25 m beyond the final sample where earthworms were found. In total we sampled 26 points. A map of the site is provided in Appendix 1.

2.2. Earthworm sampling, identification, and mapping

We chose to sample earthworms in August (Haliburton, ON, August climate data 1971–2000: mean daily temperature: 17.8 °C, daily minimum–daily maximum 1971–2000: 12.2–23.4 °C, mean
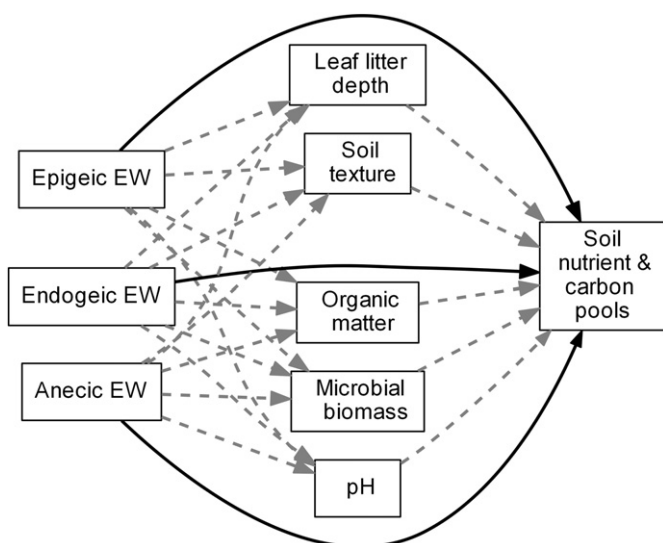
rainfall: 85.5 mm (Environment Canada, 2011)), because we observed the proportion of adults to juveniles at our site increases in late summer and early fall. On 14–19 August 2011, once at each sampling point, we collected earthworms using mustard liquid extraction (Lawrence and Bowers, 2002) from three 25 × 25 cm sub-plots within 2 m of the sample point at randomly selected cardinal directions. We first removed leaf litter from the sub-plot, searching the litter for earthworms, and then applied 2 L of mustard solution to the sub-plot (10 g of Colman's mustard powder (Unilever PLC, London UK) per L water) over a period of 10 min, collecting any emerging earthworms. This was followed by hand-searching of the sub-plot soil for remaining earthworms. Earthworms were immediately placed in a 75% isopropyl alcohol solution, transferred to a 10% formalin solution for 24–48 h for fixation, and then stored in 75% isopropyl alcohol. Biomass of earthworms was determined using preserved earthworms – earthworms were removed from the alcohol solution, dried with paper towel, and weighed. Earthworms were identified to genus, and if possible, species, using the key from Reynolds (1977). Voucher specimens were deposited in the invertebrate collection at the Royal Ontario Museum.

Earthworms were divided into three groups based on taxonomic and functional characteristics (Edwards and Bohlen, 1996): (1) Epigeic earthworms (*Dendrobaena octaedra* and *Dendrodrilus rubidus*); (2) Epi-endogeic/endogeic soil-dwelling earthworms (*Lumbricus rubellus* (epi-endogeic), *Aporrectodea rosea*, and the *Aporrectodea calignosa* complex (endogeic)): species within the *A. calignosa* complex are difficult to differentiate using morphological traits (Pérez-Losada et al., 2009); (3) Anecic soil-dwelling earthworms (*Lumbricus terrestris*): we included the immature *Lumbricus* spp. in the anecic grouping because *L. rubellus* was a very small component of the adult *Lumbricus* assemblage (3% of total biomass) compared to that of *L. terrestris* (97%) (Table 1); we predicted that a similar biomass ratio occurred in the immature pool of earthworms. Although this pooling of species has the disadvantage of obscuring the relative contribution of different species to observed effects (Hale et al., 2005), we felt this disadvantage was offset by the reduction in the number of different earthworm groups used as predictors and resulting simplification of the analytical models.

We tested for a significant gradient in earthworm biomass with distance from the disturbed areas (pastures) by regressing total earthworm biomass against the distance of each sampling point from the corner of the grid nearest to the two pastures (map in Appendix 1).

