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Effects of tree leaf litter, deer fecal pellets, and soil properties on growth of an introduced earthworm (*Lumbricus terrestris*): Implications for invasion dynamics

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ABSTRACT

Invasive earthworm communities are expanding into previously earthworm-free forests of North America, producing profound ecosystem changes. *Lumbricus terrestris* is an invasive anecic earthworm that consumes a large portion of the detritus on the soil surface, eliminating forest floor organic horizons and reducing soil organic matter. Two mesocosm experiments were used to examine the individual and combined effects of litter and soil type on the growth of *L. terrestris*. The litter type experiment tested nine different food source treatments (7 tree leaf litters, deer fecal pellets, and a control), while the soil × litter type experiment used five different soil treatments (4 soil types and one soil type with A horizon material removed) in combination with four different food source treatments. We found that leaf litter type ($p = 0.001$) and soil type ($p = 0.018$) significantly affected earthworm growth rates, with growth rates on deer pellets similar to many high quality deciduous leaf litters. Of soil variables, exchangeable Ca ($r^2 = 0.99$), sum base cations ($r^2 = 0.98$), % organic matter ($r^2 = 0.93$), %N ($r^2 = 0.89$), %C ($r^2 = 0.87$), and exchangeable Mg ($r^2 = 0.85$) were all significant predictors of earthworm growth. Litter disappearance of all litter types was linearly related to growth, suggesting similar growth efficiency on different litter types. However, chemical properties, specifically foliar C:N and a linear combination of a suite of other chemical properties predicted growth, suggesting that consumption or gut passage rates were regulated by litter quality. This information about soil and litter characteristics that regulate *L. terrestris* growth should improve models of their distribution, spread and abundance.

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1. Introduction

North American temperate forests of the northern Great Lakes Region have had no native earthworm populations since the last glacial period and are being invaded by exotic Eurasian earthworm species (James and Hendrix, 2004; Hale et al., 2005). The most dramatic effects of exotic earthworm invasions on soil processes have been in areas previously devoid of earthworms (Hendrix and Bohlen, 2002). It is important to understand controls on exotic species invasion, as they are capable of transforming entire ecosystems with long-term biological impacts (Rooney et al., 2004). As 'ecosystem engineers' earthworms can modify their environment (Jones et al., 1994) by substantially altering forest soils, nutrient storage and availability, and greatly affecting populations and communities of other forest soil inhabitants (Bohlen et al., 2004). Our understanding of the factors controlling earthworm invasion is imperfect; however, litter type and soil type are two factors likely to influence earthworm invasion.

Earthworm communities rely on organic matter food bases, which are vitally important in determining their distribution and abundance. The invasive anecic earthworm *Lumbricus terrestris* has a diet consisting of leaf litter and other organic debris (Reynolds, 1977). Characteristics such as litter quality and palatability influence feeding behavior and/or alter litter disappearance rates (Brown et al., 2000; Suárez et al., 2006). Previous studies have demonstrated the correlation of food quality (%N, tannin content, C:N ratio) with litter selection by *L. terrestris* (Satchell and Lowe, 1967; Zicsi, 1983; Hendriksen, 1990). Earthworm growth has been found to be higher on lower C:N litter types, and lower polyphenolics, and may be negatively affected by litter lignin content (e.g., Shipitalo et al., 1988; Kasurinen et al., 2007), although support

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for the latter is inconsistent (e.g., Meehan et al., 2010). Earthworm abundance and litter consumption rate has also been associated with leaf litter high in calcium (Ca; Reich et al., 2005; Holdsworth et al., 2012), suggesting that litter Ca could be positively related to earthworm growth. Yet we lack direct tests of the chemical properties of forest tree litters that regulate earthworm growth.

In addition to the analyses described above, other metrics of refractory or labile components of leaf water extracts could inform our understanding of the regulation of earthworm growth, e.g., extractable C and N, absorbance of specific wavelengths of light indicative of aromaticity or size of DOC, and excitation and emission spectra of leaf extracts indicative of specific humic-like, fulvic-like, pigment-like, and protein-like fractions (e.g., Hunt, Ohno, & Fernandez, 2008). Lability of these extractable components could influence the nutritional quality and assimilation efficiency of litter substrates. As earthworms are thought not to assimilate much structural material or phenolics but rather preferentially assimilate non-structural organic compounds (Curry and Schmidt, 2007; Fahey et al., 2013), the quality of soluble components are likely to be better predictors of earthworm growth.

In northern forests earthworm invasions are still progressing (e.g., Hale et al., 2005; Frelich et al., 2006; Shartell et al., 2013), so understanding the role of litter type and quality on invasion will assist us in predicting the landscape patterns and ecosystem consequences of earthworm invasion. Variation among tree species in leaf litter quality could affect the rate and ultimate extent of earthworm invasion. In addition to leaf litter, white-tailed deer (*Odocoileus virginianus*) fecal pellets might be a locally important source of higher quality litter available to earthworms, especially relative to eastern hemlock (*Tsuga canadensis*) litter in hemlock stands where deer yard during the winter, concentrating fecal inputs (Karberg and Lilleskov, 2008). To our knowledge no studies have examined the effects of northern forest leaf litters or fecal pellets from deer on earthworm growth.

Earthworm growth and invasion potential may also be associated with the physical and chemical properties of soils (Edwards and Bohlen, 1996) such as soil pH, base cations, and organic matter content (Chandrashekara et al., 2010; Reich et al., 2005). Earthworm abundance is typically associated with soils that contain high organic matter content, which greatly affects soil pH, soil nutrients, water-holding capacity, and aggregation (Chandrashekara et al., 2010). *Lumbricus terrestris* has been found in soils ranging from pH 4 to 8 (Reynolds, 1977) with pH ~4 the lower threshold for *L. terrestris* presence in northern Michigan forests (Shartell et al., 2013). Litter inputs from vegetation can strongly influence soil organic matter, in turn affecting nutrient inputs and pH. Tree species' litter rich in calcium (Ca) are associated with increased soil pH and exchangeable Ca, and increased earthworm abundance (Reich et al., 2005). Yet it is not clear if pH *per se*, or Ca availability is the better predictor of earthworm abundance and growth.

