Community-specific impacts of exotic earthworm invasions on soil carbon dynamics in a sandy temperate forest

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Abstract. Exotic earthworm introductions can alter above- and belowground properties of temperate forests, but the net impacts on forest soil carbon (C) dynamics are poorly understood. We used a mesocosm experiment to examine the impacts of earthworm species belonging to three different ecological groups (*Lumbricus terrestris* [anecic], *Aporrectodea trapezoides* [endogeic], and *Eisenia fetida* [epigeic]) on C distributions and storage in reconstructed soil profiles from a sandy temperate forest soil by measuring CO_2 and dissolved organic carbon (DOC) losses, litter C incorporation into soil, and soil C storage with monospecific and species combinations as treatments. Soil CO_2 loss was 30% greater from the Endogeic × Epigeic treatment than from controls (no earthworms) over the first 45 days; CO_2 losses from monospecific treatments did not differ from controls. DOC losses were three orders of magnitude lower than CO_2 losses, and were similar across earthworm community treatments. Communities with the anecic species accelerated litter C mass loss by 31–39% with differential mass loss of litter types (*Acer rubrum* > *Populus grandidentata* > *Fagus grandifolia* > *Quercus rubra* ≥ *Pinus strobus*) indicative of leaf litter preference. Burrow system volume, continuity, and size distribution differed across earthworm treatments but did not affect cumulative CO_2 or DOC losses. However, burrow system structure controlled vertical C redistribution by mediating the contributions of leaf litter to A-horizon C and N pools, as indicated by strong correlations between (1) subsurface vertical burrows made by anecic species, and accelerated leaf litter mass losses (with the exception of *P. strobus*); and (2) dense burrow networks in the A-horizon and the C and N properties of these pools. Final soil C storage was slightly lower in earthworm treatments, indicating that increased leaf litter C inputs into soil were more than offset by losses as CO_2 and DOC across earthworm community treatments.

Key words: Aporrectodea trapezoides; *community composition*; Eisenia fetida; *exotic earthworm;* Lumbricus terrestris; *soil carbon storage; temperate forest; University of Michigan Biological Station.*

Introduction

European earthworm introductions into northern U.S. temperate forests have attracted increased attention during the past decade. Although endemic earthworms have been slow to recolonize the northern U.S. temperate forests from which they were extirpated during the last glacial advance (James 1995), human activities in the past century have led to introductions of peregrine earthworm species, such as *Dendrobaena octaedra, Lumbricus rubellus, L. terrestris, Aporrectodea caliginosa* and *A. trapezoides* (Holdsworth et al. 2007). Dense earthworm invasions have shifted understory plant diversity, increased leaf litter decay rates, and diminished forest floor horizons (Bohlen et al. 2004b,

Hale et al. 2006, Frelich et al. 2006, Holdsworth et al. 2007, Sackett et al. 2012). Invasions have also been linked to decreased soil C stocks (Scheu 1997, Burtelow et al. 1998, Bohlen et al. 2004b, Marhan and Scheu 2006, Eisenhauer et al. 2007), soil C redistribution (Burtelow et al. 1998, Bohlen et al. 2004a, Wironen and Moore 2006, Straube et al. 2009), and increased soil CO_2 emissions (Marhan and Scheu 2006). While impacts on subsets of forest ecosystem functions and properties have been described, community-specific impacts of earthworm invasions on forest soil C cycling and net C storage are less understood.

Earthworm invasions in forest ecosystems can involve multiple species (Araujo et al. 2004, Fisk et al. 2004, Wironen and Moore 2006, Eisenhauer et al. 2007, Costello and Lamberti 2009) with diverse feeding, dispersal, and burrowing behaviors (Bouché 1977, Lee 1985, Jégou et al. 1998b, Hale et al. 2005, Curry and Schmidt 2006). Interspecific interactions (Jégou et al.

2000, Capowiez et al. 2001, Whalen and Costa 2003) can, in turn, mediate earthworm community impacts on forest ecosystem properties and processes (Wolters 2000, Uvarov 2009). However, direct tests of how earthworm species interactions mediate impacts on forest soil C dynamics and storage are limited (Hale et al. 2005, Postma-Blaauw et al. 2006, Straube et al. 2009).

In this study, we examined monospecific and multi-species earthworm community impacts on C loss and C redistribution in reconstructed forest soil profiles with mixed-species leaf litter (Oi) horizons representative of temperate forests on sandy soils in the Upper Great Lakes region, USA. We report the results of a mesocosm experiment in which earthworm species of three functional groups (*Lumbricus terrestris* L., *Aporrectodea trapezoides* Dugès, and *Eisenia fetida* Savigny) were combined in a factorial design. Over one year, we measured carbon dioxide (CO_2) and dissolved organic carbon (DOC) losses, and related C losses to earthworm species combinations. At the end of the experiment, we (1) assessed relationships between subsurface burrow system structure and soil C budget components, and (2) quantified net changes in soil C storage. We expected that impacts on CO_2 and DOC outputs, leaf litter C inputs, and net C storage would be mediated by earthworm community composition, and that burrow system properties would be related to C redistribution in soil profiles.