2.3. Soil sampling and laboratory analyses

2.3.1. Soil and litter sampling

Within 0.5–1 m of each of the three worm sampling quadrats we measured the depth of the LFH horizon in three locations and collected three 100 cm^2 soil sub-samples to a depth of 15 cm. Soils were separated into three horizons: 0–5 cm (roughly corresponding to the Ah horizon), 5–10 cm, and 10–15 cm, and frozen within 8 h of collection. Because we only sampled the soils on one date, our data reflect a snapshot of soil conditions and many of the factors we measure (e.g., nutrients, DOC, microbial biomass) will vary over the short-term, although we are interpreting these data in the context of a longer term response to earthworm presence.

2.3.2. Soil texture, pH, and organic matter

After thawing, soils were sieved using a 2 mm sieve to remove pebbles and large roots. The distributions of soil particle sizes in the third horizon (10–15 cm) of the samples (sand: 2 mm–50 mm; silt: 50–2 μm; clay: 0–2 μm) were determined using an adapted pipette method (Kroetsch and Wang, 2008) that uses sedimentation rates based on Stoke's law. Organic matter content (of all three horizons for each soil sample) was measured as loss-on-ignition from samples put in a muffle furnace for 4 h at 550 °C.

2.3.3. Extraction and measurement of nutrients/carbon from soil

For the three soil horizons from each sample point, approximately 10 g of soil (field moisture) were extracted with 75 mL of 0.5 M K_2SO_4 and after shaking the samples for 1 h they were filtered with 0.45 μm pore size glass fiber filters (Machery-Nagel, Düren Germany). pH was recorded from the filtered extracts; pH values from K_2SO_4 extracts are lower than those in water, but have a strong linear relationship (pH in water = 1.32 × extractable pH + 0.71; $r^2 = 1.0$; N. Basiliko pers. obs.). We measured the concentrations of six nutrient and carbon pools from the soil extracts: nitrate (NO_3^-), ammonium (NH_4^+), orthophosphate (PO_4^{3-}), and dissolved organic nitrogen, carbon and phosphorus (DON, DOC, DOP). Dissolved inorganic N (NH_4^+ and NO_3^-) and inorganic P (PO_4^{3-}) concentrations in the un-fumigated extracts were measured colorimetrically on a Lachat QuickChem 8500 flow injection analyzer (Lachat Instruments, Milwaukee, WI, USA) using Quik-Chem methods 12-107-06-2-A, 10-107-06-2-C, and 10-115-01-1-A. Total amounts of dissolved organic C and N in fumigated and un-fumigated sample extracts were measured with a Shimadzu 5050 TOC/TN analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). Total dissolved organic P was determined through the potassium persulphate oxidation (Williams et al., 1995) of soil extracts, followed by measurement of PO_4^{3-} in the Lachat flow injection analyzer.

2.3.4. Microbial biomass

For the measurement of microbial biomass carbon, another 10 g of soil from each sample was fumigated with chloroform and then extracted as for un-fumigated samples. The microbial pool of C was calculated by subtracting the total amount of C in the un-fumigated sample extracts from the amount in the fumigated sample extracts.

2.4. Statistical analyses

2.4.1. Data checking and transformation

Path analysis, like multiple regression, assumes data residuals are normally distributed, thus data were checked for normality and transformed if necessary. The transformations used for each variable are presented in Appendix 1. During data checking, measurements from multiple variables from two of the 26 sites were found to be extreme outliers (Bonferonni outlier test $P < 0.001$). These sites, which were in a rocky, low-lying area, had thick (>15 cm) A horizons primarily composed of decaying hemlock needles, which likely accounted for their unusual measurements. Data from these sites were removed from the analyses leaving data from 24 sites.

Table 1
Earthworm species, analytical groups, and mean density and biomass in 2010 collections from a forest stand at Haliburton Forest, ON. See Sub-section 2.2 for details on analytical groupings.

Earthworm species	Group for analysis	Mean abundance m^{-2} (standard deviation)	Mean biomass g m^{-2} (standard deviation)
Dendrobaena octaedra	Epigeic	23.5 (43.0)	0.68 (1.18)
Dendrodrilus rubidus	Epigeic	19.8 (43.9)	0.73 (1.77)
Aporrectodea rosea	Endogeic	1.2 (6.5)	0.07 (0.33)
Aporrectodea spp.	Endogeic	30.9 (42.0)	3.62 (4.34)
Lumbricus rubellus	Endogeic	1.3 (3.6)	0.55 (1.89)
Lumbricus terrestris	Anecic	6.4 (9.0)	17.84 (26.86)
Lumbricus spp. (immature)	Anecic	16.4 (19.4)	5.82 (6.96)

2.4.2. Analysis of horizon and organic matter changes associated with earthworm biomass

The depths of the LFH and Ah horizons were related to endogeic, anecic, and epigeic earthworm biomass using linear regressions. The relationship between earthworm biomass and soil organic matter in the three soil horizons was tested using multiple linear regression; we also tested for an interaction between earthworm biomass and horizon depth to determine if earthworms were translocating organic matter down through the soil profile. Linear models were tested using the lm function in the R package {stats} (R Development Core Team, 2010).

