Exotic Earthworm Communities as Drivers of Soil Carbon Dynamics in Northern Temperate Forests

by

Jasmine M. Crumsey

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Doctoral Committee:

Professor Knute J. Nadelhoffer, Co-chair
Professor George W. Kling, Co-chair
Professor William S. Currie
Professor Deborah E. Goldberg
Professor Donal R. Zak

DIRECTIONS TO NORTH FISHTAIL BAY[‡]

If you paddle down past
the point where the eagles
hang out, you're almost there.
It's best like this – a hint
of fog flittering across
the lake before a breeze.
No sun, sky gray, but calm,
not a ripple or a wave.
Just round the next point, where
the sand drops away fast
under luminous deep green
water . . . And you made it!
Go now. It looks like rain.

You'll hear a hermit thrush
calling, hidden in the pines
or in a cedar swamp
where, when you look hard into the dark, you will see
a profusion of iris,
almost purple and fresh
on this day, the very day you've
come alone to North Fishtail Bay.
There's thunder in the west.
Go now. It looks like rain.

[‡] Reprinted from "Directions to North Fishtail Bay" in *If the World Becomes So Bright* by Keith Taylor. Copyright © 2009 Wayne State University Press, Detroit, Michigan. Reprinted as the dissertation frontispiece with permission of the author and publisher.

DEDICATION

To my parents, Iris and Alvin Crayton, who have given me the support to explore each opportunity that comes my way; to my brothers, Brandon and Cameron, who have grown and laughed with me through all these years; to my extended family and friends for good times and many life lessons; and to a cadre of mentors that have helped me appreciate the craft of scientific inquiry.

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ABSTRACT

This research applies community and ecosystem ecology principles to answer previously unresolved questions regarding changes in forest soil carbon dynamics in response to exotic earthworm introductions. Here, I describe four separate studies completed in northern temperate forests at the University of Michigan Biological Station. These studies (1) characterized the spatial and temporal variability in the species composition of exotic earthworm communities across a forest landscape, and (2) evaluated key factors that contribute to variation in exotic earthworm community impacts on soil carbon content, chemistry, and redistribution. Specifically, this dissertation makes the following conclusions:

1. *Exotic earthworm species distributions show spatial variation associated with leaf litter and soil properties, and have expanded across upland forests over decadal time scales.*

   Using bi-annual field surveys and a re-analysis of earthworm species distribution data collected 60 years earlier in the same landscape, I infer long-term shifts in the composition of exotic earthworm communities along with relationships between earthworm species distributions and environmental factors. Historical surveys conducted by W.R. Murchie provide the earliest comprehensive records of exotic earthworm species distributions across re-growing forests and cultivated lands surrounding UMBS. Soil-dwelling species present across the landscape are of Eurasian origin and include *Lumbricus terrestris, Lumbricus rubellus, Aporrectodea trapezoides, Aporrectodea caliginosa,* and *Dendrobaena octaedra*. Historical and contemporary records indicate a long-term succession in the composition of exotic earthworm communities in upland forest soils, with increased species diversity over time and the recent establishment of *Lumbricus terrestris*. Unlike incipient earthworm invasions actively occurring in other parts of the Great Lakes region, present-day earthworm communities in Michigan's northern lower peninsula appear stable over time with no apparent invasion fronts moving across the landscape. Species-specific responses to

environmental factors in the landscape studied here are likely due to differences in: (1) dispersal limitation, (2) response to soil moisture regimes, and (3) dependence on resource quality and foraging behavior. Results provide novel insights into earthworm community dynamics following initial species introductions into previously-glaciated forests originally devoid of soil-dwelling earthworm species.

2. *Exotic earthworm species present on site represent three ecological groups, which vary in burrowing and foraging activity. Variation in the species composition of exotic earthworm communities thereby results in community-specific impacts on all major components of soil carbon budgets.*

   Using a one-year laboratory experiment with three earthworm species belonging to different ecological groups, I resolved changes in soil C content, cumulative CO_2 losses, dissolved organic C (DOC) losses, and burrow system structures to explain how variations in earthworm community composition affect soil C budgets. These three groups include endogeic (mineral soil dwelling), epigeic (surface soil dwelling), and anecic (surface-feeding, deep-burrowing) earthworm species. Soil CO_2 loss was greater from the Endogeic × Epigeic treatment than from controls (no earthworms) over the first 45 days; CO_2 losses from mono-specific 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 (either alone, or in combination with other ecological groups) accelerated litter C loss with differential mass loss of litter types 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 through leaf litter relocation to A-horizon C and N pools, as indicated by strong correlations between (1) sub-surface vertical burrows made by anecic species, and accelerated leaf litter mass losses (with the exception of *Pinus strobus*); and (2) dense burrow networks in the A-horizon and the C and N chemistry 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. Variation in the species composition of exotic earthworm communities thereby results in community-specific impacts on all major components of soil carbon budgets.

3. *Leaf litter degradation and transport are key processes by which earthworm communities influence soil carbon budget components. Leaf litter degradation is primarily driven by the foraging activity of one ecological group; while leaf litter redistribution in the soil profile is determined by the volume and connectivity of burrow systems created by the composite earthworm community. The subsequent fates of litter-derived carbon and nitrogen (gaseous and leachate loss, retention in the soil profile) in earthworm-invaded soils are strongly influenced by soil texture.*

   In a second laboratory experiment, I characterize mechanisms determining community-specific impacts of exotic earthworms on soil C budgets, by tracking the redistribution of dual-labeled (^{13}C and ^{15}N) leaf litter in sandy and sandy loam Spodosols. I combined earthworm species of three functional groups (*Lumbricus terrestris* [anecic], *Aporrectodea trapezoides* [endogeic], and *Eisenia fetida* [epigeic]) in a factorial design. Over a 150-day incubation study, I measured all major components of the soil carbon budget including leaf litter ^{13}C and ^{15}N redistribution using isotopic mass balance, and assessed sub-surface burrow system structures using X-ray computed tomography. I observed a difference in the onset of earthworm community-enhanced CO_2 release, with sandy loam soils showing a longer temporal lag prior to maximum respiration than sandy soils. Isotopic tracers revealed that A-horizons were dominant sinks for leaf litter C and N, with ^{13}C and ^{15}N transport significantly higher in (1) sandy loam than in sandy soil, and (2) in sandy soil containing both endogeic and anecic species relative to sandy soils in which these groups were absent. Together, the results demonstrate the importance of interspecific interactions and soil properties in determining observed impacts of exotic earthworm communities on the soil C processes in northern temperate forest soils.

4. *The alteration of multiple soil carbon processes by earthworm activity can lead to minor shifts in the net soil carbon budget, though shifts in soil carbon chemistry may have long-term implications for rates of soil carbon turnover.*

Using an existing field-based leaf litter manipulation experiment, I provide preliminary data on relationships between earthworm species diversity, leaf litter loading, and long-term (7-year) shifts in soil carbon chemistry and depth distribution. Earthworm biomass and species diversity increased with leaf litter loading, with significant declines in earthworm biomass observed with leaf litter removal. Over a seven-year period of leaf litter manipulations, no significant shifts in soil C and N pools or isotopic values were detected along the experimental leaf litter gradient. Soil chemical properties differed with depth and time, showing a general decline over the seven-year observation period. ^{13}C CPMAS NMR spectra from composite A-horizons suggest differences in soil carbon chemistry with a higher abundance of recalcitrant C forms (carboxyl-C and aromatic-C) with leaf litter removal. NMR spectra also suggest an accumulation of labile C forms (alkyl-C and O-alkyl C) with increased leaf litter inputs and the highest earthworm densities. These shifts are likely a function of increased leaf litter degradation and incorporation into soil organic matter pools associated with earthworm activity, and may have long-term implications for rates of soil carbon turnover.

This dissertation research answers questions concerning net changes in soil carbon budgets following exotic earthworm introductions, and community-specific impacts on soil carbon processes. Importantly, the establishment of fundamental baseline data (in the form of soil C budgets) to compare earthworm community impacts on soil C content, and evaluation of earthworm species distribution following regional spread across the landscape, will contribute to the growing literature on biological invasions in north temperate forests of the Midwestern and Northeastern U.S. and advance our general knowledge of exotic earthworm invasions and their impacts.

Chapter 1

Introduction

Across northern temperate and boreal regions in North America, there is a growing concern about exotic earthworm invasions and associated impacts on the carbon storage capacity of forest soils. Several factors, described below have, hindered the synthesis of recent results into a comprehensive and coherent understanding of exotic earthworm community dynamics and impacts on temperate forest ecosystems. In this dissertation, I use empirical observations and experiments presented in this dissertation have extended our understanding of relationships between earthworm community dynamics and forest ecosystem function. In this introduction, I provide (1) an overview of our current understanding of exotic earthworm community dynamics and impacts on northern temperate forest ecosystems, (2) a description of the experimental forests and exotic earthworm communities at the University of Michigan Biological Station (Pellston, MI) where field and laboratory experiments were conducted, (3) an outline of the research objectives and strategies developed to address existing gaps in our understanding, and (4) an overview of the dissertation chapters that follow.

1.1 Earthworm Community Dynamics and Forest Ecosystem Function

Earthworm Community Dynamics in Northern Temperate Forests

Earthworms were extirpated from northern temperate and boreal regions in North America during the last glacial maximum, and have been slow to recolonize these ecosystems for at least 10,000 years (James 1995). Earthworm species (Lumbricidae) of European-origin whose native habitats match many of those of northern temperate and boreal regions in North America previously devoid of earthworms are generally successful invaders having widespread distributions (Gates 1982, Reynolds 1995). These species vary in physiology, foraging activity, and burrowing patterns (Table 1.1), and include *Dendrobaena octaedra* (Savigny), *Lumbricus rubellus* (Hoffmeister), *Lumbricus terrestris* (Linneus), and *Aporrectodea caliginosa* (Savigny), and Aporrectodea trapezoides (Dugès) (Appendix A). Invasions into northern temperate and boreal forest ecosystems involve two essential stages: transport of organisms to a new location (i.e., introduction), and population establishment in the invaded locality (i.e., colonization) (Williamson and Fitter 1996, Mack et al. 2000).

The community dynamics of a third stage, regional spread from initial successful populations (Shigesada and Kawasaki 1997), determine (1) the species composition and distribution of established exotic ecological communities across forest landscapes and (2) the long-term impacts of exotic communities on ecosystem functions (Loreau 2000, Hooper et al. 2005, Cottenie 2005). Though studies of established earthworm communities are extensive across European northern temperate forests where human-facilitated earthworm dispersal began in the 13th – 15th centuries (Tiunov et al. 2006b), similar studies are few in northern temperate forests of North America where human-facilitated earthworm dispersal began in the 17th – 19th centuries (Stoscheck et al. 2012, Crumsey et al. 2013a).