METHODS

Experimental design

We conducted a mesocosm experiment from August 2009 to August 2010 in a belowground laboratory (Lussenhop et al. 1991) at the University of Michigan Biological Station, Cheboygan County, USA (UMBS; see Appendix A for study area description), using seven combinations of three exotic earthworm species present in forest soils as treatments and no-earthworm controls in uniform leaf litter and soil profiles. Adults of the earthworm species representing different functional groups included: *L. terrestris* [anecic, which are litter feeding and vertical burrowing], *A. trapezoides* [endogeic, mineral soil feeding and dwelling], and *E. fetida* [epigeic, litter feeding and surface dwelling]. Treatments, hereafter capitalized, included species monocultures: Epigeic Alone, Endogeic Alone, and Anecic Alone; and mixed treatments: Epigeic × Anecic, Epigeic × Endogeic, Endogeic × Anecic, and All Species. Earthworm biomass additions were higher than observed in field surveys (21 ± 2.66 g/m² fresh mass [mean ± SE]), but allowed for the scaled additions of anecic species across monocultures and mixed treatments. Earthworm biomass amounts were also within ranges of values reported in similar northern temperate forests (e.g., Hale et al. 2005, Suárez et al. 2006). Earthworm biomass was constant at 20 ± 0.5 g (fresh mass) per mesocosm. Earthworm biomass was 20 ± 0.5 g in species monocultures, 10 ±

0.5 g of each species in two-species treatments, and 6.5 ± 0.5 g of each species in the All Species treatment. Biomass additions in monocultures corresponded to 27 ± 1 endogeic earthworms per mesocosm, 31 ± 1 epigeic earthworms per mesocosm, and 3 anecic earthworms per mesocosm.

Mesocosms were contained in 20-L plastic buckets (20 cm diameter and 30 cm depth). Soil profiles were constructed by adding 25 kg (fresh mass) of sieved and homogenized B-horizon material packed to a bulk density of 2.5 g/cm³, and 5 kg (fresh mass) of sieved and homogenized A-horizon material packed to a bulk density of 1.3 g/cm³. Leaf litter additions were scaled from area-normalized leaf litter data of the UMBS AmeriFlux site in 2008 (C. S. Vogel, *unpublished data*). Leaf litter additions from overstory tree species summed to 16.5 g: 41% *Populus grandidentata*, 32% *Acer rubrum*, 21% *Quercus rubra*, 4% *Fagus grandifolia*, and 2% *Pinus strobus* (Table 1).

Mesocosm C loss measurements (CO_2 and DOC)

Soil CO_2 efflux was measured from August 2010 to June 2011 (25 times over a 320-day period). Measurements were taken daily in week one, and three times in week two when burrow production and initial soil redistribution likely occurred (Jégou et al. 1998a, 2000, Capowiez et al. 2011); weekly during early fall and spring months when earthworm activity is highest (Callaham and Hendrix 1997); and monthly during late fall and winter months when earthworm activity and soil CO_2 efflux is lowest (Toland and Zak 1994, Davidson et al. 1998). CO_2 efflux was measured using an infrared gas analyzer (IRGA, LICOR-6400; LICOR Biosciences, Lincoln, Nebraska, USA) connected to an air-tight lid placed on each mesocosm. In a 4.67-L headspace, air flowed in a closed loop to the LI-6400, temperature was measured with a type E thermocouple (Omega, Stamford, Connecticut USA), and a capillary tube was inserted for air pressure equilibration. Soil CO_2 efflux rates (F_c) were determined by measuring 10 μmol/mol change in CO_2 concentration (ΔCO_2) over a 20-s measurement period, from which CO_2-C loss rate per unit soil surface area was calculated as

$$F_c = \frac{\left(\dfrac{\Delta CO_2}{\Delta t}\right)\left(\dfrac{PV_t}{RT}\right)}{S} \qquad (1)$$

where F_c is corrected for headspace volume (V) and surface area (S) (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), t is time, P is atmospheric pressure (kPa), R is the universal gas constant, and T is temperature (°C). CO_2 efflux values were integrated to derive cumulative curves for each mesocosm.

Soil moisture was maintained at field capacity with 500 mL deionized water additions. Soil leachates collected from zero-tension lysimeters installed below each mesocosm were weighed, filtered using glass-fiber filters (Whatman, GF/F), acidified with 6 mol/L HCl,

TABLE 1.   Initial leaf litter, A-horizon, and B-horizon C and N properties.

Pool	C (g/m^2)	%C	%N	C:N
Leaf litter	128 (0.26)	47.9 (<0.01)	0.65 (<0.01)	74.2 (0.03)
Acer rubrum	39.68 (0.06)	46.5 (0.4)	0.5 (0.04)	98.8 (<0.01)
Pinus strobus	2.01 (0.01)	50.3 (0.03)	0.4 (0.01)	137.6 (<0.01)
Populus grandidentata	53.65 (0.24)	49.2 (0.6)	0.8 (0.1)	62.7 (<0.01)
Quercus rubra	27.25 (0.07)	47.9 (0.4)	0.6 (0.03)	74.2 (<0.01)
Fagus grandifolia	5.44 (0.02)	45.7 (0.5)	0.7 (0.06)	62.2 (<0.01)
Bulk soil	2882 (67.57)	1.14 (0.08)	0.05 (<0.01)	22.02 (0.13)
A-horizon	994 (42.31)	1.56 (0.07)	0.07 (<0.01)	21.7 (0.31)
B-horizon	1761 (35.53)	0.6 (0.01)	0.03 (<0.01)	22.4 (0.28)

Notes: Values represent means, with SE in parentheses; $n = 6$.

and stored at −20°C until analyzed for DOC concentration using an Aurora (Model 1030) OI Analytical TOC analyzer (OI Analytical, College Station, Texas USA). DOC loss values were integrated to derive cumulative curves for each mesocosm.