In this paper we present the results from mesocosm experiments testing the effects of litter and soil characteristics on earthworm growth. The objectives of this study were to determine whether soils and/or forest litter types affected *L. terrestris* growth (biomass increase); whether the effect of litter types on growth depended on the soil type; and to determine the characteristics of these soils and litter types that might serve as quantitative predictors of *L. terrestris* growth. We tested the following hypotheses:

1. *Lumbricus terrestris* growth will differ among forest litters and soils from northern hardwood forests.
2. The physical and chemical properties of litter species (thickness, %N, lignin, Ca, tannin, leaf extractable chemical character) will predict *L. terrestris* growth. Specifically, *L. terrestris* will grow faster on litters with thinner leaves, higher N and Ca; and lower C:N, lignin:N and tannin:N. We also present an exploratory analysis of additional extractable chemical properties that might affect growth.
3. The chemical properties of forest soils will affect *L terrestris* growth; specifically, *L. terrestris* will grow faster in soils with higher organic matter, higher percent N and C, higher pH, higher Ca, and higher base cations.

2. Materials and methods

2.1. Litter collection

In May 2008, leaf litter was collected from the litter layer in mixed northern hardwood stands located northeast of Howe Lake in the Huron Mountain Club (HMC), Marquette County, MI, USA, (N46° 53′ 37.92″, W87° 56′ 27.32″). Currently being invaded by *L. terrestris*, this site provided a range of tree leaf litters commonly encountered by invading earthworms. Collection occurred in areas just beyond the invasion front of the earthworms, which included both *Aporrectodea* spp. and *Lumbricus terrestris*. This site was also adjacent to an eastern hemlock dominated stand in which the fecal pellets and hemlock litter were collected. Most litter had fully senesced before falling. The one exception was eastern hemlock litter, which consisted mostly of green needles, because small twigs shed with green needles over winter were the dominant hemlock litter input. The litter was sorted by species and dried in a forced air oven for one week at 35 °C.

2.2. Soil collection

In May 2008, soil was collected from four different locations in Michigan's Upper Peninsula, chosen for their presence ($n = 2$) or absence ($n = 2$) of *L. terrestris* (Table 1). The O_i and O_e horizons were removed before collecting the A and B horizon material. The A horizon consisted either of true A material in sites already invaded by worms (in which case O_a and E material had been mixed by earthworms) or a mixture of the O_a and E horizons from uninvaded sites. All soils were coarsely sieved on site (12 mm mesh). The soil was homogenized and stored at room temperature for 3 weeks prior to use. Of the uninvaded soil types, Un2 (Table 1), was located within 10 m up-slope of earthworm-invaded riparian soils.

2.3. Earthworm collection and analysis

In May 2008, juvenile and mature *L. terrestris* were collected from mixed northern hardwood stands located in the HMC. Direct collection at night and chemical extraction during the day were used to obtain the earthworms (Schwert, 1990). Mustard water solution (Lawrence and Bowers, 2002; Hale et al., 2005), a temporary irritant causing earthworms to surface, was used for chemical extraction by pouring the solution onto the soil within a ring of sheet metal pressed into the soil. Worms were placed in Ziploc bags filled with soil and leaf litter from the collection site. The bags were transported back to the lab in coolers and stored at 4 °C with abundant leaf litter until used. Litter was replaced as needed.

2.4. Mesocosms

Mesocosms were constructed using 20.32 cm (OD) industrial-grade PVC pipe cut into 33 cm lengths, with 21 cm square PVC sheet plastic base secured with aquarium caulk (All-Glass Aquarium Co. Inc., Franklin, WI). One cm drainage holes were drilled into the center of the PVC sheets for drainage and plugged with rubber stoppers. Mesocosms were filled with soil (see below), saturated with water and drained for 36 h before the addition of earthworms or litter.

2.5. *Experimental design*

2.5.1. *Overview*

Two growth experiments were performed. Both experiments examined the growth rate of earthworms: the first in response to eight different litter types plus a control (no litter), and the second in response to a full-factorial combination of five different soil types and three litter types plus a no litter control. In both experiments, six grams dry weight of litter was spread evenly over each mesocosm soil surface.

One day prior to initiation of the experiments, the earthworms were extracted from the storage bags, placed in individually labeled moistened sealed plastic bags filled with air and left at room temperature for 24 h to clear their guts (Persson et al., 2007). After the starvation period each earthworm was weighed and sorted into four weight classes ranging from 0.92 to 2.70 g mean fresh weight. The worms were then identified as immature or mature (Reynolds, 1977). To control for any earthworm size effects, earthworms from the different weight classes were distributed evenly among the different litter and soil experimental treatments, with one worm placed in each mesocosm.

During the experiment, mesocosms were covered loosely by hard foam sheet insulation except during watering and soil temperature readings, which occurred once a week. Soil temperature averaged 20.9 ± 0.13 °C over the course of the experiment. The mesocosms were remoistened weekly by misting. All experiments ran for 42 days, after which earthworms were harvested by hand sorting over a 12-h period. Earthworms were then starved for 24 h and weighed using the previous procedure to obtain fresh weights. All leaf litter was removed prior to worm extraction, dried in a forced-air drying oven for 72 h at 35 °C, and weighed, except for hemlock litter which was buried by earthworm castings.

2.5.2. *Litter type experiment*

All mesocosms were filled with equal parts A and B horizon (15 cm each) soil excavated from the forest adjacent to the Forestry Sciences Laboratory, Houghton, Houghton County, MI (Inv1) (Table 1). Earthworms were subjected to 9 food source treatments, with 6 g litter added to four replicates each: no litter (control), eastern hemlock (*Tsuga canadensis*), red oak, (*Quercus rubra*), yellow birch (*Betula alleghaniensis*), American hophornbeam (*Ostrya virginiana*), red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), American basswood (*Tilia americana*), and fecal pellets from white-tailed deer. Pellets were used to further investigate the deer pellet effect on *L. terrestris*, as it was previously determined that they are readily consumed by this invading earthworm (Karberg and Lilleskov, 2008). These litter types were selected because they are common in invaded regions of our forests, were all dominant across the earthworm gradient site we chose for selection, and represented a range of litter chemical and physical characteristics likely to be important in earthworm growth.

2.5.3. *Soil-type × litter-type experiment*

Earthworms were subjected to four food source treatments using a subset of the litter types above (no litter control, sugar maple, basswood, and hemlock) under the same environmental conditions, on five different soil treatments (Table 1). Mesocosm containers were filled with equal parts A horizon and B horizon

Table 1
Location, description and properties of soils used in the present study. Soil series, texture, and available water capacity from Web Soil Survey (Natural Resources Conservation Service, 2009).