Exotic earthworm introductions into previously earthworm-free northern temperate and boreal regions of North America are also associated with shifts in forest ecosystem structure and function; of particular interest here are associated changes in soil carbon dynamics. In the Great Lakes region, net ecosystem production of forest stands is approximately 1.53 ± 1.15 Mg C ha^{-1} yr^{-1}, with net storage estimated as 180.5 ±12.8 Mg C ha^{-1}, with 44% (80 ± 12.4 Mg C ha^{-1}) stored in soil organic matter (Gough et al. 2008b). Because of its importance in C storage, alteration to pool sizes, chemistry, or turnover times of C in soil organic matter have large implications for the overall C dynamics of temperate forest ecosystems. Shifts in soil C dynamics following exotic earthworm introductions can lead to changes in C stocks (Alban and Berry 1994, Burtelow et al. 1998, Bohlen et al. 2004c, Wironen and Moore 2006, Fahey et al. 2012), C depth distribution (Burtelow et al. 1998, Bohlen et al. 2004b, Wironen and Moore 2006, Straube et al. 2009, Fahey et al. 2013a), elevated CO_2 losses (McLean and Parkinson 1997a, Li et al. 2002, Fisk et al. 2004a, Eisenhauer et al. 2007, Aira et al. 2009), and dissolved organic C losses (Haimi and Huhta 1990, Scheu and Parkinson 1994, McInerney and Bolger 2000, Bohlen et al. 2004b). The magnitude and direction of shifts in forest soil C dynamics depend on the species composition of exotic earthworm communities (Hale et al. 2005c, Straube et al. 2009) and are constrained overall by land use history (Bohlen et al. 2004, Gough et al. 2008a, Ma et al. 2013), soil properties (Scheu and Parkinson 1994, McInerney and Bolger 2000, Marhan and Scheu 2006), and leaf litter chemistry (Hobbie et al. 2006a, Melvin and Goodale 2013). Despite this recent research, not all studies have found the same results, and the net impacts of earthworm invasions on forest soil C balance are unknown due to the fact that no single study has measured all major components of a forest soil C budget (inputs and outputs of particulate, dissolved, and gaseous forms) associated with earthworm community activity (but see Chapter 3, Crumsey et al. 2013b).

Earthworm activity influences forest soil C dynamics through enhanced degradation and transfer of surface litter materials to subsurface soils (Jégou et al. 1998, Fahey et al. 2013a), and mixing of soil horizons by subsurface burrow system construction (Jégou et al. 2000, Bohlen et al. 2004b, Don et al. 2008, Crumsey et al. 2013b). These processes alter organic matter decomposition rates by increasing the availability of labile C and nitrogen (N) for microbial processing (Burtelow et al. 1998, Tiunov and Scheu 1999, Brown et al. 2000, Li et al. 2002, Groffman et al. 2004). Earthworms also alter organic matter stabilization by increasing aggregate formation and organo-mineral associations (Scheu 1987, McInerney and Bolger 2000, Lavelle et al. 2004, Bossuyt et al. 2005, Marhan and Scheu 2006). These results have led to a prediction that exotic earthworm introductions will result in an initial decline in soil C storage with the elimination of forest floor horizons, followed by a subsequent increase in soil C storage as soil C is stabilized by earthworm activity (Lavelle et al. 1998, 2004). Understanding changes in forest soil carbon dynamics in response to exotic earthworm introductions thereby necessitates (1) short-term studies that explicitly test of how earthworm species interactions and soil properties control earthworm community impacts on total soil carbon budgets, soil structure, and organic matter redistribution; coupled with (2) long-term studies that evaluate earthworm community impacts on soil carbon dynamics over time (Crow et al. 2009a, Fahey et al. 2013a, Melvin and Goodale 2013).

1.2 Dissertation Overview

To extend our understanding of exotic earthworm impacts on forest soil structure and processes, I completed four studies at the University of Michigan Biological Station (UMBS) Forest Ecosystem Study (FEST), a long-term site for research on forest succession and ecosystem processes in northern Lower Michigan, USA (45°35.5’N, 84°43’W). The 30-hectare study area functions as an experimental forest in which the secondary successional forests are currently dominated by bigtooth aspen (*Populus grandidentata*), and lie on outwash plains with well-drained soils classified as mixed, frigid Entic Haplorthods of the Rubicon series (National Resources Conservation Service 1991). Tree species composition, forest age, and disturbance history of the aspen-dominated forest represents a regionally dominant forest type (USDA Forest Service 2002). Across this landscape, five earthworm species of European origin representing different ecological groups (Bouché 1977) dominate earthworm communities across the FEST research area (Crumsey et al. 2013a). The species include *Dendrobaena octaedra* (Epigeic = litter feeding, surface–dwelling), *Aporrectodea caliginosa*/*Aporrectodea trapezoides* (Endogeic = mineral soil feeding and dwelling), *Lumbricus rubellus* (Epi-endogeic), *Lumbricus terrestris* (Anecic = litter feeding, vertical burrowing). These communities were first described in the early 1900's through studies that provide a historical context for the study of exotic earthworm communities in a previously earthworm-free northern temperate forest (Smith and Green 1916, Murchie 1954, 1956). The overarching research objectives and overview of the four studies comprising this dissertation are described below.

Objectives

The focus of this work is to describe the community dynamics of exotic earthworms that were first documented in aspen-dominated forests of northern-lower Michigan 60 years ago, and earthworm community-specific impacts on soil biogeochemical processes. I address the knowledge gaps outlined above through the following research objectives:

- Characterize spatial and temporal variability of established exotic earthworm communities in a north temperate forest.
- Establish fundamental baseline data (in the form of soil C budgets) to compare earthworm community impacts on soil C content.
- Investigate the role of earthworm-mediated litter degradation and redistribution as compensatory mechanisms maintaining short-term carbon balance across dominant soil types of a north temperate forest.
- Describe long-term impacts of exotic earthworm communities and litter loading on soil C content and chemistry.

To accomplish these objectives, I used long-term field experiments previously established at the University of Michigan Biological Station, and I conducted two laboratory mesocosm experiments to collect fundamental baseline data describing earthworm community effects on all major components of soil carbon budgets. I also developed extensive collaborations with researchers at the University of Michigan Biological Station, University of Michigan School of Radiology, the French National Institute for Agricultural Research, and Queens College City University of New York. Analysis of earthworm community responses to environmental conditions and effects on soil properties necessitated the application of multivariate statistical techniques widely used in community ecology, in conjunction with elemental and isotopic mass balance approaches used in ecosystem ecology.

Overview of the Dissertation Chapters

In this dissertation, articles are compiled that have been or will be published independently in scientific journals. Chapters 2 – 5 describe the design and outcomes of four studies that characterize exotic earthworm species distributions in a northern temperate forest landscape and describe community-specific impacts on soil carbon and nitrogen dynamics. In Chapter 2, I present an analysis of historical earthworm species distribution data, and use these findings to inform contemporary associations between earthworm species distributions and environmental factors. Chapter 3 details earthworm community-specific impacts on soil structure and the net soil carbon balance using a one-year laboratory experiment in which earthworm species representing different ecological groups were combined in a full-factorial design. In Chapter 4, I used results of the study described in Chapter 3 to design a second laboratory experiment in which isotopically labeled leaf litter and two major forest soil types that differ in organic matter content were used to quantify community-specific impacts on the flux of leaf litter carbon and nitrogen into soil pools, and how earthworm community impacts on this process are mediated by soil texture. Preliminary data on the long-term implications on soil carbon storage and chemistry in response to exotic earthworm activity and leaf litter manipulations, using a long-term leaf litter manipulation experiment, is presented in Chapter 5. In the final chapter, I summarize conclusions from the previous chapters and discuss implications of my findings for future research.

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Chapter 2

Using historical patterns of exotic earthworm distributions to inform contemporary associations with soil physical and chemical factors across a northern temperate forest [2]

Abstract

Understanding environmental factors related to exotic earthworm distributions across invasion stages (i.e., introduction, colonization, regional spread) is critical for assessing long-term impacts on previously earthworm-free forests. Studies following earthworm community establishment in North America, however, remain limited. We address this by characterizing historical and current exotic earthworm distributions in a regionally representative aspen-dominated forest, where their presence was first documented in the early 1900s. We map historic earthworm distribution records in a 360-km^2 area surrounding our current study site, and re-analyze data collected nearly 60 years ago to inform contemporary associations between species densities and environmental factors. Field surveys were conducted over two years (2008 – 2010) using 10 permanent plots, with concurrent measurements of environmental 'effect factors' determined by large-scale ecosystem processes (leaf litter inputs, soil physical properties, soil C and N content), and environmental 'response factors' likely impacted by earthworm activity over short time scales (annual litter mass loss and soil isotopic values). Present-day communities included five exotic species with varying densities: *Lumbricus rubellus* ≥ *Lumbricus terrestris* >> *Dendrobaena octaedra* ≥ *Aporrectodea* spp. (*Aporrectodea trapezoides* + *Aporrectodea caliginosa*). These species were also present in the landscape in the early to mid-1900s though shifts in species composition, particularly the movement of *L. terrestris* into upland forest soils, were evident. Over two years, earthworm community composition did not show strong temporal or spatial trends characteristic of incipient invasions. However, species-specific associations with environmental factors were observed: *L. terrestris* and *L. rubellus* densities were positively associated with soil C and N content, *Acer rubrum* (red maple) inputs, and soil moisture; and were negatively associated with *Pinus strobus* (white pine) inputs. *D. octaedra*, and *Aporrectodea* spp. densities were positively associated with % sand; and negatively associated with plot-to-road distance. Soil moisture and texture were significant drivers of earthworm species abundance in historical surveys, though associations with soil C were only evident for *Aporrectodea* spp. Contemporary associations between earthworm species and soil C and N content suggest greater nutrient limitation in upland forest soils, while the importance of plot-to-

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road distance suggests the persistence of dispersal limitation and repeated introductions as a mechanism maintaining population densities. Species-specific associations with environmental response variables were also observed, where: surface soil $\delta^{13}C$ depletion was associated with *Aporrectodea* spp. and *D. octaedra* biomass; $\delta^{15}N$ enrichment was associated with total earthworm biomass, but negatively associated with *L. rubellus* biomass; and increased leaf litter mass loss was associated with *L. terrestris* and juvenile biomass. As soil C and N pools were not higher in plots with higher earthworm biomass, these results suggest earthworm activity may influence soil element cycling by decreasing turnover times of nutrient pools over the long-term. Our results characterize exotic earthworm distributions at scales relevant to forest ecosystem processes, and allow for future extrapolation of laboratory and controlled field studies assessing impacts on soil nutrient cycling across northern temperate forests.

2.1 Introduction

Quaternary glaciations resulted in the removal of earthworms from northern temperate and boreal regions in North America and northeastern Europe, and northward migrations of endemic earthworm species following glacial retreat have been limited (James 1995). The reintroduction of earthworm species into northern temperate and boreal regions has instead been facilitated by European human migration and disturbance; beginning in Europe in the 13th – 15th centuries, and in North America in the 17th – 19th centuries (Tiunov et al. 2006a). Interestingly, European migrations into these regions have resulted in a common suite of what are now considered “peregrine” earthworm species of the Lumbrucidae family, including *Aporrectodea caliginosa, Dendrobaena octaedra, Lumbricus rubellus*, and *L. terrestris* (James and Hendrix 2004, Holdsworth et al. 2007). The relatively recent establishment of exotic earthworm populations (i.e. within the last 150 years) in previously earthworm-free North American landscapes has been facilitated by similar climate regimes of northeastern European where these species have been naturalized (Reynolds 1995, Tiunov et al. 2006a). Although climatic drivers determine macro-scale distributions of earthworm species (Lavelle 1983, Edwards and Bohlen 1996a, Tiunov et al. 2006a), regulation of earthworm community composition and density at smaller regional and landscape scales is largely determined by mechanisms of reintroduction and environmental factors.