2.4.3. Path analyses

In addition to soil horizon depth and earthworm biomass (endogeic, anecic, and epigeic) there were five predictors that we hypothesized would be important in predicting soil nutrient and carbon pools: proportion of organic matter, pH, soil texture (i.e. proportion of sand), average depth of the LFH horizon, and microbial biomass carbon. Soil moisture is also an important predictor, but sporadic showers during the sample collections may have biased the soil-moisture measurements so we did not include them. Because some of the predictors are ecologically (and statistically) correlated, we used path analysis to determine significant direct predictors of nutrient and carbon pools, as well as indirect pathways by which earthworms affect the response variables via changes to soil factors. Path analysis is a simple type of structural equation modeling, which in which more advanced analyses can incorporate latent, or unmeasured variables (Mitchell, 1992; Quinn and Keough, 2002).

Our *a priori* path analysis models included the three earthworm functional groups as predictors of the measured response variables, in addition to LFH depth, pH, organic matter, soil texture (proportion sand), and microbial biomass carbon. The subsequent development of the path analysis models was exploratory (Petraitis et al., 1996) because we did not have *a priori* hypotheses on the relative significance or magnitude of potential predictors, but rather used the observed data to determine these relationships between the pathways and predictors, including the *a posteriori* trimming of non-significant pathways from the initial model. The initial path analysis models for each nutrient/carbon pool were fully identified, including all possible causal links between observed predictors and the response variable (nutrients/DOC), and all correlations among predictors: these models provided estimates and significance tests for all potential paths among variables. We were primarily interested in the potential direct and indirect effects of the earthworm groups (anecic, endogeic, and epigeic), in addition to direct effects due to other predictor variables. Thus, we trimmed the initial models by retaining only significant direct predictors of the response variable from the non-earthworm predictors of litter depth, organic matter, microbial biomass C, pH, or sand. For the earthworm group predictors, we retained any of the three earthworm groups that were significantly correlated with the direct non-earthworm predictors of the response variable (thus allowing us to calculate the indirect effects of the earthworm group via the non-earthworm predictor variable). Significance and goodness-of-fit of the trimmed models were assessed using three indices: a χ^2 test (note that a non-significant χ^2 statistic is desired, as it indicates that the observed data is not-significantly different from the proposed model), Bentler's comparative fit index (CFI), which is recommended for small samples and where a value of >0.95 indicates a good fit (Byrne, 1998), and the standardized root mean residual (SRMR), where a value below 0.08 indicates a good fit. We constructed path diagrams using standardized path coefficients (β) between the predictors and response variable, and correlation coefficients (r) between predictors. We also included a path for error variance (ε) to the response variable not measured by our predictors. The indirect effects of earthworms through other predictors were calculated by multiplying the value of the correlation between the earthworm variable and the non-earthworm predictor variable (r) with the value of the path coefficient (β) between the non-earthworm predictor and the response variable. The SEM function in the R package {sem} was used to calculate path coefficients and correlations (R Development Core Team, 2010).

Although we hypothesized that relationships between earthworms and the other predictors yield indirect effects on soil biogeochemistry, we could not determine cause and effect relationships between earthworms and edaphic factors such as pH and organic matter in this observational study, because earthworm communities and populations can themselves be affected by these same factors (Edwards and Bohlen, 1996). In addition, our relatively small sample size ($n = 24$) could decrease the precision of the path coefficients, particularly in models with higher numbers of pathways (Petraitis et al., 1996).

3. Results

3.1. Earthworm communities and relationship to forest floor and soil horizon depths

We collected 440 earthworms from six species (Table 1). Density and biomass ranged from 0 to 298 individuals/m^2 and 0 to 144 g/m^2, respectively. A gradient was observed across the site, with the highest earthworm biomass at the corner of the grid nearest to the two pastures, and significantly decreasing densities with increasing distance from this corner ($F_{1,22} = 38.4$, $R^2 = 0.62$, $P < 0.001$).