ID	Collection location	Latitude and longitude	Forest type	Soil series	Earthworm invaded	Texture	Available water capacity	pH	Drainage
Un1; Un1 (NoA)[a]	Atlantic Mine, Houghton County, MI	N47° 5′ 51.85″, W88° 39′ 40.68″	Red pine plantation	Keweenaw–Kalkaska complex	No	Keweenaw: Gravelly loamy sand, fine sandy loam, loamy sand, Kalkaska: all sand	Low	5.2 No-A: 6[a]	Keweenaw: well drained; Kalkaska: excessively drained
Un2	Huron Mountains, Marquette County, MI	N46° 51′ 10.27″, W87° 48′ 36.49″	Northern hardwood stand	Kalkaska sand	No	All sand	Low	4.5	Somewhat excessively drained
Inv1	Huron Mountains, Marquette County, MI	N46° 52′ 18.62″, W87° 51′ 20.26″	Sugar maple dominated northern hardwood stand	Yellowdog very channery sand	Yes	Very channery sand, unweathered bedrock	Very low	4.9	Excessively drained
Inv2	Houghton, Houghton County, MI: Soil excavated from U.S. Forest Service Northern Research Station Rhizotron site	N47° 6′ 52.50″, W88° 32′ 52.38″	Mixed oak-northern hardwood stand	Arcadian-Michigamme-Rock outcrop complex	Yes	Arcadian: very cobbly fine sandy loam with unweathered bedrock Michigamme: cobbly fine sandy loam, fine sandy loam, cobbly sandy loam, unweathered bedrock.	Arcadian: Very Low Michigamme: Low	5.01	Moderately well drained

[a] Refers to No-A treatment, which was B horizon material only.

(15 cm each) from each of the four collection sites. Soils were chosen for their current presence or absence of earthworms, with two soils collected from each (Table 1). For the two uninvaded soils the Oa and E horizons were mixed to create an A for comparison with the invaded soils. To test for the effect of A horizon material on growth, the fifth soil type (Un1-no A) was created by using 30 cm of B horizon soil from the uninvaded Keweenaw–Kalkaska complex (Un1) (Table 1). By removing the A horizon we can control for other chemical or physical variables that might influence earthworm growth among soils, allowing insights into growth impacts of different horizons. Although the primary food source for *L. terrestris* is fresh litter, forest A horizons are typically more organic rich than B horizons, and hence could be used as a potential supplementary food source for *L. terrestris*, either on its own or co-metabolized with fresh litter. Additionally, A-horizon could have non-nutritional effects on earthworms, e.g. by buffering availability of metals in the soil. Each soil-type by litter-type combination was replicated three times.

2.6. Analyses

2.6.1. Litter analysis

Foliar C and N were determined using a Fisons NA 1500 Elemental Analyzer calibrated with atropine. Foliar cations and P were determined using the dry ash method on a Perkin Elmer Optima 7000DV ICP-OES. Foliar lignin was determined using pyrolysis molecular beam mass spectrometry, and corrected with NIST 8492.

To determine litter extractables, 0.1 g of ground, dry foliar tissues were extracted in 10 ml of deionized water via vigorous shaking for three hours at 21 °C. Extracts were centrifuged and the supernatant collected. Supernatant was analyzed for foliar water soluble tannin using the Hach Tannin/Lignin method (Hach #193701) on a Molecular Devices Spectramax M2 Plate Reader. To develop indicators of DOC quality, absorbance of the extracts in the UV and visible spectrum was determined at four wavelengths important for describing organic matter quality: 254 nm, 365 nm, 465 nm, and 665 nm. Extracts were also analyzed for total organic carbon and total nitrogen on a Shimadzu TOC-V analyzer. From these data the following qualitative indicators were determined: specific UV absorbance at 254 nm relative to total DOC ($SUVA_{254}$) as an index of aromaticity (Weishaar et al., 2003), absorbance at 254 nm relative to emission at 365 nm (E2:E3) as an index of molecular size (Lou and Xie, 2006), and absorbance at 465 nm relative to absorbance at 665 nm (E4:E6) as an index of humification (Worrall et al., 2006).

For additional characterization of dissolved organic matter quality excitation-emission matrices (EEMS) for the leaf extracts were determined on an Aqualog C Benchtop Fluorometer (Horiba Corporation, Edison, NJ, USA). EEMS, combined with parallel factor analysis (PARAFAC) have been used to identify potentially labile and recalcitrant components of dissolved organic matter, such as humic-like, fulvic-like, and protein-like components (e.g., Stedmon et al., 2003) that could influence earthworm assimilation and growth. Run parameters were excitation: 240–600 nm by 3 nm increments; emission: 212–608 nm by 3 nm bandpass; integration time = 1.0 s. Samples were run neat, as preliminary exploration indicated that these samples exhibited low fluorescence despite characteristic absorbance bands in the UV–Vis region. Further preprocessing steps followed the scheme presented in Lawaetz and Stedmon (2009) and Singh et al. (2013). The resulting EEM fluorescence intensities were normalized to the integral of the water Raman peak (Ex 350/Em 371–428) of each sample. We normalized these resulting fluorescence intensities to an external, ultrapure Raman peak water standard (Starna Cells, Atascadero, CA, USA), integrated similarly to the sample Raman peak. Parallel Factor Analysis (PARAFAC) was used to decompose sample fluorescence EEMs from these extracts into discrete fluorescence components following the methods of Stedmon and Bro (2008) and Kothawala et al. (2014).

Leaf thickness was measured for all litter types except deer pellets. Ten leaves from each litter type were measured randomly at two points with a microcaliper; the overall average was then calculated for each litter type.

2.6.2. Soil analyses

Soil C and N were determined using the same protocol as for leaf C and N above. Soil pH_{H2O}; Bray P; exchangeable K, Ca, Mg using the ammonium acetate method on a Technicon Autoanalyzer; nitrate-N and ammonium-N on a Lachat 8500; and loss on ignition (LOI) were obtained using standard methods (Brown, 1998).

2.6.3. Statistical analysis

Statistical analyses were carried out in SPSS 14.0 for Windows, JMP (version 9.0.2, from SAS Institute Inc., Cary, NC, U.S.A.), Primer 6+, and Sigmaplot 12.5. All experiments were analyzed with a univariate analysis of variance (ANOVA). A one-way ANOVA was used for the litter type experiment, with each litter type as an independent variable. A full-factorial two-way (4 litter type × 5 soil type) ANOVA was used for the soil by litter type experiment. For both ANOVAs the dependent variable was absolute earthworm growth rate, because initial size had no effect on absolute growth rate ($r^2 = 0.0015$, NS). Multiple comparisons were performed using Fisher's protected LSD (Ott, 1988).