In North America, the earliest mechanisms of reintroduction were both passive, such as when earthworms were introduced through the removal of soils used as ballast for ships, and active, following the purposeful reintroduction or earthworm species for agriculture (Gates 1942, Schwert 1980, Edwards and Bohlen 1996a, Eijsackers 2011). The latter mechanism is still operative, but is accompanied by a suite of other mechanisms that include the inclusion and release of peregrine earthworm species as fishing bait, and passive dispersal facilitation through road construction, vertebrate transport, and imports of soil-containing materials (Gates 1982, Ehrenfeld and Scott 2001, Cameron et al. 2007, Hendrix et al. 2008, Cameron and Bayne 2009). Although the presence of exotic earthworm species in northern temperate and boreal forests were first documented in the early 1900s, historical patterns of earthworm species abundance and distributions are largely unknown for most areas where incipient invasions are occurring.

Characterization of earthworm community association with environmental factors in European forests accompany a larger understanding of top-down controls such as predation and disease (though this is still limited) (Curry 1994), the importance of earthworm activity in the remediation of acidified forest soils (Deleporte and Tillier 1999, Potthoff et al. 2008, Hirth et al. 2009), and the importance of human disturbance and land management in regulating earthworm community establishment (Muys and Granval 1997, Tiunov et al. 2006a, Eisenhauer et al. 2009). In northern temperate and boreal forests of North America, these associations have largely been used to understand the likelihood of invasion success given active mechanisms of introduction. For example, previous work has determined that the primary factors determining invasion success in northern temperate forests include propagule pressure (Hale et al. 2005a), seasonal abundance dynamics (Callaham and Hendrix 1997), and environmental factors including soil pH, temperature, soil moisture, and litter palatability (Decaëns and Rossi 2001, Whalen 2004a, Reich et al. 2005b, Sackett et al. 2012, Fisichelli et al. 2013). Further, in areas where human-mediated dispersal of earthworm species occurs, distance from roads and cabins within forested areas have also been reported as major predictors of earthworm species densities (Hale et al. 2005, Holdsworth et al. 2007). What remains is a limited understanding of how these associations might change as earthworm communities become established across landscapes. Better understanding of environmental controls on exotic earthworm distributions following regional establishment is essential to extrapolating from laboratory and small plot studies to ecosystem scales at which nutrient dynamics are studied (Whalen and Costa 2003).

Similarly, earthworm impacts on temperate forest ecosystems have primarily been characterized during incipient invasions in North America, and impacts on some ecosystem processes and properties are better understood than others. Areas of dense earthworm invasion have shifted understory plant diversity and diminished forest floor horizons (Bohlen et al. 2004c, Hale et al. 2006). These invasions have also been linked to decreased soil C stocks (Burtelow et al. 1998, Lachnicht and Hendrix 2001, Bohlen et al. 2004c, Marhan and Scheu 2006, Eisenhauer et al. 2007, Sackett et al. 2012), soil C redistribution (Burtelow et al. 1998, Lachnicht and Hendrix 2001, Bohlen et al. 2004b, Wironen and Moore 2006, Straube et al. 2009), and increased soil CO_2 exports (Marhan and Scheu 2006). However, limited information is available on how different earthworm species affect leaf litter disappearance in temperate forest ecosystems (cf. Suárez et al. 2006, Holdsworth et al. 2008, 2012) and particulate organic matter chemistry in surface soils (Marhan et al. 2007, Crow et al. 2009b, Crumsey et al. 2013b, Fahey et al. 2013a). Therefore, it is necessary to characterize impacts on leaf litter mass loss and soil chemistry following earthworm community establishment to enhance understanding of the ecological consequences of exotic earthworm distributions.

In this study, we analyze two historical data sets to map approximate geographic locations of earthworm species collections in a regionally representative hardwood forest, and characterize relationships between the relative abundance of earthworm species and soil physical and chemical properties qualitatively evaluated approximately 30 years after major logging and fire disturbances. We then report the results of field surveys conducted over two years, nearly 60 years following previous studies, examine contemporary patterns of exotic earthworm species densities, and characterize environmental correlates of species distributions; we consider site distance from potential introduction sites (lake shore and roads) and environmental factors. We divided environmental factors into ‘effect factors’ determined by large-scale ecosystem processes (leaf litter inputs, soil physical properties, soil C and N content), and ‘response factors’ likely impacted by earthworm activity over short time scales (litter decomposition, soil isotopic values). We address the following questions; (1) How have the relative abundance of earthworm species and interaction with environmental variables changed as communities become established across the landscape? (2) Considering earthworm species biomass as a proxy for earthworm activity, to what extent do earthworm species distributions affect ecosystem processes (specifically, decomposition processes characterized by leaf litter mass loss rates and soil isotopic values)? We expected long-term shifts in the relative abundance of earthworm species, with leaf litter production and soil properties functioning as bottom-up controls to determine earthworm community distributions. We also expected strong functional consequences of earthworm communities for measured ecosystem processes.

2.2 Materials and Methods

2.2.1 Site description

Our study was conducted at the University of Michigan Biological Station (UMBS) in northern Lower Michigan, USA (45°35.5’N, 84°43’W); located within a landscape from which glaciers receded approximately12,000 years BP (Fig. 1a). Secondary successional forests are currently dominated by bigtooth aspen (*Populus grandidentata*), with northern red oak (*Quercus rubra*), red maple (*Acer rubrum*), paper birch (*Betula papyrifera*), American beech (*Fagus grandifolia*) and eastern white pine (*Pinus strobus*) occurring as co–dominants (Curtis et al. 2005). The presettlement white pine, red pine, hemlock forest was cut around 1880, and the study area was disturbed repeatedly and uniformly by fire until 1923; there is no history of agriculture (Gough et al. 2007). UMBS forests lie on outwash plains with well-drained soils (92.9% sand, 6.5% silt, 0.6% clay, pH 4.8) classified as mixed, frigid Entic Haplorthods of the Rubicon series (National Resources Conservation Service 1991). Seasonal average temperature and precipitation regimes (1980 – 2010) are as follows: Spring (April – May) temperature is 8.9 ± 0.7°C, and precipitation is 6.9 ± 0.4 cm; Fall (September – October) temperature is 11.7 ± 0.7°C, and precipitation is 8.9 ± 0.5 cm (Vande Kopple 2012). Tree species composition, forest age, and disturbance history of the aspen-dominated forest represents a regionally dominant forest type (USDA Forest Service 2002).

2.2.2 Secondary analysis of historical earthworm survey data (1916 – 1954)

The presence of exotic earthworm species in Michigan was first documented in the early 1900s (Smith and Green 1916, Gates 1942). Comprehensive surveys of earthworm species richness and relative abundance across various habitat types were later conducted by W.S. Murchie (1954, 1956). Historical earthworm distributions were mapped across a 360 km^2 area surrounding present-day research sites for the dominant soil-dwelling species: *D. octaedra*, *L. rubellus*, *L. terrestris*, and *A. caliginosa* (Smith and Green 1916, Murchie 1954; Fig 1b). Site locations were georeferenced in ArcGIS 10.1 (ESRI 2011) using (1) state of Michigan public lands section grid system (Michigan Department of Natural Resources [MDNR] and Rockford Map Publishers, Inc. 1998), (2) Michigan Spring 2012 Digital Orthophoto Quadrangle aerial imagery (MDNR and U.S. Geological Survey 2012), and (3) historical descriptions of earthworm sampling locations indexed by township, range, and section for earthworm species surveys completed by Smith and Green (1916) and extended by Murchie (1954).

In a second historical data set compiled by Murchie (1954), the abundance of earthworm species per ~ 2L of soil were classified in semi-quantitative ranks as follows: 0 = absent; 1 = rare, 1 – 3 specimens; 2 = few, 3 – 15 specimens; 3 = common, 15 – 25 specimens; 4 = abundant, 25+ specimens. Statewide collections occurred across 86 terrestrial sites, and were classified as hardwood forests (n=44), stream banks (n=2), mixed grass and shrub cover (n=30), and open fields (n=3). Six cases were excluded due to missing data. Site observations for each species were as follows: *A. caliginosa*/*A. trapezoides* (n = 56), *L. rubellus* (n = 25), *L. terrestris* (n = 8), and *D. octaedra* (n = 5). Originally, densities of each species were qualitatively related to measures of soil physical and chemical properties including: pH (3.9 – 7.75), percent water-holding capacity (28.5 – 100%), percent organic C (0.03 – 3.18%), and percent sand (12.5 – 98.5%).

In our secondary analysis of this historical data set, we treated relative abundance ranks of earthworm species as ordinal dependent variables, and assessed relationships with soil physical and chemical properties by species-specific ordinal logistic regressions with stepwise variable selection. Measures of soil physical and chemical properties were standardized to a zero mean and unit variance. We used binomial logistic regression with stepwise variable selection for presence-absence of *D. octaedra* as all reported cases had an abundance rank value = 1. An ordinal logistic regression with stepwise variable selection was then carried out using as the dependent variable the *total* number of earthworm species recorded in each site (i.e. inclusion of presence data for rare soil-dwelling species: *Bimastos longicinctus* and *Diplocardia singularis*; and log-dwelling species: *Bimastos beddardi*, *Bimastos tenuis*, and *Bimastos zeteki*). We applied Akaike's Information Criterion (AIC) as a measure of model performance and the complexity of each regression model (Akaike 1974, Burnham and Anderson 2004), and assessed the significance of coefficients via the likelihood ratio test. Statistics were performed in R version 2.15.2 (R Development Core Team 2012) on RStudio version 0.96.331 (http://www.rstudio.org/), using the packages: MASS (Venables and Ripley 2002), Hmisc (Harrell 2012), and aod (Lesnoff and Lancelot 2012).

2.2.3 Characterization of present-day soil-dwelling earthworm communities

Present-day measurements of earthworm communities and soil properties were conducted in 10 permanent 0.08 ha plots distributed across ~140 ha of a forested area nested within the footprints of two eddy covariance towers: the UMBS AmeriFlux tower (AmeriFlux code: US-UMB) and the FASET eddy-flux tower (AmeriFlux code: US-UMd) (Fig. 1c). Each tower is surrounded by a circular 1.1 ha plot and smaller plots (0.08 ha) established along transects radiating out from the towers at 100 m intervals. Principal components analysis was previously used to identify a subset of plots from the overall pool ($n = 73$), that encompassed landscape-level variation in the relative abundance of overstory tree species using litterfall data (85 to 220 g C m^{-2}), and variation in productivity levels using plot-level leaf area index data (LAI range: 2 to 7) (Gough et al. 2008b, Nave et al. 2011a). Using ArcGIS 10.1 (ESRI 2011), we measured distances between plot centers and two potential sites of earthworm introduction: Douglas Lake shore (~ 200 to 930m), and a road bisecting the study area (~ 660 to 3700m).