Epigeic and anecic earthworm biomasses were negatively associated with depth of the LFH layer, although anecic more strongly so (Epigeic: $F_{1,22} = 4.34$, $R^2 = 0.13$, $P = 0.05$; Anecic: $F_{1,22} = 25.3$, $R^2 = 0.51$, $P < 0.001$). Endogeic and anecic biomasses were positively associated with the depth of the Ah horizon (Endogeic: $F_{1,22} = 6.3$, $R^2 = 0.19$, $P = 0.02$; Anecic: $F_{1,22} = 7.0$, $R^2 = 0.21$, $P = 0.015$). Proportion of organic matter was negatively predicted by horizon depth and endogeic earthworm biomass ($F_{3,68} = 22.0$; adjusted $R^2 = 0.47$, $P < 0.001$), but there was a significant positive interaction between horizon and earthworm biomass. Separate linear regressions of organic matter and endogeic earthworm biomass in each horizon indicated the interaction was due to a significant negative relationship between earthworm biomass and organic matter in the 0–5 cm mineral soil horizon ($F_{1,22} = 7.53$, adjusted $R^2 = 0.22$, $P = 0.012$), but no significant relationship in lower horizons ($P > 0.05$). Therefore there was no detectable earthworm translocation of organic matter to lower horizons.

3.2. Path analyses

We present results for the surface (0–5 cm) mineral horizon data only (summary statistics in Table 2). Correlations among earthworms and predictor variables were weak or non-significant in the lower two horizons (data not shown), indicating that at our site, the effects of earthworms were confined to the top (Ah) horizon.

After model trimming, path analyses of the 0–5 cm horizon data yielded significant models for all response variables (Table 3). We did not find many direct effects of earthworms, but endogeic, and in some cases anecic, earthworms had significant indirect effects on soil nutrients and carbon via relationships with pH, organic matter, and microbial biomass carbon (Figs. 2 and 3). Sand was a significant negative direct predictor for NO$_3^-$ concentrations (Fig. 2), and because it was not associated with earthworm biomass there were

Table 2
Summary of soil and litter measurements in 2010 samples from Haliburton Forest. Units for the components of nitrogen, phosphorus, and carbon pools are all in $\mu g \, g^{-1}$ dry soil. Abbreviations are defined in Section 2.3.3.

Soil/litter measurement	Mean	Standard deviation
LFH (cm)	2.7	1.5
Ah (cm)	7.2	4.1
pH (K_2SO_4)	4.5	0.6
Microbial biomass carbon	492	269
Proportion organic matter	0.27	0.14
Proportion sand	0.72	0.10
NO_3^-	1.2	2.5
NH_4^+	64.1	21.0
PO_4^{3-}	6.9	14.5
DON	94.2	52.7
DOP	9.5	9.6
DOC	706	438

no indirect effects of earthworms via sand. The average depth of the forest floor (LFH horizon) was not a significant predictor in any models, and there were no significant indirect or direct effects of epigeic earthworms. The raw data and correlation matrix (of transformed variables) used in the path analyses are presented in Appendix 1.

Organic matter consistently had a positive relationship with nutrient and carbon concentrations, and because endogeic earthworm biomass was associated with a decrease in organic matter, there was a negative indirect effect of endogeic earthworms on NH_4^+ and the extractable dissolved organic matter components (DON, DOP, DOC). pH was positively associated with NO_3^- and NH_4^+, and negatively associated with PO_4^{3-}, DON, and DOC. As a result, the endogeic and anecic associated increases in soil pH yielded both negative and positive indirect effects on nutrient and carbon pools. Microbial biomass carbon was positively associated with NH_4^+ and PO_4^{3-} concentrations, and negatively associated with DOC concentrations. The endogeic associated decrease in microbial biomass carbon therefore also yielded both negative and positive indirect effects on nutrient and carbon pools.

4. Discussion

Our path analyses suggest that the indirect effects of exotic earthworms via changes to abiotic and biotic soil parameters are larger and affect more nutrient and carbon pools than do direct effects.