To avoid excessive numbers of comparisons of all the leaf variables against earthworm growth, and to determine whether linear combinations of our leaf chemical variables did a better job of predicting growth than individual variables, we ran PCA to reduce data dimensionality using Primer 6+. First we carried out PCA on a core set of variables previously found to influence *L. terrestris* abundance, growth or food palatability (Ca, N, C:N, lignin, lignin:N, tannin, tannin:N). Next we performed PCA on the total set of hypothesized and exploratory normalized litter data to display litter type differences in chemical factors (%N, C:N, Ca, P, Mg, lignin, lignin:N, extracted TOC, TN, water soluble tannins, SUVA254, E2:E3, E4:E6, five EEMS PARAFAC component scores).

We next carried out simple linear regressions in Sigmaplot of earthworm growth against various aspects of litter that have been hypothesized or previously found to be important (leaf thickness, lignin, %N, lignin:N, extractable tannins, Ca concentration) as well as the axis scores from the two PCAs of leaf chemistry. We repeated the analysis with and without hophornbeam included, because as a result of mortality our estimate of earthworm growth on this litter type was based on only two earthworms, the lowest sample size of any litter type. We also carried out regressions of earthworm growth vs. soil quality metrics (soil texture, pH, organic matter, %C, %N, Ca). To account for multiple comparisons we estimated significance under different false discovery rates (FDR) using the Benjamini–Hochberg test (McDonald, 2014). The number of false positives expected is indicated by the FDR value. For example, an FDR of 0.1 is equivalent to an estimated one false positive out of ten tests deemed significant under the FDR criteria.

To determine whether litter disappearance was a good proxy for earthworm growth independent of litter type, we performed regressions of litter disappearance against earthworm growth. Eastern hemlock litter was not included in statistical analysis for the litter disappearance due to unattainable final litter weights caused by burial of the fine leaf litter in earthworm middens.

3. Results

3.1. Earthworm mortality

Of 84 earthworms, eight died (9.5% mortality). Four died in the soil-type × litter-type experiment (2 control and 2 eastern hemlock), and four in the litter type experiment (1 basswood, 1 deer pellet, and 2 hophornbeam). Only surviving worms were used in the growth analyses.

3.2. Litter type and litter property effects on growth

Litter type significantly ($p < 0.001$) affected earthworm growth. Mean earthworm growth responded to litter as follows, in descending order of mean growth rate: basswood, sugar maple, deer pellets, red maple, hophornbeam, yellow birch, eastern hemlock and red oak (Fig. 1a). The only treatment where earthworms exhibited mean weight loss was the control.

The higher sample size for each litter type in the soil-type × litter-type experiment allowed us more power to resolve significant differences in growth rate. Earthworm growth rates differed among all four litter types used ($p < 0.05$) with basswood > sugar maple > hemlock > control, again with weight loss exhibited only in the control (Fig. 1b).

The PCA of our hypothesized set of important predictors shows that leaf thickness, tannin, and tannin:N are positively related to each other, and negatively related to leaf Ca. These traits are largely orthogonal to lignin:N and C:N. Species to the left in the PCA supported lower growth. However, when all litter types were included the PCA did not resolve between species that supported higher and moderate growth. Most notably, basswood and hophornbeam litter were convergent in the PCA (Fig. 2a), reflecting their chemical and physical trait similarity (Supplemental Table 1), yet hophornbeam supported lower growth than basswood (Fig. 1). As a result, there were no significant relationships between individual litter characteristics or the PCA axes and earthworm growth, although the sign of the slope of the relationship generally matched our hypotheses (Table 2). A PCA of the extended set of leaf chemical traits exhibited a similar clustering between species (Fig. 2b) and lack of significant prediction of earthworm growth. When hophornbeam (which had only two surviving earthworms contributing to its growth estimate) was removed from the model r^2 for hypothesized relationships universally increased, and p values decreased despite the loss of power (Table 2). C:N became significant, with higher growth at lower C:N, as did the PC1 of the simple PCA and PC2 of the extended PCA at $p < 0.05$ (Table 2). Leaf Ca and N concentrations were only significant under a false discovery rate of 0.2. For the PC axes that were predictive of growth, the loadings were concordant with the individual regressions, with positive loadings for Ca and N, and negative loadings for C:N, lignin:N and tannin:N (Supplemental Table 2). In the extended PCA EEMS C3 (a humic-like component; component C of Coble, 1996) loaded negatively on PC2, whereas EEMS C5 (a humic-like component; FH4 of Singh et al., 2013) loaded positively on this axis (Table 2).

When deciduous leaf litter types were compared on a common soil, mean earthworm growth was well-predicted by mean litter disappearance (Fig. 3), both with control included ($r^2 = 0.89$, $p < 0.0014$) and excluded ($r^2 = 0.90$, $p < 0.0034$). The y-intercept was significantly greater than zero with the control excluded ($p = 0.03$), but not when the control was included ($p = 0.14$).

3.3. Soil type and soil property effects on growth

Soil type significantly ($p = 0.018$) affected earthworm growth. Earthworm growth was the greatest on Inv2. All other soils were not significantly different (Fig. 4). There was no significant interaction of litter type and soil type ($p = 0.168$). When the effect of A horizon removal alone was examined (Un1 vs Un1 noA), there was no effect on growth on the higher quality basswood and sugar maple litter types, but there was a significant negative effect of removal on *L. terrestris* growth for the hemlock ($p = 0.006$) and control ($p = 0.002$) litters (Fig. 5).

We found strong significant positive regression relationships between mean earthworm growth and the following soil characteristics indicative of presence of soil organic matter and base cations, in descending order of variance explained: soil exchangeable Ca (Fig. 6), sum base cations, % organic matter, soil %N, soil %C, and soil exchangeable Mg (Table 3).