Earthworm communities were first sampled in October 2008, and biannually in May and October from 2009 – 2010. Within each plot, we established five randomly distributed 0.25m^2 subplots. We removed and sorted the Oi horizon to collect surface-dwelling earthworms, and used an electroshock extraction method to sample sub-surface earthworms (Thielemann 1986, Bohlen et al. 1995). Electroshocking probes were sets of eight steel rods (50 cm long × 6 mm diameter), installed in a 4 × 4 array at 6.25 cm apart. Eight-wire delivery cables were connected to a gasoline-powered generator (Honda EU 2000i) at one end, and split at the other end where alligator clips were connected and attached to probes. We applied 120 V A.C. for 20 minutes, and collected all earthworms that surfaced. A 1m^2 buffer was placed around each subplot for subsequent sampling. Adult earthworms were identified to species, and juvenile earthworms were identified to genus according to Schwert (1990). Each specimen was measured field moist, and frozen at -80°C until freeze-dried for archiving. Earthworm freeze-dried weights from the October 2008 collection were used to calculate a fresh-to-dry weight ratio and estimate species-specific earthworm biomass across plots for each sampling period.

2.2.4 Annual leaf litter inputs and mass loss

Total and species-specific leaf litter production in each plot was estimated using leaf litter traps (0.264 m^2) deployed annually. Traps were emptied weekly during leaf abscission (September – November), and monthly otherwise. Leaf litter was separated by species (*A. rubrum, B. papyrifera, F. grandifolia, P. strobus, P. grandidentata*, and *Q. rubrum*), dried at

60°C, and weighed. Leaf litter %C was measured by a CN elemental analyzer (Costech Elemental Analyzer 1030), and multiplied by dry leaf litter mass to determine total and species-specific leaf litter production (g C/m^2). Annual leaf litter mass loss was measured using leaf litter bags (13 mm nylon mesh netting; 25 × 25 cm) filled to quantitatively represent plot-level leaf litter species mix and mass inputs (n = 3 per plot). After one year, remaining leaf litter was dried at 60°C, weighed, and the % mass remaining calculated. Though the 13mm mesh size permitted earthworm–mediated leaf litter degradation, this mesh size also allowed for particulate transport, leaf litter data were thereby interpreted as an estimation of plot-level annual leaf litter mass loss rather than a direct measure of annual leaf litter decomposition.

2.2.5 Soil physical and chemical properties

In 2008, we sampled surface soils (Oa-horizon) 1m from each subplot using a 15cm × 15cm monolith. After removing roots, soils were dried at 60°C, and pulverized. Soil %C, %N, δ^{13}C and δ^{15}N were measured by continuous flow isotope ratio mass spectrometry (Thermo Finnigan Delta Plus XL) after sample combustion to CO_2 and N_2 at 1000°C by an on-line elemental analyzer (Costech Elemental Analyzer 1030). Instrument error determined by repeated internal standards was ± 0.19‰ for δ^{13}C and ± 0.16‰ for δ^{15}N. Dry soil mass was multiplied by %C and %N to determine soil C and N content. Extractable Ca^{2+} was determined after extraction of dried soil samples with 0.4M LiCl (Husz 2001) using an inductively coupled plasma optical emission spectrometer (ICP-OES Perkin-Elmer Optima 2000 DV). Soil texture (% sand, % clay, and % silt) was determined using a 40g subsample following the hydrometer procedure (Gee et al. 1986). Soil volumetric water content (%) was recorded at each subplot using a soil moisture sensor (CS620 HydroSense, Campbell Scientific) during earthworm sampling.

2.2.6 Statistical analyses

Plot-level variations in earthworm density and biomass by species were assessed by Kruskal-Wallis tests with nonparametric multiple comparisons (statistic = H, $n = 46$, $\alpha = 0.05$). Pairwise comparisons of earthworm community composition across plots were computed as Bray–Curtis distances, and visualized using non-metric multidimensional scaling (NMDS). The significance of the NMDS ordination was determined using a Monte Carlo permutation test (999 permutations; $P < 0.05$). The multi-response permutation procedure (MRPP, statistic = δ)

(McCune et al. 2002), was used to determine significant differences in earthworm community composition across plots. Two separate permutation-based multivariate analysis of variance tests (PerMANOVA; statistic = F) (Anderson 2001) were used to determine significant differences in earthworm community composition nested by season (fall vs. spring) and year (2008 – 2010).

Earthworm density and biomass were related to environmental factors by co-inertia analysis (CoIA), which identifies co-relationships between transformed species and environmental data matrices (Doledec and Chessel 1994, Dray et al. 2003). CoIA is complementary to canonical correspondence analysis, but is recommended when the number of measured variables is greater than sites sampled (Doledec and Chessel 1994, Borcard et al. 2011). Species-specific earthworm density and biomass measures were first standardized to a zero mean and unit variance. Data matrices of environmental factors and standardized earthworm species densities were each transformed by principal components analysis. We expected earthworm activity to be proportional to biomass due to large differences in the average size and weight of earthworm species present (Bouché 1977), and have the strongest influence on environmental response variables; variation in species-specific earthworm density would more likely indicate responses to environmental effects variables. We thereby applied two co-inertia analyses, measuring (1) relationships between earthworm species abundance and environmental effect variables, and (2) relationships between earthworm species biomass and environmental response variables. Statistical significance of each CoIA was assessed by Monte Carlo permutation tests (999 permutations; $P < 0.05$). All statistics were done in R version 2.15.2 (R Development Core Team 2012) on RStudio version 0.96.331 (http://www.rstudio.org/), using the packages: ade4 (Dray and Dufour 2007), pgirmess (Giraudoux 2012), and vegan (Oksanen et al. 2012).

2.3 Results

2.3.1 Historical patterns of earthworm species abundance and relation to environmental factors

Historical distribution records of exotic earthworm species first completed by Smith and Green (1916) and extended by Murchie (1954), show that *A. caliginosa*, *L. rubellus*, and *D. octaedra*, and were distributed throughout the landscape surrounding the current study area (Figure 1bc). *A. trapezoides* was considered a “form” of *A. caliginosa* when earthworm surveys were conducted (Murchie 1954, 1956), but is now considered a distinct species (c.f. Pérez-Losada et al. 2012); therefore, records of *A. caliginosa* presence likely include distributions of *A. trapezoides* and are referred to as *Aporrectodea* spp. here. *L. terrestris* was present in the region, though only one record from an agricultural site was reported within the 360 km^2 survey area. Statewide surveys (119 total sites) showed similar patterns of species presence, where the most widely distributed species were *Aporrectodea* spp. (all sites) and *L. rubellus* (46% of sites), *D. octaedra* (16% of sites) was present in the western and northern parts of the state, and *L. terrestris* (18% of sites) was primarily located in the southern part of the state (Murchie 1954).

Soil water holding capacity, percent sand, and the interaction between these two variables had significant effects on the relative abundance of earthworm species, and were stronger drivers of overall earthworm species richness than pH or percent organic carbon (Table 1). Percent sand was negatively associated with earthworm species densities and total earthworm species richness, whereas the opposite was observed for soil water holding capacity; these effects were strongest for *L. terrestris* and *D. octaedra*. *L. rubellus* abundance was positively associated with pH, but was not explained by any remaining soil variables. *Aporrectodea* spp. abundance was also positively associated with pH, and was the only group whose abundance was associated with percent soil organic carbon (Table 1).

2.3.2 Present-day patterns of earthworm distribution and abundance

Five species of European origin representing different ecological groups (Bouché 1977) dominate earthworm communities sampled in our present study (Figure 2). The species include *D. octaedra* and *L. rubellus* (Epigeic = litter feeding, surface–dwelling), *L. terrestris* (Epi-anecic = litter feeding, vertical burrowing), *A. caliginosa* and *A. trapezoides* (Endogeic = mineral soil feeding and dwelling). Spatial patterns of earthworm biodiversity showed variation in total earthworm density, biomass, and community composition. Earthworm density across plots, averaged across sampling dates, ranged from 7 ± 1 to 92 ± 56 individuals m^{-2}, with *L. rubellus* and *L. terrestris* densities being significantly higher than those of *Aporrectodea* spp. and *D. octaedra* (Kruskal-Wallis tests, $P < 0.05$; Table A1). Plot-level earthworm biomass (fresh weight) averaged across sampling dates ranged from 2 ± 0 to 47 ± 15 g m^{-2}, with *L. terrestris* biomass significantly higher than those of all other species (Kruskal-Wallis tests, $P < 0.05$; Table A2).

The NMDS ordination was reliable (Stress = 0.173) and clearly discriminated earthworm communities across plots (Figure 3). The MRPP showed that earthworm community dissimilarity across plots was highly significant, indicating spatial dependence of earthworm community composition (δ = 0.2795, P = 0.001). Pairwise comparisons of earthworm community composition over time did not show strong trends; neither season nor year was significant in the PerMANOVAs (Table 2). We thereby excluded time from subsequent analyses of relationships between earthworm species distributions and environmental factors, and used sampling times as replicate measures of plot-level earthworm species densities and biomass.

2.3.3 Earthworm species abundance and environmental effect factors

Plots exhibited significant differences in most environmental effect factors measured. Soil moisture, extractable Ca^{2+}, and species-specific leaf litter inputs differed significantly across plots, but variations in soil texture, C content, and N content were only marginally significant; soil C:N, soil bulk density, and total leaf litter C inputs were similar across plots (Kruskal-Wallis tests; Table 3). The fact that soil C and N pools were not significantly lower on sites with high earthworm abundance suggests that the effects of these properties on earthworm habitat quality override any responses of soil C and N to earthworm activity across the landscape, and justify their classification as environmental effects variables in the present analysis. The first two axes (F1 and F2) of the co-inertia analysis explained 75.1% of the total variability in the species-specific earthworm density and environmental effect data co-structure (Monte Carlo permutation test, P = 0.001). Earthworm species projections in the co-inertia factorial plane highlight unique responses of earthworm species to environmental effect factors (Figure 4): Densities of *L. terrestris* and *L. rubellus* were positively associated with soil C and N content, *A. rubrum* inputs, and soil moisture; and negatively associated with *P strobus* inputs. Densities of *D. octaedra,* and *Aporrectodea* spp. were positively associated with % sand, and negatively associated with plot-to-road distance. *L.* juvenile density was positively associated with % silt, *F. grandifolia* inputs, and plot-to-road distance, and negatively associated with % sand. Total earthworm density and biomass were strongly correlated with each other and positively associated with soil moisture and litter inputs including *A. rubrum*, *Q. rubra*, and total leaf litter inputs.

2.3.4 Earthworm species biomass and environmental response factors

All three environmental response factors (i.e. soil $\delta^{13}C$ and $\delta^{15}N$ values, and annual leaf litter mass loss) exhibited significant variation across our study's footprint (Kruskal-Wallis tests; Table 4). The first two axes (F1 and F2) of the co-inertia analysis explained 94.0% of the total variability in the earthworm species biomass and environmental response data co-structure; the overall ordination was marginally significant (Monte Carlo permutation test, $P = 0.08$; Figure 5). Surface soil $\delta^{13}C$ depletion was associated with *D. octaedra* and *Aporrectodea* spp. biomass; surface soil $\delta^{15}N$ enrichment was directly associated with total earthworm biomass, and negatively associated with *L. rubellus* biomass. Increased leaf litter mass loss rates were associated with *L. terrestris*, juvenile and total earthworm biomass.