Three-dimensional reconstruction and quantification of burrow systems

Soils containing earthworm treatments were imaged using X-ray computed tomography (X-ray CT; GE Discovery CT 750 HD scanner [General Electric Healthcare, Waukesha, Wisconsin USA], 140 kV, 500 mA, 1 s, 0.984:1 pitch, 1.25-mm slice interval, 1.25-mm slice thickness, 0.78 mm X and Y resolution, 40 cm field of view, Bone reconstruction filter) at the School of Radiology, University of Michigan Hospital (Ann Arbor, Michigan, USA). The sequential analysis of two-dimensional binarized images enables three-dimensional tracking of earthworm burrows and subsequent three-dimensional volumetric reconstructions of the burrow systems (Fig. 1). Image preparation and quantification of burrow continuity, volume, and size distribution followed methods previously described (Capowiez et al. 2001, Pierret et al. 2002, Bastardie et al. 2005).

Litter and soil sampling, C and N content

Mesocosms were destructively harvested by first collecting intact leaf litter remaining on the soil surface. Soils were excavated by first removing A-horizon soil, followed by removal of B-horizon soil that was separated into burrow and non-burrow soil (i.e., soil not visibly altered by earthworm burrowing activity or ingestion). Separation of burrow and non-burrow soil in the A-horizon was not feasible due to highly dense burrow networks across treatments (Fig. 1). Pool subsamples were weighed fresh, dried at 60°C, weighed again to obtain dry-mass corrections, and pulverized for C and N analyses using a CN elemental analyzer (Elemental Analyzer 1030; Costech Analytical Technologies, Valencia, California, USA). Species-specific leaf litter mass losses were used in calculating a weighted average of composite leaf litter C and N properties expressed at the end of the experiment.

Soil C mass storage

We used an elemental mass balance equation to calculate net changes in soil C storage as follows:

$$\Delta C = (L_c + E) - \left(\int_0^{320} F_c(t)dt + \int_0^{320} \text{DOC}_{EX}(t)dt \right) \tag{2}$$

where ΔC is the net storage of C inputs to soil as leaf litter mass loss (i.e., from the soil surface) across control and earthworm treatments (L_c) plus earthworm biomass not recovered at the end of the experiment (E), minus C outputs via 320-d cumulative CO_2 efflux (F_c in Eq. 1), plus dissolved organic C export (DOC_{EX}). Minor C fluxes occurring in aerobic upland forest soils, including CH_4 consumption (Castro et al. 1995, Le Mer and Roger 2001), and dissolved inorganic C export (Kaiser and Zech 1998), were not measured in this study.

Statistical analyses

We used Kruskal-Wallis H tests (H, df = 7, $n = 32$, $\alpha = 0.05$) with nonparametric multiple comparisons to assess treatment differences in soil C budget components, burrow system variables, and soil C storage. To assess treatment effects on CO_2 and DOC loss over time, we used a general linear model (GLM) with repeated measures, followed by Bonferroni-corrected pairwise comparisons of cumulative curves. We used Spearman rank correlations (ρ, $n = 32$, $\alpha = 0.05$) to characterize relationships among soil C budget components and among burrow system variables. Soil C budget component and burrow system variable associations were characterized using co-inertia analysis (CoIA), which identifies co-relationships between two ecological data matrices first transformed, in this case, by principal component analysis (Doledec and Chessel 1994, Dray et al. 2003). Statistical significance of the CoIA was assessed by Monte Carlo permutation tests (999 permutations; $P < 0.05$). Statistics were done in R v2.15.2 (R Development Core Team 2012) on RStudio v0.96.331 (*available online*),[5] using the packages ade4
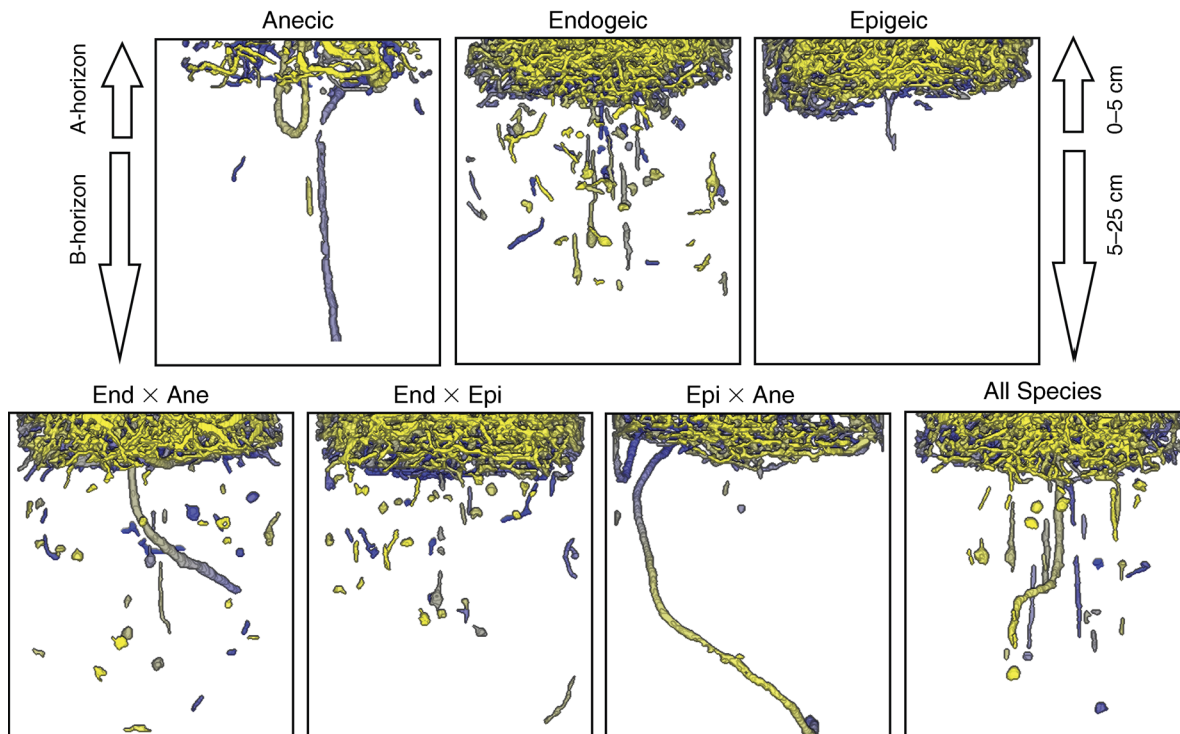
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[5] www.rstudio.com