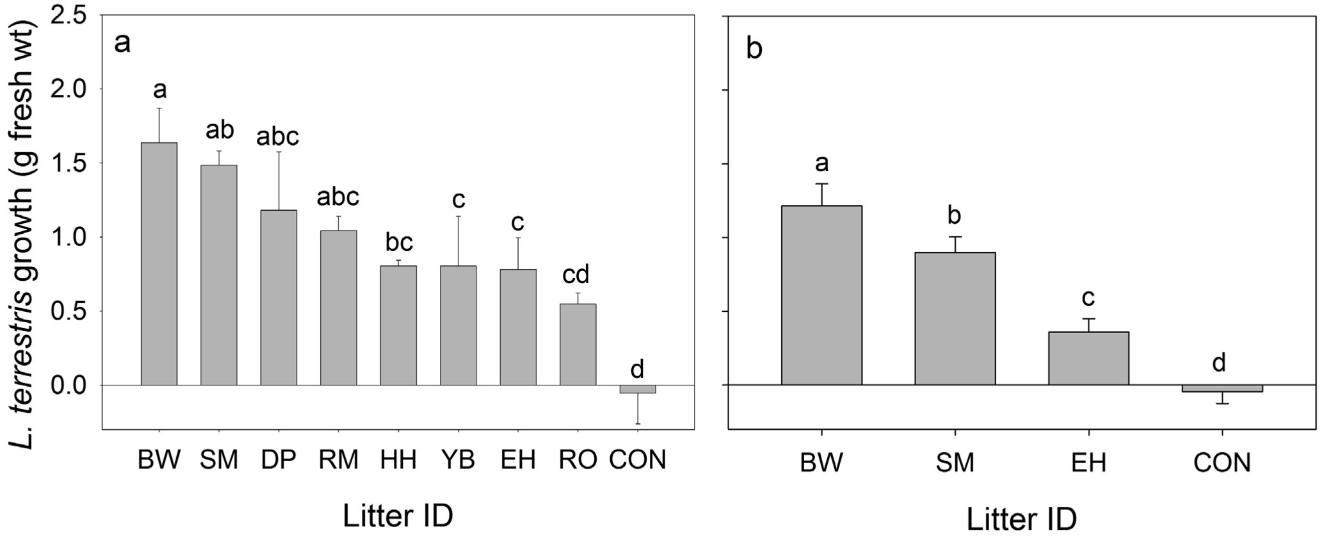

Fig. 1. *Lumbricus terrestris* net growth on different litter types for a) litter type experiment b) the soil × litter type experiment. Levels not labeled with the same letter are significantly different ($\alpha = 0.05$). Litter ID: BW = basswood, SM = sugar maple, DP = deer pellets, RM = red maple, HH = hophornbeam, YB = yellow birch, EH = eastern hemlock, RO = red oak, CON = no litter control.

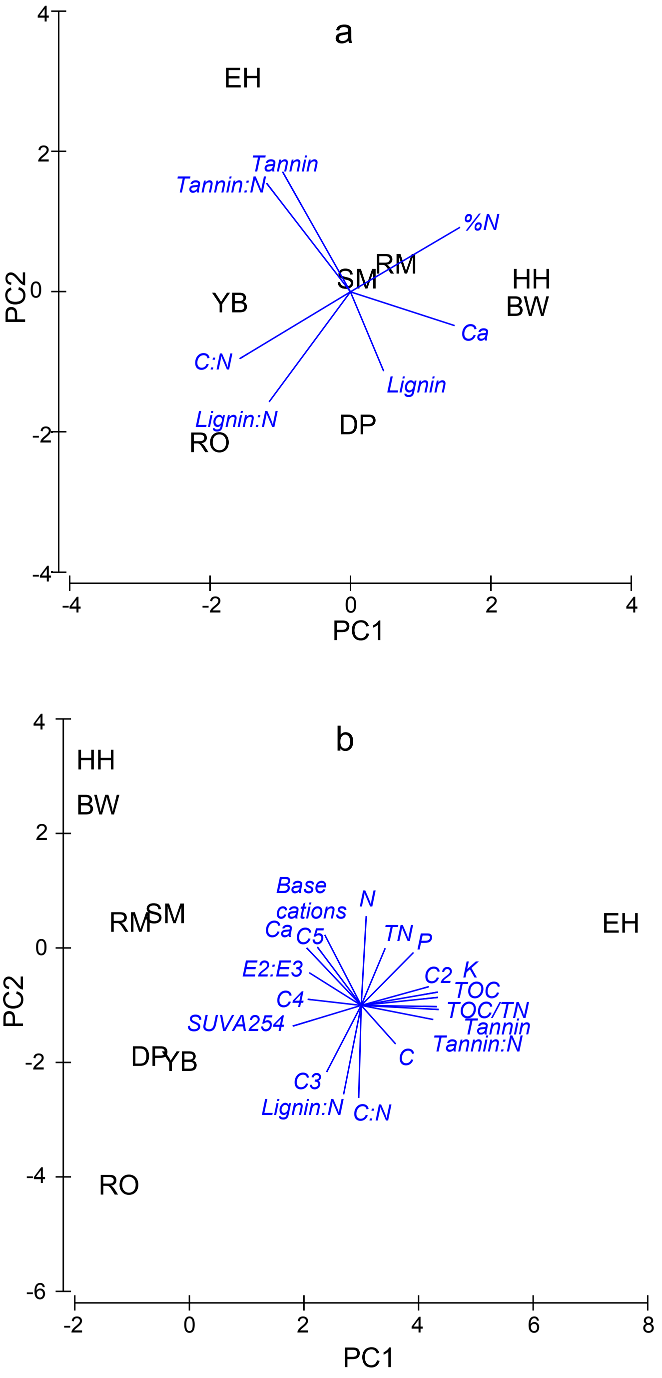

Fig. 2. PCA of normalized litter chemical traits, a) hypothesized chemical variables of importance based on prior literature, b) exploratory analysis of larger set of chemical traits. In a) PC1 and 2 explain 46.5% and 36.3% of the variation respectively; in b) PC1 and 2 explain 38.9%and 25.2% of the variation, respectively. Litter type abbreviations are as in Fig. 1, chemical traits as in Supplemental Table 2. Shortest vectors (Mg, lignin, C1, E4:E6, and %ash) were removed for clarity of presentation.

4. Discussion

Our experiments support the hypotheses that both litter and soil characteristics from northern hardwood forests affect *L. terrestris* growth, and measured chemical characteristics of soil, and perhaps leaves, can explain significant portions of variation in growth. Assuming that earthworm growth positively influences invasion success, areas with litter or soil types supporting the most earthworm growth will have a higher invasion potential and higher earthworm impacts.