4.1. Earthworm functional groups

We observed that different earthworm functional groups had different effects on the soil nutrient and carbon pools we measured. Endogeic earthworm biomass was associated with more effects than anecic earthworm biomass. Epigeic earthworm biomass,
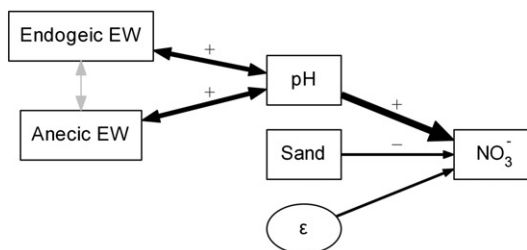
Table 3
χ^2 values and significance, and Bentler CFI and RSMR goodness-of-fit indices of final path analysis models for predicting concentrations of inorganic nutrients (NO_3^-, NH_4^+, PO_4^{3-}) and dissolved organic matter (DON, DOP, DOC) extracted from the 0–5 cm mineral soil horizon at an earthworm invaded forest stand at Haliburton Forest. Abbreviations are defined in Section 2.3.3.

Path analysis model	χ^2; df; P	Bentler CFI	RSMR
NO_3^-	7.17; 5; 0.21	0.954	0.099
NH_4^+	0.99; 2; 0.61	1	0.015
PO_4^{3-}	0.99; 1; 0.32	1	0.012
DON	1.54; 2; 0.46	1	0.023
DOP	1.95; 2; 0.38	1	0.041
DOC	3.88; 2, 0.14	0.98	0.031

despite its influence on litter layer depth, was not associated with changes to soil nutrients and carbon. Differences in the ecological effects of earthworm functional groups is not unexpected considering the different feeding and burrowing behaviors of species in these groups. Hale et al. (2005), by comparing samples from soils with different population densities of earthworm functional groups, similarly found that endogeic species were most highly associated with changes to organic matter. However, they found that nutrient concentrations changed the most in soils that included *L. terrestris* in addition to endogeic and epigeic species, as opposed to soils with endogeic and epigeic species alone. The importance of endogeic species at our site can be attributed to the changes to pH, organic matter, and microbial biomass carbon associated with endogeic biomass, whereas anecic earthworm biomass was only associated with changes to pH.

4.2. Direct and indirect effects of earthworms

In general, the total effects of endogeic and anecic earthworms on soil nutrient and carbon pools were negative, although earthworm biomass was positively associated with NO_3^- (endogeic and anecic) and NH_4^+ (anecic only) concentrations. The partitioning of the total effects into direct and indirect effects provides insight into the mechanisms of earthworm changes to nutrient and carbon pools through shifts in organic matter, pH, and microbial biomass carbon.

The only direct effect of earthworms found in our path analysis models was the negative effect of endogeic earthworms on PO_4^{3-}. Earthworm burrowing and feeding behavior may lead to an increase in PO_4^{3-} fixation to aluminium and iron hydroxides or leaching (Suárez et al., 2004). Plant uptake of phosphorus may also affect PO_4^{3-} pools more than nitrogen pools: phosphorus, not nitrogen, is the limiting nutrient for sugar maple at the site (Gradowski and Thomas, 2008), and fine root production by trees at Haliburton Forest is strongly negatively related to soil phosphorus availability (Peng and Thomas, 2010).

Strong negative indirect effects on soil concentrations of NH_4^+ and the three components of DOM (DOC, DON, and DOP) were related to decreases in organic matter associated with endogeic earthworm biomass. Both soil organic matter and leaf litter are sources of nutrients and DOC, but we found soil organic matter was more important because there was no significant relationship between leaf litter depth and these variables. Leaf litter may be a more important source of DOM directly after leaf fall (Park et al., 2002), whereas we collected our samples in late August. Increases in mineral soil organic matter due to the incorporation of large amounts of forest floor materials into the soil is seen in early stages of invasion (Alban and Berry, 1994; Hale et al., 2005) and at sites with greater masses of forest floor (Bohlen et al., 2004a). However, we observed a decrease in mineral soil organic matter, suggesting increased mineralization and perhaps a longer history of invasion. Although it is thought that earthworms increase the stability of soil carbon in the long term (Scheu and Wolters, 1991), our results imply a loss of total soil carbon from the mineral soil, and not only from the LFH layer (Alban and Berry, 1994; Bohlen et al., 2004a).