FIG. 1. Examples of three-dimensional reconstructions of earthworm community burrow systems imaged by X-ray computed tomography (CT). Color gradations represent the distance of burrows relative to the viewer's perspective (yellow for the foreground to blue for the background). Earthworm species of different functional groups included: *Lumbricus terrestris* (anecic [Ane]), *Aporrectodea trapezoides* (endogeic [End]), and *Eisenia fetida* (epigeic [Epi]).

(Dray and Dufour 2007), Hmisc (Harrell 2012), lattice (Sarkar 2008), and pgirmess (Giraudoux 2012).

RESULTS

Cumulative CO_2-C and DOC loss

Rates of respiratory CO_2 loss decreased after the first six weeks of the experiment as winter temperatures decreased (Fig. 2A). We found no significant differences in total CO_2 loss across treatments at the end of the one-year incubation period (Kruskal-Wallis H test, $P >$ 0.05). When mesocosms were destructively harvested at the end of the experiment, we found no adult earthworms and juvenile biomass accounted for <1% of initial earthworm biomass. To evaluate differences in cumulative CO_2 and DOC loss over time, we thereby restricted data analysis to the first 45 days of the experiment when temperature was above 20°C, earthworm mortality and reproduction were likely low, and treatment variance was uniform. Earthworm treatments significantly affected CO_2 loss over the first 45 days (GLM with repeated measures, $P = 0.042$). The Endogeic × Epigeic treatment lost significantly more CO_2 than the control, Endogeic Alone, and Epigeic Alone treatments (Bonferroni test, $P < 0.05$). The Epigeic Alone and Endogeic Alone treatments had the lowest rates of CO_2 loss, and were similar to CO_2 loss in controls (Bonferroni test, $P > 0.05$). Total CO_2 loss was

4.54–6.16% of total C, and was similar across treatments (Kruskal-Wallis H test, $P > 0.05$). DOC loss increased over time (GLM with repeated measures, $P = 0.049$), though no significant effects of earthworm treatments were detected (Kruskal-Wallis H test, $P > 0.05$). Total DOC loss was three orders of magnitude lower than CO_2 (Fig. 2B), and represented <0.01% of total initial C.

Leaf litter and Soil C

Earthworm community composition significantly affected leaf litter C loss (Fig. 3; Appendix B: Table B1). Leaf litter C remaining in treatments including anecic species was 33–39% less than in controls, but only 4–9% less where anecic species were absent. Two leaf litter types lost significant C: *A. rubrum* and *P. grandidentata* (Kruskal-Wallis H tests, $P < 0.05$). Treatment and controls lost similar *F. grandifolia*, *P. strobus*, and *Q. rubra* leaf litter C (Kruskal-Wallis H tests, $P > 0.05$). Across treatments, the morphology of decayed leaf litter remaining at the soil surface was primarily petioles and mid-veins of *A. rubrum* and *P. grandidentata* litter, largely intact *F. grandifolia* and *Q. rubra* litter (i.e., most soft tissue, mid-veins, and petioles remained), and fully intact *P. strobus* litter.