Table 2
Regression of earthworm growth against individual litter variables and PCA axis scores for all litter types, and with hophornbeam excluded.

Litter variable	All litter types			All litter types minus HH		
	Reg. Slope	r^2	P[a]	Reg. Slope	r^2	P[a]
Ca (mg/g)	0.04	0.26	0.20	0.05	0.48	0.08
N (%)	0.60	0.17	0.31	1.14	0.46	0.09
C:N	−0.02	0.30	0.16	−0.04	0.64	**0.03***
Lignin:N	−0.05	0.12	0.39	−0.08	0.25	0.26
Tannin:N	−0.02	0.12	0.40	−0.02	0.22	0.29
Leaf Thickness (mm)	−3.01	0.25	0.25	−4.04	0.42	0.16
PC1 simple litter chem[b]	0.12	0.33	0.13	0.22	0.80	**0.01****
PC2 simple litter chem[b]	0.00	0.00	0.98	0.00	0.00	0.99
PC1 full litter chem[b]	−0.03	0.07	0.53	−0.04	0.12	0.45
PC2 full litter chem[b]	0.08	0.26	0.20	0.14	0.62	**0.04***

For variables included in the PCA analyses see Supplemental Table 2.

[a] Bold indicates significant under a FDR = 0.1 (**) or 0.15 (*).

[b] PC1 and PC2 are the principle component analysis axis scores for PCA axis 1 and 2 respectively.

4.1. *Controls on L. terrestris growth*

Our soil type experiment supports the hypothesis that even relatively subtle variation in soil characteristics can influence the distribution and abundance of earthworms. Both soil organic matter and exchangeable Ca were excellent predictors of earthworm growth on different soils. Although the correlation of soil organic matter and base cations makes it difficult to separate their relative influences on *L. terrestris* growth, it is likely that both of these factors contribute. Future studies should be designed to test the relative importance of these factors in regulating *L. terrestris* growth.

4.1.1. *Soil organic matter*

Soil organic matter metrics (LOI and %C) were excellent predictors of earthworm growth, and removal of the organic-rich A horizon negatively impacted growth under poor quality or no litter. Although leaf litter is the primary food of *L. terrestris* in forests, the nutrients obtained from soil organic matter may be enough to sustain earthworms for prolonged litter-less periods or complement their regular diets. The *L. terrestris* mass loss on eastern hemlock and no litter controls only when the A horizon material is removed (Fig. 5) point to the importance of the more organic-rich A horizon material to *L. terrestris* growth under poor or sparse litter. The fact that removal of A horizon material only affected growth on lower quality litter types suggests that the benefit was nutritional rather than ameliorative, e.g., by reducing exposure to toxic metals. Interestingly, the non-zero intercept of the linear fit between leaf litter disappearance and growth only when control is excluded from the regression combined with the absence of growth in the control (Fig. 3), suggests that access to litter increases *L. terrestris*' ability to use mineral soil organic matter for growth. This possible co-metabolism effect might explain the fact that litter-feeding worms prefer to ingest a mixture of mineral soil and litter over pure litter (Doube et al., 1997). Although alternative explanations exist, as mineral soil consumption can improve grinding in the gizzard (Marhan and Scheu, 2005), there was still plenty of mineral soil available in the A horizon removal treatments so that is unlikely to explain the enhanced growth with A horizon present.

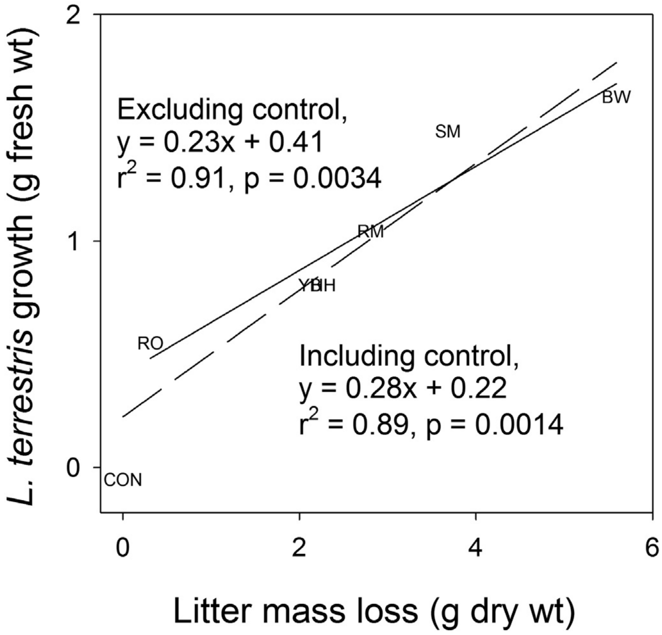

Fig. 3. Regressions of *Lumbricus terrestris* net growth (g) as a function of litter disappearance, including (dashed line) and excluding (solid line) the control. Litter ID as in Fig. 1.

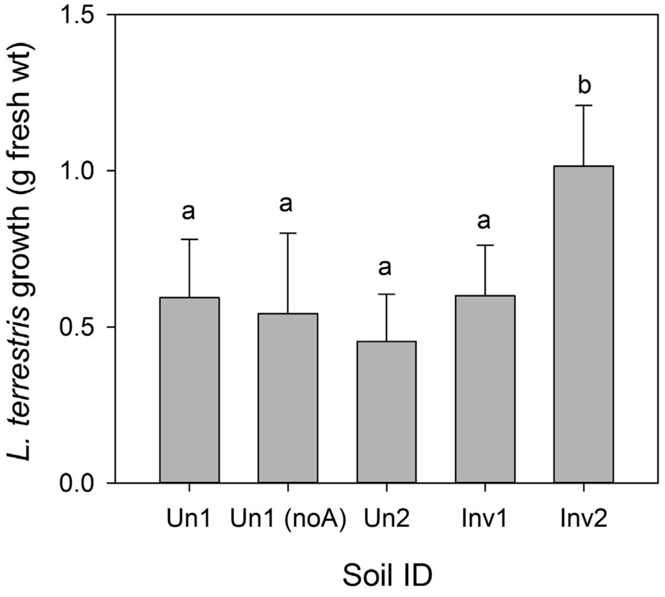

Fig. 4. *Lumbricus terrestris* net growth (g) for different soils in the soil × litter type experiment. Columns not sharing the same letter are significantly different ($\alpha = 0.05$). See Table 1 for soil ID.