The negative association of earthworms with organic matter may increase competition for soil carbon between earthworms and the microbial community, producing the negative relationship we observed between endogeic earthworm biomass and microbial biomass C. Thus, the indirect effects of earthworms on NH_4^+, PO_4^{3-}, and DOC may be caused by earthworms reducing microbial mineralization of organic matter and microbial consumption of DOC. Both increases and decreases in microbial biomass have been observed in forest mineral soils in response to earthworms (Li et al., 2002; Scheu et al., 2002; Groffman et al., 2004; Eisenhauer et al.,

a Path diagrams

b Direct and indirect effects

(i) NO_3^-

Earthworm group	Direct effects	Indirect effects via pH	Total effects
Endogeic	0	0.41	0.41
Anecic	0	0.42	0.42

(ii) NH_4^+
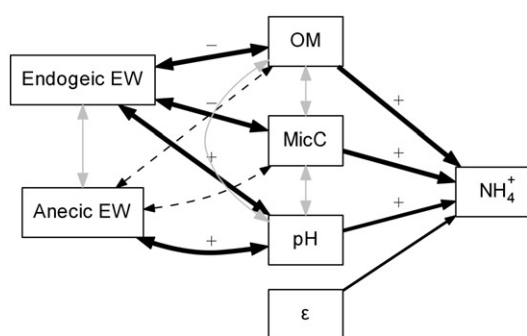
Earthworm group	Direct effects	Indirect effects			Total effects
		via OM	via MicC	via pH	
Endogeic	0	-0.29	-0.32	0.23	-0.38
Anecic	0	0	0	0.23	0.23

(iii) PO_4^{3-}
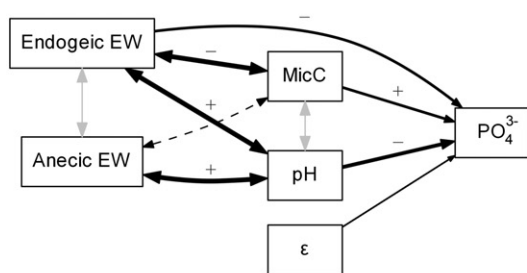
Earthworm group	Direct effects	Indirect effects		Total effects
		via MicC	via pH	
Endogeic	-0.34	-0.21	-0.23	-0.78
Anecic	0	0	-0.23	-0.23

Fig. 2. (a) Path analysis diagrams and (b) direct and indirect effects of earthworms for path analysis models of earthworm biomass and soil predictors on inorganic nutrients (i) NO_3^-, (ii) NH_4^+, and (iii) PO_4^{3-} in soils from Haliburton Forest. In diagrams, single-headed arrows represent the path coefficients, double-headed arrows are correlations among the predictors. Black solid arrows indicate significant relationships with arrow thickness representing the magnitude of the estimate and the sign (+ or −) above the arrow indicating the direction. Black dotted arrows indicate non-significant relationships. Gray arrows between predictors indicate that the correlations were included in the model, but were not used to calculate indirect effects, and ε represents error variance. In tables, direct effects are equal to path coefficients (β) and indirect effects are calculated as $\beta \times r$ (correlation coefficients) as described in Subsection 2.4.3. Abbreviations as in Subsection 2.3.3.

2007, 2011), and differences are likely due to multiple factors including earthworm functional group, time since invasion, and available soil carbon (McLean et al., 2006; Huang et al., 2010). However, a decrease in microbial biomass C may not necessarily reflect decreases in rates of all microbial processes, and may actually reflect a smaller but more active microbial community (McLean and Parkinson, 1997; McLean et al., 2006).

The indirect effects of endogeic and anecic earthworms on nutrient and carbon pools via changes to pH were complex, as there were positive indirect effects on NO_3^- and NH_4^+, but negative indirect effects on PO_4^{3-} and dissolved organic nitrogen and carbon. Soil pH may have been highly correlated with variability in nutrient and carbon concentrations because the soils are normally acidic at our site (pH = 4.2–5.1; (Gradowski and Thomas, 2006)), so changes in pH could markedly affect microbial communities and processes (Rousk et al., 2009; Dempsey et al., 2011). Fungal microbial communities are more active than bacterial at low pH values (Blagodatskaya and Anderson, 1998;

a Path diagrams

b Direct and indirect effects

(i) DON

Earthworm group	Direct effects	Indirect effects		Total effects
		via OM	via pH	
Endogeic	0	-0.33	-0.21	-0.54
Anecic	0	0	-0.21	-0.21

(ii) DOP

Earthworm group	Direct effects	Indirect effects via OM	Total effects
Endogeic	0	-0.39	-0.39
Anecic	0	0	0