A-horizon and B-horizon C mass, %C, %N, and C:N did not change significantly (Kruskal-Wallis H tests, P
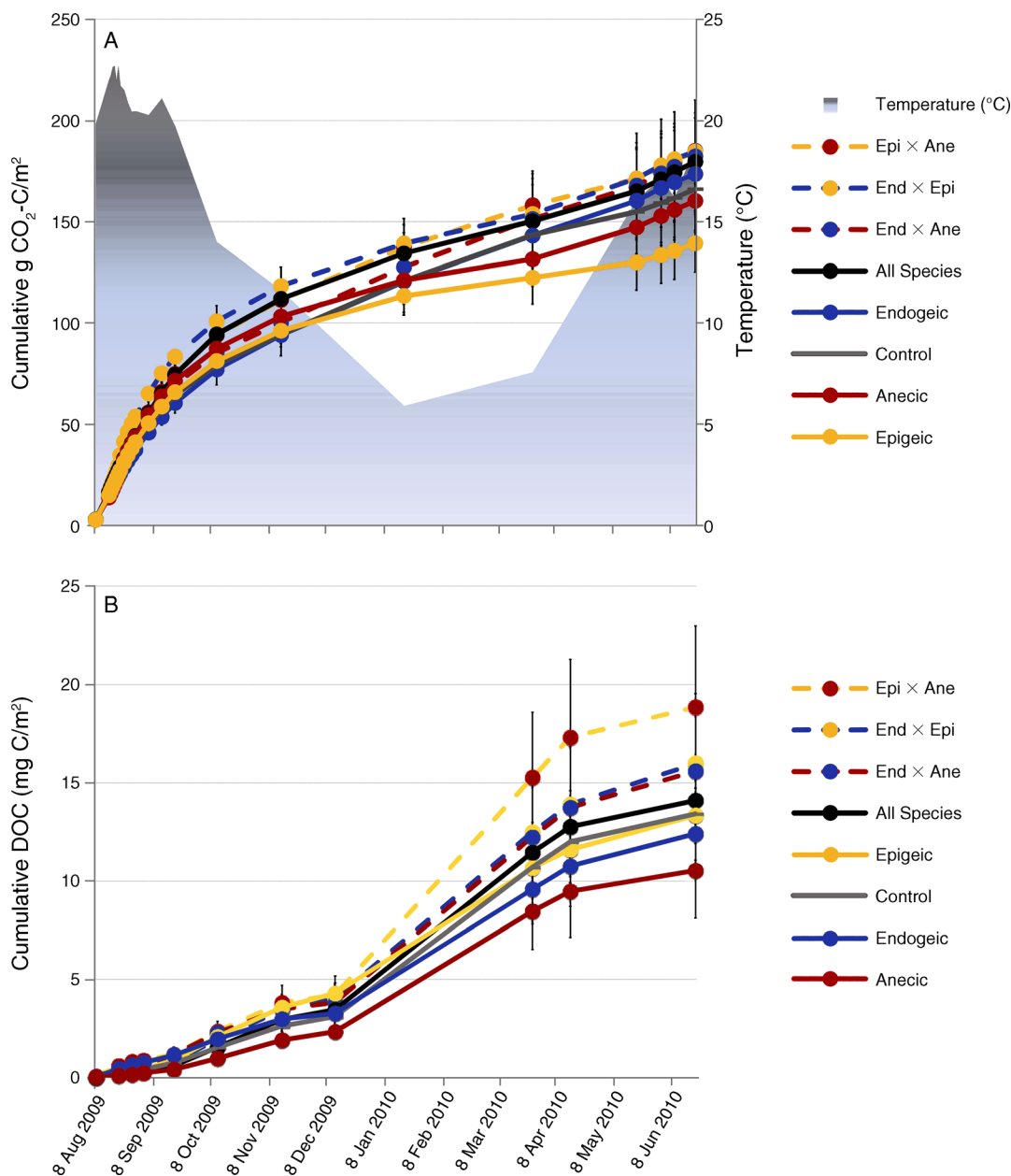
Fig. 2. (A) Cumulative soil CO_2-C efflux and (B) cumulative dissolved organic carbon (DOC) efflux across earthworm treatments (see Fig. 1 for abbreviations). Values represent means, and vertical bars show ±SE. Temperature (°C) is shown in blue shades behind soil CO_2-C efflux curves.

> 0.05). Burrow soil, which accounted for 2–5% of total soil C mass (Fig. 4), showed significantly higher %C and %N values than non-burrow soils (Appendix B: Table B2). Burrow soil C content and %C was positively correlated with A-horizon and leaf litter C content and %C. Total soil C, A-horizon C mass, A-horizon %C, and were positively correlated with total CO_2 loss. No significant correlations between soil C properties and DOC loss were observed (Appendix B: Table B3).