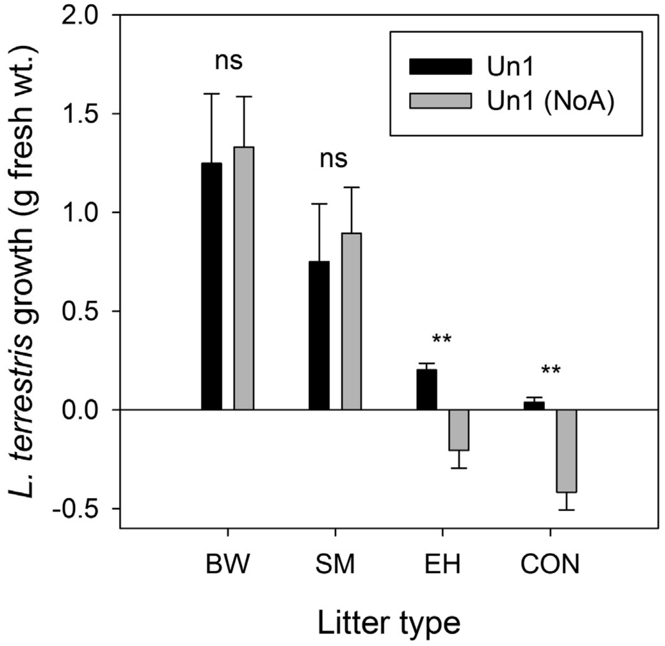

Fig. 5. Effect of A horizon removal on earthworm growth for hemlock and control treatments. NS = not significant. ** = $p < 0.01$.

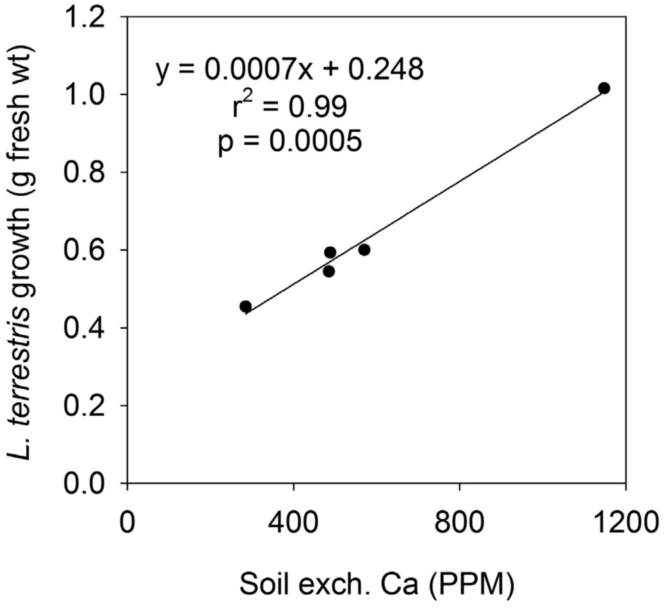

Fig. 6. Regression of *Lumbricus terrestris* net growth as a function of soil exchangeable Ca.

4.1.2. Calcium and pH

Our results support the hypothesis that soils rich in Ca or base cations may be more susceptible to earthworm invasion. Reich et al. (2005) found that leaf litter Ca concentrations affect soil acidity, soil pH, exchangeable Ca, and percent base saturation and fertility, with native earthworm abundance increasing with Ca concentrations. However, in our study pH was not a good predictor of growth rate, perhaps because of the effect of soil organic matter on pH. Accumulation of soil organic matter tends to acidify soils (Brady and Weil, 2002). In this study, the soil with the highest pH had the lowest organic matter content. Despite this acidifying effect, soil organic matter could have positive effects on *L. terrestris* by adding cation exchange capacity or as a supplementary food resource. Soil Ca was weakly predicted by soil pH ($r^2 = 0.14$), but strongly predicted by soil organic matter ($r^2 = 0.92$). Given the stronger relationship between Ca and worm growth than pH and worm growth, this suggests that Ca and/or soil organic matter, rather than pH, may be a more important regulator of *L. terrestris* growth. In another study in similar soils we observed that pH was a good predictor of *L. terrestris* presence but not of abundance, suggesting a threshold rather than continuous pH effect (Shartell et al., 2013).

Although the positive relationship of *L. terrestris* growth with foliar Ca was weak, together with our results indicating positive effects of soil Ca it suggests that Ca from soil and/or litter

Table 3
Measured values for all chemical variables for each soil used in the experiment, and regressions against earthworm growth. See Table 1 for soil ID.

Measured soil variable	Soil ID					r^2	p-value[a]
	Un1 no A-horizon	Un1	Un2	Inv1	Inv2		
Net *L. terrestris* growth	0.544	0.593	0.454	0.600	1.015	NA[b]	NA
Ca (ppm)	485	488	284	570	1148	0.99	**0.0005**
Sum base cations (ppm)	547	566	419	704	1388	0.98	**0.002**
LOI (%)	1.58	2.15	1.75	2.17	5.73	0.96	**0.003**
N (%)	0.025	0.055	0.045	0.06	0.145	0.89	**0.016**
C (%)	0.71	1.025	1.02	1.015	2.61	0.87	**0.022**
Mg (ppm)	36	43	51	76	148	0.85	**0.026**
K (ppm)	25	30	54	55	89	0.56	0.1482
C/N	28.4	18.6	22.6	16.9	18	0.23	0.4136
pH	6.1	5.2	4.5	4.9	5.5	0.11	0.5821
P (ppm)	49	46	21	130	46	0.01	0.909

[a] Bold indicates significant under a BH FDR = 0.05.
[b] NA indicates not applicable.

contributes to *L. terrestris* growth. This might help to explain the observation that tree litter rich in Ca is associated with increased exchangeable soil Ca, increased earthworm abundance (Reich et al., 2005), and increased leaf litter consumption by earthworms (Holdsworth et al., 2012). For example, we have observed islands of intense *L. terrestris* activity under basswood trees in forests of the Upper Peninsula of Michigan. Basswood is known to produce Ca-rich litter (e.g., Page and Mitchell, 2008; this study) and has been found to enrich the mineral soil with Ca (Fujinuma et al., 2005; Page and Mitchell, 2008). Hence litter Ca could have indirect effects on earthworm persistence via its effect on soil Ca.