(iii) DOC

Earthworm group	Direct effects	Indirect effects			Total effects
		via OM	via MicC	via pH	
Endogeic	0	-0.37	0.18	-0.28	-0.47
Anecic	0	0	0	-0.28	-0.28

Fig. 3. (a) Path analysis diagrams and (b) direct and indirect effects of earthworms for path analysis models of earthworm biomass (endogeic and anecic) and soil predictors on dissolved organic matter (i) DON, (ii) DOP, and (iii) DOC in soils from Haliburton Forest. In diagrams, single-headed arrows represent the path coefficients, double-headed arrows are correlations among the predictors. Black solid arrows indicate significant relationships with arrow thickness representing the magnitude of the estimate and the sign (+ or −) above the arrow indicating the direction. Black dotted arrows indicate non-significant relationships. Gray arrows between predictors indicate that the correlations were included in the model, but were not used to calculate indirect effects, and ε represents error variance. In tables, direct effects are equal to path coefficients (β) and indirect effects are calculated as $\beta \times r$ (correlation coefficients) as described in Subsection 2.4.3. Abbreviations as in Subsection 2.3.3.

Bååth and Anderson, 2003) and fungi are the most important decomposer group for DOM production (Kalbitz et al., 2000). Soil microbial communities involved in nitrification and mineralization can also be sensitive to variations in pH, with increased pH leading to increased rates (Nyborg, 1978; Högberg et al., 2007). Szlavecz et al. (2006) also found N-cycling rates in invaded forests were more correlated with pH than with earthworm biomass. We observed a negative effect of pH on PO_4^{3-}, although increasing pH is normally thought to increase PO_4^{3-} solubility. However, a decrease in phosphate availability with liming has been frequently observed, and may be due to adsorption of PO_4^{3-} to polymeric hydroxyl-Al precipitates formed in acidic soils with increases in pH (Haynes, 1982).

In some cases, indirect effects of earthworms worked in opposing directions, reducing the total effect of the earthworm group on the nutrient or carbon pool. For example, endogeic earthworm associated increases in pH had a positive effect on NH_4^+, offsetting a certain amount of the negative indirect effects via decreases to organic matter and microbial biomass carbon. The positive indirect effect via pH did not completely mitigate the negative indirect effects however, and the total effect of endogeic earthworms on NH_4^+ was negative. The partitioning of earthworm effects into the direct and indirect components allows for an understanding of nutrient and carbon pool shifts and their potential mechanisms that may be obscured when earthworm biomass and response variables are modeled directly.

Although in this study we interpret relationships between earthworms, soil factors, and nutrient and carbon pools as resulting from the effects of earthworms, and not *vice versa*, experimental manipulations are needed for causal evidence of our hypotheses. In addition, a large component of the direct effects of earthworms (and the error variances) in our models will be due to predictors not measured in this study. In particular, the effect of earthworms on microbial community composition and activity is of great importance in understanding indirect effects of earthworms on nutrient pools and carbon sequestration, and forest community composition (Dempsey et al., 2011).

4.3. Conclusions

Nutrient availability in forest soils is influenced by the relative strength of a network of biological and geochemical sinks and sources (Attiwill and Adams, 1993). Quantifying the indirect effects of earthworms via edaphic factors and microbial biomass can provide a better understanding of the mechanisms behind earthworm-induced changes to soil biogeochemistry. Here we found that pH, organic matter, and microbial biomass are important intermediaries of endogeic and anecic earthworm effects, particularly in nutrient poor, acidic forests. We found that endogeic or anecic earthworm biomasses were negatively associated with all nutrient and carbon pools measured, excepting NO_3^-, which had very low concentrations and probably plays a relatively minor role in N cycling. Negative earthworm associations with PO_4^{3-} are of particular concern, as high rates of N deposition over the last half-century in eastern North America may be shifting some forest areas from N-limitation to P-limitation (Casson et al., 2011), including forests in our area (Gradowski and Thomas, 2006). Additionally, we found an earthworm associated loss of organic matter and dissolved organic carbon in the mineral soil, not just the forest floor horizons. Alban and Berry (1994) estimated carbon losses of 0.5 Mg/ha/year due to earthworm elimination of the forest floor; additional carbon loss from mineral soil horizons could yield higher numbers. Including measurements of indirect effects of earthworms may provide a more precise picture of the impacts of earthworms in forest soils, and may help explain inconsistent patterns associated with earthworms at different sites.

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Appendix 1. Supplementary material

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.soilbio.2012.08.015.

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