Burrow system structure

Across treatments, burrow system structure differed significantly in total macroporosity, A-horizon burrow volume, the continuity of burrows with vertical lengths >3.75 cm (i.e., 0–15% of core length) and burrow size classes (Kruskal-Wallis H tests, $P < 0.05$; Appendix C: Table C1). Measures of burrow system structure, with the exception of burrow continuity classes characteristic of vertical burrowing activity by anecic species (25% to
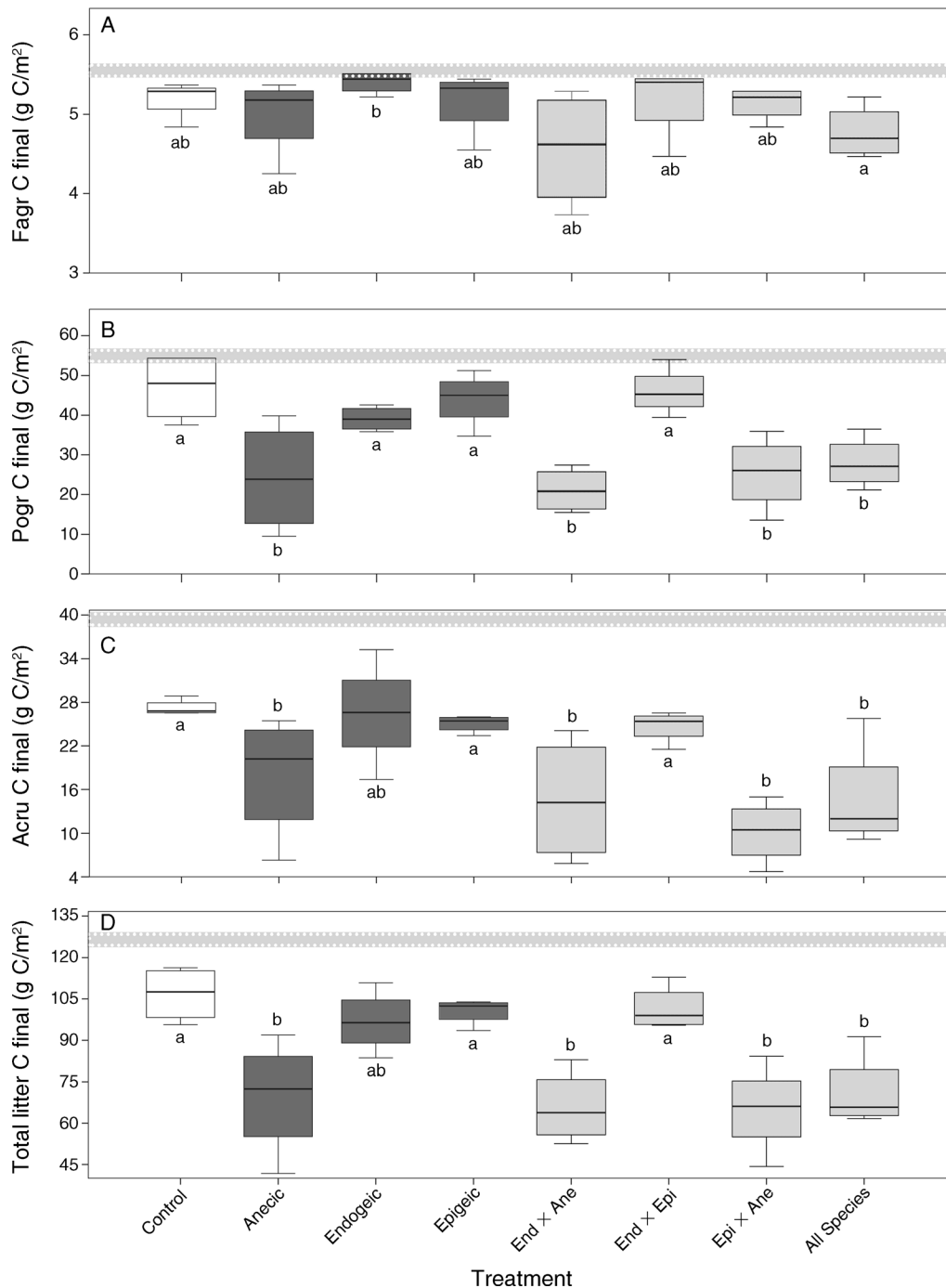
FIG. 3. Final *Fagus grandifolia* (Fagr), *Populus grandidentata* (Pogr), *Acer rubrum* (Acru), and total leaf litter C mass across control, Anecic, Endogeic, Epigeic treatments (dark gray boxes), and multispecies earthworm treatments (light gray boxes). Horizontal gray bars show initial leaf litter C mass. Horizontal lines within boxes indicate median mass values for each leaf litter type; the first and third quartiles of the data (the interquartile range; IQR) are indicated by the top and bottom edges of each box; and extreme mass values (within 1.5 times the upper or lower quartile) are indicated by the ends of the lines extending from the IQR. Different lowercase letters indicate significant differences determined by Kruskal-Wallis H tests with nonparametric multiple comparisons ($P < 0.05$). *Pinus strobus* and *Quercus rubra* losses are not shown. See Fig. 1 for abbreviations.
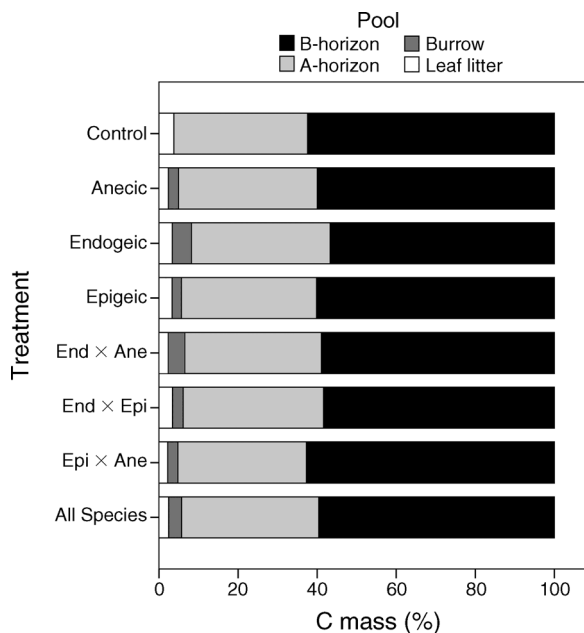
Fig. 4. Leaf litter and soil C pools expressed as the percentage of total C across the control and treatments (see Fig. 1 for abbreviations).

>50% of core length), were highly correlated (Appendix C: Table C2).

Two axes (F1 and F2) of the co-inertia analysis explained 85.0% of the total variability in the burrow system structure and soil C budget components data co-structure (Monte Carlo permutation tests, $P = 0.007$;

Fig. 5). In the co-inertia factorial plane, projections of burrow system structure variables discriminated between burrow structure in the A-horizon and subsurface burrow structures in the B-horizon; projections of soil C budget components discriminated between leaf litter mass losses, A-horizon and burrow soil properties, and C losses as CO_2 and DOC. Along F1 (59.2% of total inertia), total macroporosity and burrow structures in the A-horizon (surface connectivity, burrow size classes, and burrow continuity classes <25% of core length) were correlated with A-horizon and burrow soil C and N properties (C and N content, %C, and %N). Along F2 (25.8% of total inertia), burrow structures in the B-horizon, characteristic of anecic species presence (i.e., burrow continuity classes 25–50% and >50% of core length), were correlated with leaf litter mass losses (with the exception of *P. strobus*). CO_2 and DOC losses were not correlated with burrow system properties.