2.4 Discussion

Species distribution surveys first conducted by Smith and Green (1916) along stream and river banks, and later extended by Murchie (1954, 1956, 1960) provide the earliest documented presence of the five exotic earthworm species collected in the present study. These species are also included in those now actively spreading across forests of the upper Great Lakes region (Reynolds 1995, James and Hendrix 2004, Tiunov et al. 2006a, Holdsworth et al. 2007). Interestingly, *Aporrectodea* spp. were the only earthworm species reported within the footprint of the present study area in these earlier studies, and only two records of *L. terrestris* were reported in northern-lower Michigan. Our recent surveys show the presence of all five earthworm species (*Aporrectodea* spp., *L. terrestris*, *L. rubellus*, and *D. octaedra*) across plots, indicating that there have been shifts in earthworm community composition over the past 60 years. Studies of exotic earthworm community dynamics in Canadian and northern U.S. temperate forests have primarily focused on incipient invasions, characterized as 'invasion fronts' (i.e., a succession of earthworm species across a visible leading edge due to different patterns of colonization). These studies show increases in earthworm abundance and diversity with time or distance from introduction sites (Hale et al. 2005a, Suárez et al. 2006a, Cameron et al. 2007, Addison 2009b). Here, the long time over which these organisms have been present across the landscape (i.e. >60 years), limited forest disturbance, and the lack of strong recent temporal trends in earthworm community composition, suggest currently stable communities, unlike those documented for incipient earthworm invasions (Curry 1994). Shifts in the relative densities of species inferred from historical data, however, suggest a long-term succession of earthworm species, and potential facilitation of *L. terrestris* establishment through the preceding activity of epigeic and endogeic earthworm species (Hale et al. 2005a).

Though quantitative measures of earthworm species densities cannot be derived from the historical data, current patterns of earthworm densities suggest strong bottom-up controls of environmental conditions in regulating earthworm communities. Our recent surveys found that total earthworm densities were strongly correlated with increasing leaf litter inputs and soil carbon content, but were in the lower range of the 10 to 1000 individuals m^{-2} reported for active exotic earthworm invasions into temperate forest stands of similar over-story tree composition and leaf litter inputs (Whalen and Costa 2003, Whalen 2004, Hale et al. 2005, Suárez et al. 2006, Eisenhauer et al. 2007, Holdsworth et al. 2007, Shartell et al. 2013). These forests, however, differ markedly from the forest studied here in (1) soil texture (overlying clay-rich soils higher in nutrient content and base saturation than the spodosols studied here); (2) surrounding human land-use patterns (numerous active mechanisms of earthworm species introductions by human activities); and (3) stand-age and land-use history; factors which constrain both earthworm distributions and forest structure (Reynolds 1995, Bohlen et al. 2004b, Gough et al. 2008a, Fahey et al. 2012). The present study also found strong associations between road distance and earthworm species densities in the first co-inertia analysis, which suggests the long-term persistence of roads as sources of earthworm species immigration into forests landscapes. Given the findings of species presence in areas immediately surrounding the study area, this result may point to the importance of repeated introductions in maintaining exotic earthworm species population densities in soils of northern upland temperate forests (Gundale et al. 2005, Hale et al. 2005a, Cameron et al. 2007, Holdsworth et al. 2007). Together, these results highlight the importance of forest structure and dispersal mechanisms in determining earthworm densities.

Previous work has described spatial-structuring of earthworm communities in response to soil moisture patterns, pH (which co-varies strongly with Ca^{2+} content), texture, soil organic matter content, and vegetation patterns; these observations have primarily been made in managed ecosystems or in natural systems with a history of both agriculture and logging (Decaëns and Rossi 2001, Rossi 2003, Whalen 2004a, Reich et al. 2005b, Jiménez et al. 2006, 2012, Stoscheck et al. 2012). Similarities with the findings of these studies and with the historical data analyzed here, provide key insights into the responses of earthworm communities to soil physical and chemical properties. Across our study area, mineral soil feeding and dwelling (endogeic) *Aporrectodea* spp., the litter dwelling (epigeic) *D. octaedra*, and the litter and surface soil-dwelling (epi-endogeic) *L. rubellus* had the strongest associations with environmental factors, which could be attributed to dispersal limitation, response to soil moisture regimes, and high dependence on resource quality (Whalen 2004, Hale et al. 2005, Suárez et al. 2006). The litter feeding, vertical burrowing (anecic) *L. terrestris* showed associations with soil moisture and *P. grandidentata* and *A. rubrum* litter inputs, but was weakly related to environmental heterogeneity overall. Weak relationships between leaf litter inputs and *L. terrestris* distributions may be attributed to active foraging for palatable leaf litter (Hendriksen 1990, Reich et al. 2005b, Curry and Schmidt 2006). *L. terrestris* generally show low dispersal rates (1 to 6 m yr^{-1}), a long period for maturation, low reproductive rate, and intense intraspecific competition (Satchell 1980, Nuutinen et al. 2006, Uvarov 2009). These life history traits may explain the limited distributions of *L. terrestris* in earlier surveys, while highlighting the potential importance of species interactions in facilitating *L. terrestris* establishment across the landscape. Our secondary analysis of historical data and co-inertia analysis outcomes thereby indicate unique associations between earthworm species distributions and environmental effect factors, likely related to species trait diversity in feeding, dispersal, and burrowing behaviors (Bouché 1977, Lee 1985, Jégou et al. 1998b, Hale et al. 2005, Curry and Schmidt 2006, Stoscheck et al. 2012).

Earthworm species associations with leaf litter inputs and annual leaf litter mass loss are directly relevant to understanding shifts in soil C and N processes resulting from earthworm activity, as several recent studies have shown that through the enhanced vertical transport of surface litter, earthworms influence soil processes in a species-dependent manner by way of contrasting burrowing and casting activities, priming of recalcitrant soil organic matter (SOM), stabilization of SOM in soil aggregates, and alteration of the soil microbial community (Xia et al. 2011, Fahey et al. 2012, 2013a, Holdsworth et al. 2012, Crumsey et al. 2013b). Earthworm-mediated litter decomposition is determined by food preference and rates of leaf litter comminution, consumption, and translocation into sub–surface soils (Shipitalo and Protz 1989, Edwards and Bohlen 1996a). In this study, surface-dwelling epigeic species (*D. octaedra* and *L. rubellus*) showed no strong positive associations with annual leaf litter mass loss, though previous lab and field studies have measured enhanced litter decomposition in response to higher densities of exotic epigeic species in northern temperate and boreal forests (Scheu and Parkinson

1994, McLean and Parkinson 1997a, 1997b). Enhanced leaf litter decomposition associated with earthworm invasions, and particularly that of *L. terrestris* foraging activity also seen here, has been widely observed in temperate forests (Scheu and Wolters 1991, Araujo et al. 2004, Ashton et al. 2005, Suárez et al. 2006, Zicsi et al. 2011). Species-specific associations to leaf litter inputs thereby inform interpretations of community-specific effects on soil biogeochemical processes in both field and lab studies (Reich et al. 2005b, Suárez et al. 2006a, Holdsworth et al. 2008, Crumsey et al. 2013b, Fahey et al. 2013a).

Our results partially support the prediction that earthworm communities would be associated with depleted soil C and N isotopic values. Together with the anecic species (*L. terrestris*), endogeic species (*Aporrectodea* spp.) were strongly associated with surface soil δ^{13}C depletion. Continuous burrowing and casting activity of endogeic species generally increases C and N mineralization, and can enhance leaf litter C and N incorporation in the presence of earthworm species of other ecological groups (Jégou et al. 1998a, Marhan and Scheu 2006). Anecic species, which feed at the surface but burrow vertically into the soil profile, also forage selectively for leaf litter, leaving structural tissues at the surface and increasing soil C:N ratios through sub-surface casting (Suárez et al. 2006b, Filley et al. 2008, Crow et al. 2009b, Fahey et al. 2013a). Soil δ^{13}C depletion resulting from the activity of earthworm species belonging to these functional groups may be indicative of shifts in soil C chemistry to less recalcitrant forms with faster turnover times (Nadelhoffer and Fry 1988, Nadelhoffer et al. 1999, Gaudinski et al. 2000, McFarlane et al. 2011). Forest soil δ^{13}C depletion following earthworm introductions has been observed previously. For example, Bohlen et al. (2004a) observed δ^{13}C depletion of surface soils (c.a. 2‰) following earthworm invasion into a north temperate forest. In contrast to weakly pronounced C isotope fractionation in forest soils, substantial N isotopic fractionation occurs during leaf litter degradation and soil N processing in temperate forest soils (Nadelhoffer and Fry 1988, Melillo et al. 1989, Martinelli et al. 1999, Gaudinski et al. 2000, Robinson 2001). Soil δ^{15}N enrichment with increased earthworm abundance may be associated with increased N mineralization, discrimination against ^{15}N during mineralization and nitrification, and the subsequent uptake of ^{14}N-enriched mineral N by plant roots and microbes, which would result in ^{15}N accumulation in surface SOM and observed δ^{15}N enrichment (Scheu 1987, Nadelhoffer and Fry 1994, Alban and Berry 1994, Bohlen et al. 2004b, Wironen and Moore 2006). Our results suggest that the impacts of exotic earthworms on decomposition patterns (i.e., increasing leaf litter mass loss, soil $\delta^{13}C$ depletion, and soil $\delta^{15}N$ enrichment) are influenced by earthworm biomass, with species differentially affecting decomposition processes.

In this study, we have characterized historical and current exotic earthworm species distributions in relation to environmental factors at scales relevant to forest ecosystem processes (i.e., 100m – 1000m) and with earthworm communities that have become established across the landscape over the last 60 years; until recently, these patterns have only been described during incipient earthworm invasions into northern temperate forest ecosystems (c.f. Stoscheck et al. 2012). Species distribution surveys first conducted by Smith and Green (1916) along stream and river banks, and later extended by Murchie (1954, 1956, 1960) provide the earliest documented presence of the five exotic earthworm species collected in the present study. These species are also included in those now actively spreading across forests of the upper Great Lakes region (Reynolds 1995, James and Hendrix 2004, Tiunov et al. 2006a, Holdsworth et al. 2007). Interestingly, *Aporrectodea* spp. were the only earthworm species reported within the footprint of the present study area in these earlier studies, and only two records of *L. terrestris* were reported in northern-lower Michigan. Our recent surveys show the presence of all five earthworm species (*Aporrectodea* spp., *L. terrestris*, *L. rubellus*, and *D. octaedra*) across plots, indicating that there have been shifts in earthworm community composition over the past 60 years. Studies of exotic earthworm community dynamics in Canadian and northern U.S. temperate forests have primarily focused on incipient invasions, characterized as ‘invasion fronts‘ (i.e., a succession of earthworm species across a visible leading edge due to different patterns of colonization). These studies show increases in earthworm abundance and diversity with time or distance from introduction sites (Hale et al. 2005a, Suárez et al. 2006a, Cameron et al. 2007, Addison 2009b). Here, the long time over which these organisms have been present across the landscape (i.e. >60 years), limited forest disturbance, and the lack of strong recent temporal trends in earthworm community composition, suggest currently stable communities, unlike those documented for incipient earthworm invasions (Curry 1994). Shifts in the relative densities of species inferred from historical data, however, suggest a long-term succession of earthworm species, and potential facilitation of *L. terrestris* establishment through the preceding activity of epigeic and endogeic earthworm species (Hale et al. 2005a).