The presence of calciferous glands in Lumbricidae has been known for over a century (Darwin, 1881). While their function continues to be debated, recent studies support the earlier hypotheses (reviewed in Piearce, 1972) that they are involved in CO_2 regulation (e.g., Briones et al., 2008; Versteegh et al., 2014). Whatever their function, they indicate a unique role for Ca in the physiology of Lumbricidae that may lead to dependence on sources of environmental Ca for growth.

4.1.3. Litter types and chemistry

Our results confirm that tree leaf litter type significantly affects *L. terrestris* growth, which should in turn affect spatial patterns of survival and invasion. All else being equal, litter types supporting higher growth rates are likely to support higher *L. terrestris* populations, increasing impacts on ecosystem processes in these forest types. As a result, forest management for litter types that differ in their effect on earthworm growth could have significant impacts on the distribution and size of earthworm populations and their resultant impacts on forest ecosystems.

Our observation that removal of hophornbeam from the analysis greatly improved regressions and significance with a broad range of our hypothesized variables suggests that either hophornbeam has unmeasured traits regulating *L. terrestris* growth, or our *L. terrestris* growth estimate for hophornbeam litter was inaccurate. In support of the latter interpretation, the hophornbeam data are the weakest in the litter trial, based on only two replicates because worms died in the other two replicates.

In the significant *a priori* and full litter chemistry PCA analysis without hophornbeam it appeared that a suite of traits was associated with higher earthworm growth: higher foliar Ca, base cations, N, and fulvic like extractables; and lower C:N, lignin:N, tannin-N, and certain humic-like extractables. These data are consistent with the findings of Kasurinen et al. (2007) in which a suite of correlated traits describing quantity and quality of C and N were significant predictors of earthworm growth. In particular, they found that higher N, lower C:N, higher C:P, lower lignin, lower lignin + polyphenolic:N, and lower condensed tannins, were all correlated with each other and with higher *L. terrestris* growth. As in our study, C:N was one of the strongest loadings on a highly predictive PCA axis.

C:N had the strongest support of any single variable, with both a significant linear regression against growth as well as strong loading on both predictive PCA axes. Other studies indicate that litter C:N regulates food palatability to *L. terrestris* (Edwards and Bohlen, 1996; Schönholzer et al., 1998). Thus litter C:N appears to be predictive of both palatability and growth.

Our support for other individual factors was weak. We did not find a significant relationship of *L. terrestris* growth with lignin, consistent with the experimental findings of Hartenstein (1982) for *Eisenia foetida*. Our support for a negative effect of foliar tannins is weak, based on the negative loading on axis 1 in the simple litter chemistry PCA. Others have pointed to possible relationships of earthworm feeding and growth rates with tannins, phenolics, and polyphenolics (e.g., Hartenstein, 1982; Hendriksen, 1990). Our finding in the regression of the PCA axis that the predictive axis had a positive loading of one EEMS component for extractable humics and a negative association with another EEMS for another suite of humics points may point to potential water soluble organic matter quality regulators of growth, indicating an area in need of further investigation. While Hartenstein (1982) did not find an effect of added humics on *E. foetida* growth, we are not aware of a study testing effect of humic acid fractions on *L. terrestris* growth.

4.2. Deer–hemlock–earthworm interactions

Whatever the cause of litter effects on growth variation, it is worth noting that although hemlock needles supported lower growth rates than most litters, they did support greater growth rates than the controls. This indicates that even pure hemlock stands might not be immune from *L. terrestris* invasion, and supports our observation of earthworm establishment in hemlock-dominated stands in the Huron Mountains of Michigan (Karberg and Lilleskov, 2008). However, these stands typically have some other sources of litter, e.g., subdominant hardwoods and deer fecal pellets (Karberg and Lilleskov, 2008). It is not clear whether pure hemlock stands are capable of supporting viable *L. terrestris* populations, because both quantity and quality of litter should affect earthworm invasion, and populations might require high density to persist in the absence of sufficient immigration. Hemlock not only supports lower earthworm growth per unit biomass added, but also produces less litter biomass than northern hardwoods such as sugar maple (Karberg and Lilleskov, 2008). This would likely reduce the carrying capacity of *L. terrestris* in hemlock stands.

Given the above, any additional sources of higher quantity and quality of litter should increase the invasibility of these stands. Our finding that earthworms grow as well or better on deer pellets vs. hemlock litter, when combined with our previous observation that these deer pellets are present and readily consumed by earthworms in hemlock stands that serve as winter deer yards (Karberg and Lilleskov, 2008), supports the hypothesis that inputs of deer pellets into hemlock stands by yarding deer should increase the carrying capacity for *L. terrestris* in hemlock stands. Given that yarding behavior by deer leads to significant inputs of deer pellets into hemlock stands (Karberg and Lilleskov, 2008; Jensen et al., 2011), high deer density could facilitate earthworm invasion into these stands. In turn, earthworm-mediated changes in soil properties could have significant impacts on tree growth and regeneration in these stands.

4.3. Litter disappearance as a proxy for growth

Our finding of a positive linear relationship between litter disappearance and *L. terrestris* growth has implications for modelers. Accelerated litter disappearance in invaded relative to uninvaded sites could be used to estimate *L. terrestris* growth in models of earthworm population dynamics and ecosystem impacts. The linear slope of this relationship (Fig. 3) suggests that *L. terrestris* litter consumption rates have more to do with rate limiting factors for consumption, e.g., gut passage time or physical ability to consume litter, rather than the growth efficiency on the litter once consumed, because if growth efficiency increased on higher quality litters then the best fit line should be concave upwards, not linear as seen here. If this is true, it is possible that as thicker leaved species (which also tend to be tougher) become more physically accessible and lower in C:N and extractable tannins after partial decomposition, *L. terrestris* consumption rates of these litters will increase. Greater earthworm densities under partially decomposed litters than under fresh litters of selected tree species (Hendriksen, 1990) are consistent with this hypothesis. Additional tests of growth on older litter would test this hypothesis.

5. Conclusion

Litter and soil properties significantly affect earthworm growth rates in northern hardwood-hemlock forests. Soil Ca and organic matter content, combined with the availability of palatable litter, are predictors of growth and by extension, perhaps invasibility of an ecosystem by *L. terrestris*. Litter C:N and a linear combination of a suite of litter chemical variables also were predictive of *L. terrestris* growth, and should be tested for predictive power across a broader range of litter types. By understanding more about soil and litter effects on invasive earthworm species, we will be better able to model their distribution, spread and abundance in forests, and the resultant earthworm effects on forest ecosystem processes and management.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.11.030.

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