Soil C storage

Inputs of C to soils from litter in control mesocosms (25.0 ± 4.59 g C/m² [mean ± SE]) were less than C outputs as CO_2 and DOC (153 ± 6.73 g C/m²). As a result, soil C storage (ΔC) in controls was negative, representing a baseline net loss from the soil system (−128.52 ± 11.31 g C/m²; Fig. 6). Litter C inputs to soils were higher in all treatments with anecic species (Mann-Whitney U tests, $P < 0.05$), though C outputs did not differ significantly across control and earthworm treatments (Kruskal-Wallis H test, $P > 0.05$). Significant shifts in ΔC were not detected, though a trend of greater

Fig. 5. Relationships between burrow system (dashed arrows, italicized text) and C budget measures (solid arrows, plain text) according to relative positions on the Fl × F2 co-inertia plane. Burrow system structure measures are: macroporosity (MR), surface connectivity (SC), size class (BS, defined as: 2, 0.17 to <0.34 cm²; 3, >0.34 cm²), continuity class (BC, defined as: 1, 0–15%; 2, 15–25%; 3, 25–50%; 4, >50%). C budget components are: *A. rubrum* (Acru), *F. grandifolia* (Fagr), *P. strobus* (Pist), *P. grandidentata* (Pogr), *Q. rubra* (Quru), and total leaf litter C loss; A-horizon (A) and burrow (Br) C and N properties (C:N, %N, %C, and Cmass); and CO_2 and DOC loss. Co-inertia axis eigenvalues are F1 = 4.27 and F2 = 1.99.
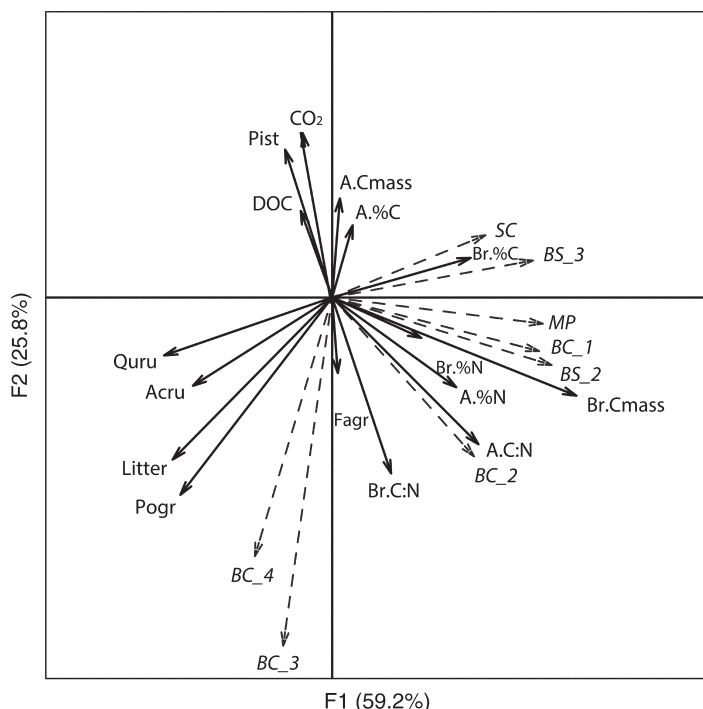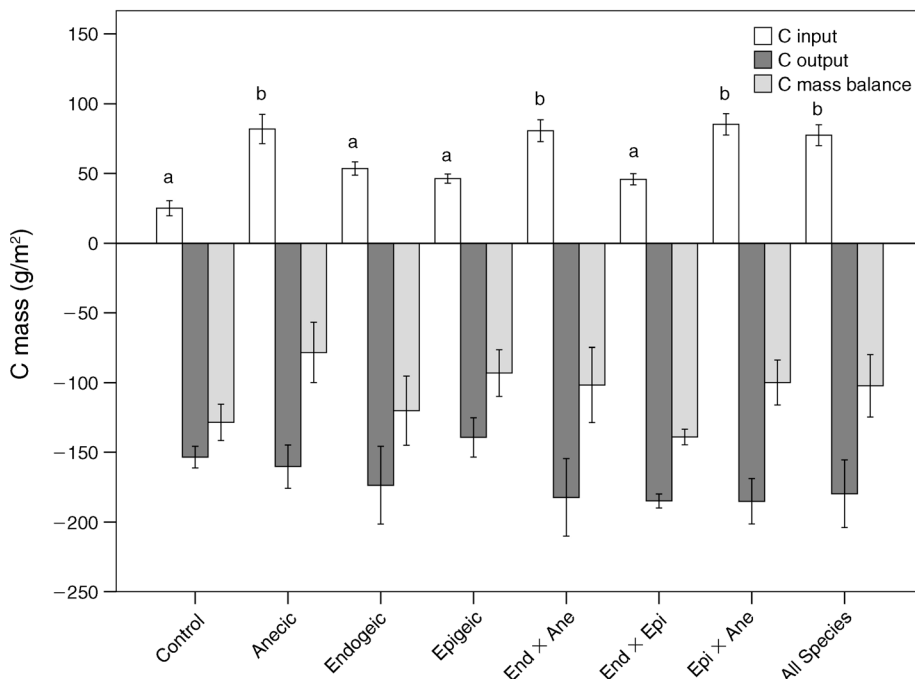
Fig. 6.   C inputs, C outputs, and net C mass storage (ΔC; Eq. 2) across the control and treatments. Lowercase letters represent significant differences determined by Kruskal-Wallis H tests with nonparametric multiple comparisons ($P < 0.05$). C output and ΔC are similar across treatments.

ΔC occurred across earthworm treatments (Kruskal-Wallis H test, $P > 0.05$).