Understanding factors controlling earthworm species distributions across invasion stages (i.e., introduction, colonization, regional spread) are critical for assessing long-term impacts on northern temperate forests ecosystems. For example, the lack of differences in soil C and N pools on sites with high earthworm abundance suggests that the effects of these properties on earthworm habitat quality override any responses of soil C and N to earthworm activity across the landscape, but may also support the proposed trajectory that following earthworm invasion into previously earthworm-free forest soils, carbon storage should initially decline with the elimination of the forest floor horizons and subsequently increase as soil organic matter is stabilized by earthworm activity (Lavelle et al. 1998), and presumably as earthworm communities become established in the landscape. Together with environmental factors, ecological constraints including assimilation efficiency and interspecific interactions are known to strongly influence earthworm growth rates, activity, and fecundity (Phillipson and Bolton 1976, Satchell 1983, Hendriksen 1990, Capowiez 2000, Lowe and Butt 2002, Uvarov 2009). These effects would be further constrained by the land use history and legacy of forest disturbances (Lee 1985, Bohlen et al. 2004b, Gough et al. 2007, Nave et al. 2011a). Our results highlight the need for further experiments and controlled field studies that include comprehensive measurements of changes in soil biogeochemical processes in response to earthworm communities, and characterize long-term patterns in exotic earthworm community dynamics across previously earthworm-free forest landscapes.

Acknowledgements

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Figure 2.1A: Hendrix, P.F. and P.J. Bohlen, "Exotic Earthworm Invasions in North America: Ecological and Policy Implications," in *BioScience*, vol. 53, no. 9, September 2002. © 2002 by the American Institute of Biological Sciences. Published by the University of California Press. Reproduced with permission.

Tables

Table 2.1: Logistic regression models for each earthworm species in relation to soil physical and chemical properties (ordinal models = relative abundance of *A. caliginosa*, *L. rubellus*, and *L. terrestris*, and total earthworm species richness; binary model = *D. octaedra* presence/absence) from historical data (Murchie 1954). Coefficients in bold text contribute to the overall significance to the optimal regression model identified by the lowest AIC value (See text.). Coefficients that do not independently explain a significant proportion of variation are reported in brackets [ ]; (*** $P < 0.01$, ** $P < 0.05$, * $P < 0.1$).

Predictors	Earthworm (EW) Species				Total EW sp. richness
	A. caliginosa	*L. rubellus*	*L. terrestris*	*D. octaedra*	
% sand	**-0.53** (0.25)**	[-0.05] (0.26)	**-1.21** (0.51)**	**[-0.27] (0.54)**	**[-0.35] (0.23)**
pH	**+0.85*** (0.26)**	**+0.53* (0.32)**	-0.34 (0.51)	+ 0.31 (0.72)	[-0.07] (0.23)
H_2O	**[+0.15] (0.25)**	[+0.19] (0.27)	**[+0.22] (0.50)**	**[+ 0.29] (0.59)**	**+0.59** (0.26)**
% organic C	**+0.55** (0.28)**	[-0.01] (0.24)	[-0.24] (0.44)	[+ 0.22] (0.69)	[+0.16] (0.21)
% sand × H_2O	**+0.61** (0.28)**	[+0.37] (0.31)	**-1.02* (0.60)**	**+ 1.43* (0.80)**	[+0.24] (0.28)

Table 2.2: Statistical outcomes of multiresponse permutation procedure (MRPP, statistic = δ, chance-corrected within group agreement = A) comparing earthworm community composition across 10 plots and two separate permutational multivariate analyses of variance (PerMANOVA; statistic = F) comparing plot-level earthworm community composition nested by season (fall vs. spring) and year (2008 - 2010), indicated by brackets [ ]; ($P < 0.05$, n = 47). Present-day comparisons use the Bray–Curtis distances between earthworm community assemblages, visualized by the NMDS in Figure 4.

EW Community × Plot				
MRPP	Observed δ	Expected δ	A	P
	0.2795	0.4293	0.3489	0.001
EW Community × [Plot]Season				
PerMANOVA	*SS*	*MS*	*F*	*P*
Similarity	0.2118	0.21180	1.943	0.112
Residuals	4.9054	0.10901		
Total	5.1172			
EW Community × [Plot]Year				
PerMANOVA	*SS*	*MS*	*F*	*P*
Similarity	0.2118	0.21180	1.943	0.123
Residuals	4.9054	0.10901		
Total	5.1172			

Table 2.3: Present-day environmental effect factors. Soil physical properties: texture (% sand, silt, clay), bulk density (BD, g cm^{-3}), and % moisture; soil chemical properties: C mass (kg C ha^{-1}), N mass (Mg N ha^{-1}), C:N, and extractable Ca^{2+} (cmol(+) kg^{-1} soil); leaf litter inputs (g C m^{-2}): *A. rubrum* (Acru), *B. papyrifera* (Bepa), *F. grandifolia* (Fagr), *P. strobus* (Pist), *P. grandidentata* (Pogr), and *Q. rubra* (Quru). Values represent means ± 1 SE, n = 4. Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = *H*) with nonparametric multiple comparisons (*** $P < 0.01$, ** $P < 0.05$, * $P < 0.1$).

Plot	Soil Physical Properties					Soil Chemical Properties				Leaf Litter C Inputs						
	Sand	Clay	Silt	BD	H_2O	C	N	C:N	Ca^{2+}	Acru	Bepa	Fagr	Pist	Pogr	Quru	Total
1	85.9^{a} (1.55)	5.89^{a} (0.03)	8.19^{a} (1.53)	0.78^{a} (0.51)	10.0^{a} (5.89)	765^{a} (479)	29.3^{a} (18.3)	26.0^{a} (0.94)	7.97^{a} (3.02)	17.6^{ab} (1.36)	1.87^{ab} (1.87)	0.32^{ab} (0.05)	33.5^{b} (1.71)	16.3^{a} (1.30)	17.7^{ab} (1.59)	87.3^{a} (4.46)
2	88.6^{a} (0.38)	5.36^{a} (0.45)	6.01^{a} (0.71)	0.59^{a} (0.19)	17.1^{ab} (5.45)	1269^{a} (206)	49.1^{a} (8.17)	25.9^{a} (1.30)	0.89^{ab} (0.36)	31.7^{ab} (2.56)	12.24^{b} (0.8)	0.57^{ab} (0.07)	0.04^{a} (0.04)	41.7^{ab} (6.32)	24.3^{ab} (3.74)	111^{a} (12.6)
3	86.7^{a} (0.49)	7.09^{a} (1.17)	6.22^{a} (1.3)	0.67^{a} (0.2)	16.6^{ab} (3.02)	806^{a} (202)	33.0^{a} (6.87)	24.2^{a} (1.71)	6.88^{a} (5.18)	29.3^{ab} (3.29)	2.42^{ab} (1.89)	0.61^{ab} (0.19)	0^{a} (0)	11.2^{a} (3.94)	58.9^{a} (6.05)	114^{a} (12.9)
4	89.7^{a} (1.35)	5.34^{a} (0.73)	4.97^{a} (1.59)	0.68^{a} (0.12)	20.3^{ab} (0.29)	487^{a} (341)	24.5^{a} (9.16)	19.0^{a} (10.7)	0.99^{ab} (0.48)	34.6^{ab} (2.72)	6.37^{ab} (1.19)	1.66^{ab} (0.38)	0^{a} (0)	54.8^{b} (2.57)	4.97^{b} (0.79)	111^{a} (2.47)
5	85.9^{a} (0.62)	7.85^{a} (1.96)	6.22^{a} (1.53)	0.81^{a} (0.24)	14.8^{ab} (3.57)	740^{a} (417)	34.2^{a} (9.99)	21.3^{a} (10.6)	5.06^{a} (3.66)	24.5^{ab} (1.64)	0.12^{a} (0.12)	0.19^{a} (0.10)	2.02^{a} (0.42)	22.2^{ab} (5.08)	50.5^{ab} (5.93)	99.5^{a} (9.08)
6	84.7^{a} (1.87)	7.57^{a} (2.05)	7.75^{a} (1.14)	0.79^{a} (0.07)	17.1^{ab} (4.38)	1021^{a} (402)	36.7^{a} (10.2)	27.3^{a} (3.40)	4.52^{a} (4.65)	42.4^{ab} (4.49)	0.08^{a} (0.08)	1.17^{ab} (0.42)	1.03^{a} (0.18)	40.6^{ab} (7.16)	36.1^{ab} (3.63)	121^{a} (11.9)
7	90.0^{a} (3.24)	4.86^{a} (1.04)	5.19^{a} (2.3)	0.89^{a} (0.28)	18.1^{ab} (4.66)	811^{a} (377)	34.0^{a} (16.6)	24.1^{a} (1.09)	0.20^{ab} (0.47)	34.4^{a} (1.11)	1.32^{ab} (0.8)	1.57^{a} (0.40)	0.08^{a} (0.05)	12.8^{a} (1.72)	54.9^{a} (3.95)	107^{a} (8.48)
8	86.5^{a} (2.86)	5.05^{a} (1.19)	8.41^{a} (1.84)	0.61^{a} (0.16)	14.3^{ab} (6.56)	558^{a} (98)	21.0^{a} (5.79)	27.1^{a} (2.98)	0.26^{ab} (0.3)	19.4^{b} (0.83)	6.98^{ab} (0.99)	0.91^{ab} (0.27)	0.81^{a} (0.11)	18.0^{ab} (0.69)	50.1^{a} (7.00)	99.4^{a} (7.72)
9	87.4^{a} (2.52)	5.51^{a} (2.03)	7.11^{a} (0.99)	0.78^{a} (0.23)	14.5^{ab} (5.31)	845^{a} (244)	29.6^{a} (6.85)	28.4^{a} (4.76)	0.02^{b} (0.01)	17.0^{ab} (5.39)	14.59^{b} (0.33)	0.10^{ab} (0.10)	3.65^{a} (0.32)	44.5^{ab} (1.96)	26.3^{ab} (1.45)	106^{a} (3.89)
10	82.9^{a} (3.3)	7.5^{a} (0.5)	9.56^{a} (3.8)	0.77^{a} (0.1)	28.2^{b} (4.04)	776^{a} (360)	40.7^{a} (17.9)	18.8^{a} (2.66)	0.76^{ab} (0.34)	58.7^{c} (2.16)	0.33^{a} (0.93)	3.24^{b} (0.55)	0^{a} (0)	0.33^{c} (0.17)	6.44^{ab} (0.50)	136^{a} (26.6)
H	16.16	15.34 *	12.90 *	7.68	21.16 **	15.41*	16.45 *	11.72	37.21 ***	25.35 ***	23.14 ***	23.14 ***	26.49 ***	26.49 ***	25.63 ***	8.14

Table 2.4: Present-day environmental response factors. Surface soil (i.e., Oa-horizon) isotopic composition, expressed as $\delta^{13}C$ and $\delta^{15}N$ (‰), and annual leaf litter mass loss rate (%). Values represent means ± 1 SE. Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = *H*) with nonparametric multiple comparisons (*** $P < 0.01$, ** $P < 0.05$, * $P < 0.1$).