Discussion

Our results suggest earthworm communities have important nonadditive effects on processes including soil CO_2 loss and mediate leaf litter redistribution, soil C budget components, and soil physical structure. First, soil CO_2 loss rates were highest during the first weeks of the experiment, though no differences in total CO_2 or DOC loss were observed at the end of the incubation. As species monocultures had the lowest CO_2 efflux rates, significant increases in CO_2 efflux rates in multispecies treatments suggests enhanced access to C resources by functional groups. Previous studies show increased soil CO_2 losses of 7–58%, following earthworm invasions in forest soils (e.g., Borken et al. 2000, Speratti et al. 2007) attributed to leaf litter incorporation into soil, highly localized organic matter redistribution, and increased microbial respiration in casts and burrow soils (Scheu 1987, Wolters and Joergensen 1992, Tiunov and Scheu 1999, Brown et al. 2000). DOC loss represented <0.01% of total C and showed no response to earthworm treatments, in contrast to a 50% reduction in DOC loss from earthworm-invaded forest soils observed by Bohlen et al. (2004a). In our study, low DOC losses could be due to root exclusion, which removed root exudates and decay as sources of DOC outputs, and possible adsorption of DOC transported from A-horizon to B-horizon soils (Currie et al. 1996, Kaiser

and Zech 1998, Kalbitz et al. 2000). It is unlikely that C redistribution and burrow system differences were generated during winter months when earthworm activity is lowest and differential mortality and reproduction occur (Lee 1985, Edwards and Bohlen 1996, Callaham and Hendrix 1997, Uvarov et al. 2011). Observed patterns of early, rapid C losses are thereby consistent with burrow system production and organic matter redistribution in the first weeks of our experiment. Further, lower rates of C losses and increased variability within treatment replicates with time are consistent with differential mortality, reproduction, or activity during fall and winter months.

Earthworm-mediated litter decomposition is determined by rates of litter comminution, consumption, and translocation into soils (Shipitalo and Protz 1989, Edwards and Bohlen 1996), and constrained by leaf litter chemistry and earthworm food preference (Reich et al. 2005, Hobbie et al. 2006, Suárez et al. 2006, Holdsworth et al. 2008). Our results showed leaf litter C loss increased by 33–39% in communities containing the anecic species, and differential mass loss and morphology of decayed leaf litter types (*A. rubrum* > *P. grandidentata* > *F. grandifolia* ≥ *Q. rubra* > *P. strobus*). Enhanced leaf litter decomposition with earthworm invasions has been widely observed in temperate forests (Scheu and Wolters 1991, Suárez et al. 2006, Holdsworth et al. 2008, Zicsi et al. 2011). Higher losses reported in field studies may be due to higher earthworm densities, longer observation periods, and the larger

community of soil invertebrates. For example, Suárez et al. (2006) observed leaf litter remaining in earthworm-invaded plots was 1.7–3.0 times less than in reference plots in a hardwood forest after 540 days. Holdsworth et al. (2008) observed increased litter mass loss from coarse-meshed litter bags, which allowed enhanced access and leaf litter translocation by the broader soil invertebrate community.

In contrast to our prediction, significant changes in C storage were not linked to earthworm community composition, although C storage generally decreased across treatments. Lack of significant changes in soil C storage could be attributed to earthworm density and activity (because burrow soils only accounted for up to 5% of soil C mass), incubation time, and land use history. For example, Alban and Berry (1994) observed earthworm density increases over a 13-year period, the concurrent development of an A-horizon, and increased mineral soil %C. Bohlen et al. (2004a) demonstrated land-use history as a factor constraining earthworm invasion impacts on soil C pools, finding no influence of earthworm invasions on soil C storage at a previously cultivated forest site with low forest floor accumulation rates. A 28% reduction in soil C storage and reduced soil C:N ratios were, however, observed in undisturbed forest sites of similar earthworm density (Bohlen et al. 2004a). Past disturbances of logging and wildfires constrain soil C storage rates in these forests (Gough et al. 2008), and with earthworm density and time, may constrain the impact of earthworm communities on soil C budgets.

Our results partially support the prediction that burrow system properties would be directly related to shifts in C redistribution. Burrow system structures differed significantly across earthworm treatments and were in agreement with the known behavior of the different ecological groups (Bastardie et al. 2005). Somewhat surprisingly, burrow systems did not affect CO_2 or DOC loss, showing no evidence of increased soil C losses with greater soil porosity. This may be attributed to the well-drained nature of these soils, where C losses are controlled by production rather than diffusion or infiltration rates. However, subsurface burrow systems were associated with vertical redistribution of litter-derived organic material into the A-horizon, as indicated by strong correlations between (1) subsurface burrows characteristic of vertical burrowing by anecic species, and leaf litter mass losses (with the exception of *P. strobus*); and (2) dense burrow networks in the A-horizon and the C and N properties of these pools.

In sandy soils, it appears earthworm community composition and associated burrow system structures mediate litter translocation and soil physical structure, altering soil organic matter inputs while having modest impacts on C losses in the short term. This outcome suggests the net effects of earthworm communities on the primary carbon pools and fluxes in these soils is moderate, with the expected increases in leaf litter translocation and burrow system formation, but with minimal or no significant effects on carbon outputs and annual carbon storage. However, as our experiment excluded plant and root exudates, both significant drivers of belowground forest C cycling (Nadelhoffer and Raich 1992, Andrews et al. 1999, Gaudinski et al. 2000), our ability to extrapolate to earthworm invasions impacts under in situ conditions is limited. Overall, this work contributes to the process-level understanding of how earthworm species interactions modify factors that ultimately determine soil C storage across forest ecosystems. Future studies with increased observation times and comparative studies that manipulate both earthworm species diversity and forest soil types would build on this baseline understanding of the net impacts of earthworm communities on forest soil C storage.

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Supplementary Material

Appendix A

Description of the study area (*Ecological Archives* E094-261-A1).

Appendix B

Tables showing numerical values of soil C budget variables (leaf litter mass loss, post-treatment soil C and N properties) across treatments, and a figure showing Spearman rank correlations (*Ecological Archives* E094-261-A2).

Appendix C

Tables showing numerical values of burrow system structure properties (macrostructure, continuity, size distribution) across treatments, and a figure showing Spearman rank correlations (*Ecological Archives* E094-261-A3).