Plot	Oa-horizon $\delta^{13}C$	Oa-horizon $\delta^{15}N$	Litter Mass Loss
1	-27.38^{ac} (0.44)	0.48^{ab} (1.48)	0.40^{a} (0.02)
2	-26.94^{b} (0.3)	-0.88^{a} (1.05)	0.41^{a} (0.02)
3	-26.98^{b} (0.38)	-0.37^{ab} (1.13)	0.46^{ab} (0.06)
4	-28.05^{a} (0.05)	-0.41^{ab} (1.16)	0.43^{ab} (0.03)
5	-26.81^{b} (0.49)	0.04^{ab} (0.46)	0.41^{ab} (0.07)
6	-27.65^{ab} (0.63)	0.1^{ab} (0.23)	0.46^{ab} (0.05)
7	-27.63^{ab} (0.22)	-0.52^{ab} (0.77)	0.51^{bc} (0.04)
8	-27.41^{ab} (0.16)	0.22^{ab} (0.53)	0.43^{b} (0.03)
9	-27.46^{ab} (0.43)	-0.71^{ab} (1.06)	0.71^{c} (0.07)
10	-28.1^{ac} (0.3)	1.77^{b} (0.54)	0.51^{b} (0.04)
H	31.54***	14.62*	21.00**

Figures

Figure 2.1: (a) The southern extent of the last glacial maximum (bold line), approximate present-day distributions of Nearctic earthworm species in the eastern region of North America (shaded area), and location of UMBS (modified from Reynolds 1995, Hendrix and Bohlen 2002).. (b) historical earthworm distribution records mapped for *Aporrectodea* spp., *D. octaedra*, *L. rubellus*, and *L. terrestris* (Smith and Green 1916, Murchie 1954). (c) Current study area: transects and 0.08 ha plots surround two atmospheric towers. Sample plots (numbered squares) are distributed around potential sites of exotic earthworm species introductions (i.e., lake shore and roads).

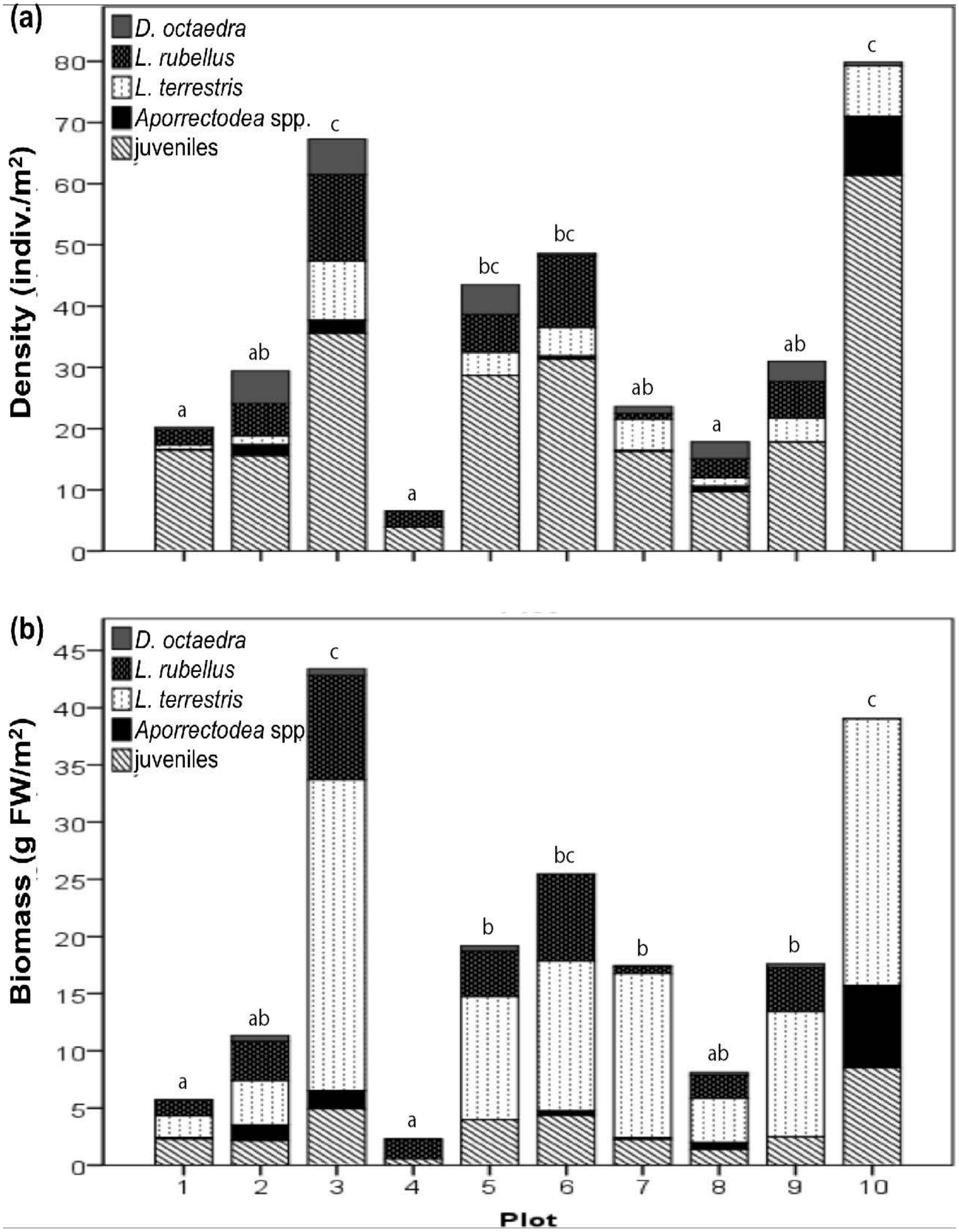

Figure 2.2: Present-day species-specific (a) earthworm species density (individuals m^{-2}) and (b) earthworm species biomass (g FW m^{-2}) across ten sampling plots, averaged across sampling dates (i.e. October 2008, May and October from 2009 to 2010). Different lower case letters above bars represent significant plot-level differences in the average total earthworm density and biomass, determined by Kruskal-Wallis *H* tests with nonparametric multiple comparisons ($P < 0.05$).

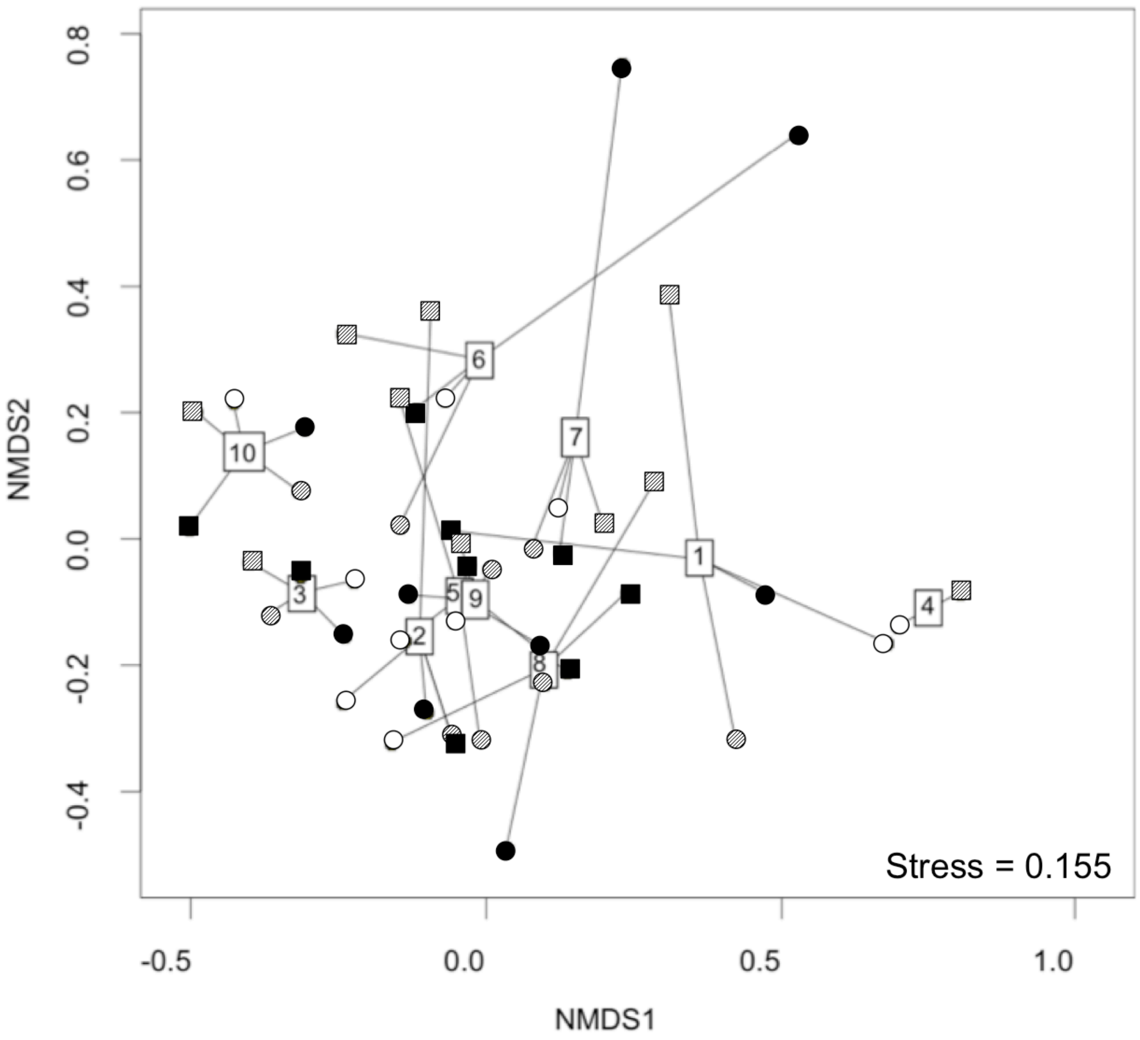

Figure 2.3: Non-metric multidimensional scaling (NMDS) analysis based on Bray-Curtis dissimilarity measures of present-day earthworm community composition. Numbers represent the weighted plot centroid of earthworm community composition over fall and spring 2008 - 2010. Each point represents a plot-level earthworm community for each season (circle = fall, square = spring) and year (white = 2008, grey = 2009, black = 2010), with communities of similar composition being located close together in the NMDS ordination space.

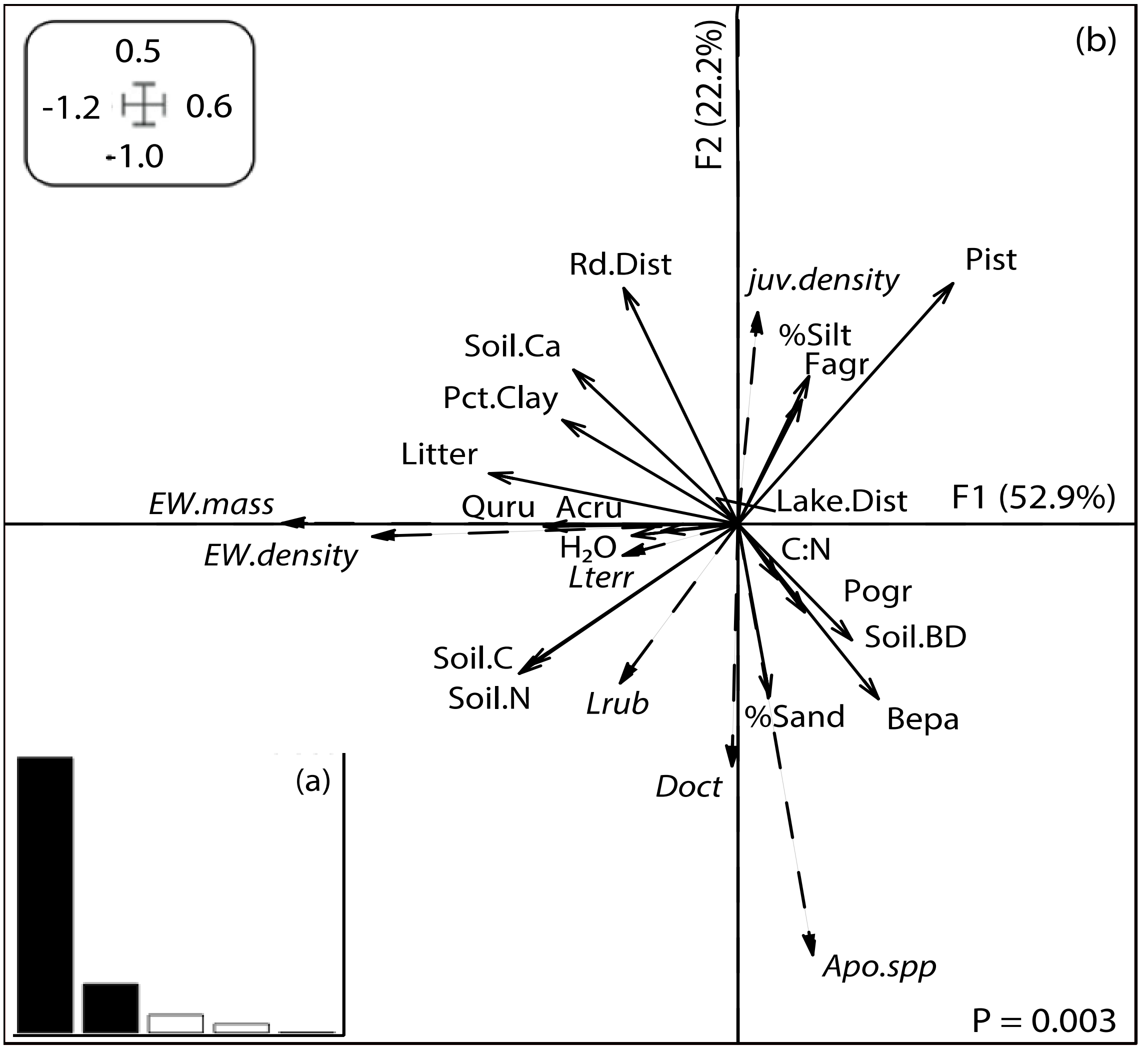

Figure 2.4: Present-day relationships between environmental effect factors and earthworm species densities. (a) Histogram of the eigenvalues corresponding to the two co-inertia axes, which are equal to 1.25 and 0.69. (b) Associated vectors of earthworm species densities (dashed vectors, italicized text) and environmental effect factors (solid vectors, plain text) according to relative positions on the Fl × F2 co-inertia plane. The % of total inertia explained by the first (F1) and second (F2) co-inertia axes are given in parentheses, and axis scales are given in top left corner of the plot. Earthworm density measures include *L. terrestris* (*Lterr*), *L. rubellus* (*Lrub*), *D. octaedra* (*Doct*), *Aporrectodea* spp. (*Apo.spp*), and juvenile (*juv*) density; total earthworm density (EW.density) and biomass (EW.mass). Environmental effect variables include plot-to-road and -lake distance (Rd.Dist and Lake.Dist); soil physical properties: texture (i.e., % sand, silt, and clay), bulk density (BD, g cm^{-3}), and % moisture; soil chemical properties: C (kg C ha^{-1}), N (Mg N ha^{-1}), C:N, and Ca^{2+} (cmol(+) kg^{-1} soil); leaf litter C inputs (g C m^{-2}): *A. rubrum* (Acru), *B. papyrifera* (Bepa), *F. grandifolia* (Fagr), *P. strobus* (Pist), *P. grandidentata* (Pogr), and *Q. rubra* (Quru), and total leaf litter C loading.

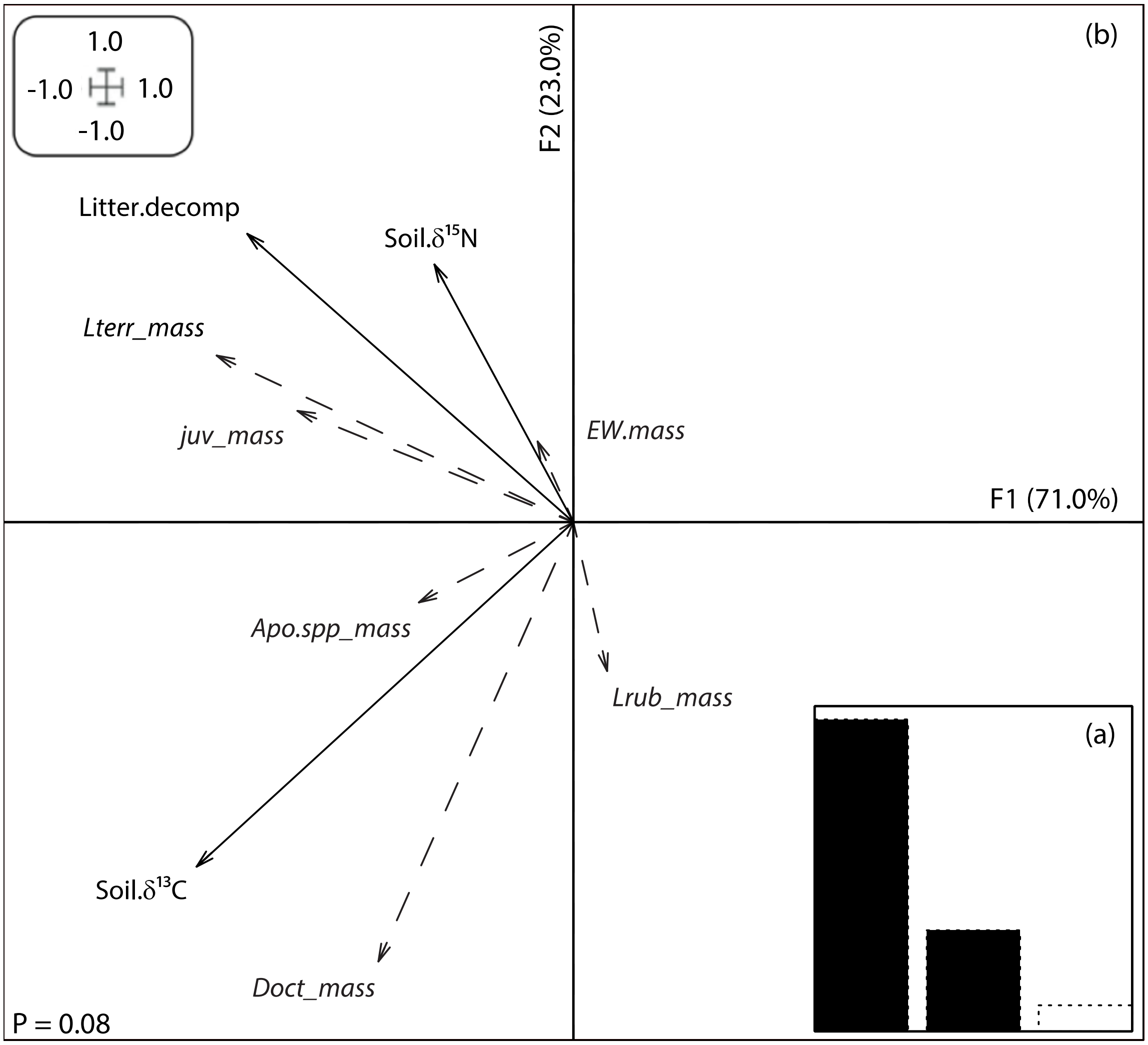

Figure 2.5: Present-day relationships between environmental response factors and earthworm species densities. (a) Histogram of the eigenvalues corresponding to the two co-inertia axes, which are equal to 0.33 and 0.11. (b) Associated vectors of earthworm species densities (dashed vectors, italicized text) and environmental response factors (solid vectors, plain text) according to relative positions on the F1 × F2 co-inertia plane. The % of total inertia explained by the first (F1) and second (F2) co-inertia axes are given in parentheses, and axis scales are given in top left corner of the plot. Earthworm biomass measures include *L. terrestris* (*Lterr_mass*), *L. rubellus* (*Lrub_mass*), *D. octaedra* (*Doct*), *Aporrectodea* spp. (*Apo.spp_mass*), and juvenile (*juv_mass*) biomass; total earthworm biomass (*EW.mass*). Environmental response variables include annual leaf litter mass loss (litter.decomp); surface soil (Oa-horizon) δ^{13}C and δ^{15}N.

Chapter 2 Appendices

Appendix A: Numerical values for plot-level earthworm species density and biomass.

Table A1: Present-day average earthworm species abundance (individuals m^{-2}) ± 1 standard error (SE). Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = H) with nonparametric multiple comparisons (** $P < 0.01$, * $P < 0.05$).

Plot	EW Abundance ± SE					
	L. rubellus	*L. terrestris*	*Aporrectodea spp.*	*D. octaedra*	juveniles	Total
1	3 ± (1)^{ab}	1 ± 0^{a}	1 ± 0^{a}	1 ± 0^{a}	16 ± 5^{ab}	20 ± 5^{a}
2	5 ± 2^{ab}	1 ± 0^{a}	2 ± 0^{a}	5 ± 1^{ab}	16 ± 3^{ab}	29 ± 3^{a}
3	14 ± 3^{a}	10 ± 1^{b}	2 ± 1^{a}	6 ± 2^{b}	36 ± 11^{ab}	67 ± 14^{b}
4	3 ± 0^{ab}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	4 ± 1^{a}	7 ± 0^{c}
5	6 ± 2^{ab}	4 ± 1^{ab}	0 ± 0^{a}	5 ± 2^{ab}	29 ± 8^{ab}	43 ± 9^{a}
6	12 ± 5^{a}	5 ± 1^{ab}	1 ± 1^{a}	0 ± 0^{a}	31 ± 9^{ab}	49 ± 11^{a}
7	1 ± 0^{b}	5 ± 1^{ab}	1 ± 0^{a}	1 ± 0^{ab}	16 ± 5^{ab}	24 ± 5^{a}
8	3 ± 1^{ab}	1 ± 0^{a}	1 ± 1^{a}	3 ± 1^{ab}	10 ± 1^{a}	18 ± 2^{a}
9	6 ± 1^{ab}	4 ± 1^{ab}	0 ± 0^{a}	3 ± 1^{ab}	18 ± 4^{ab}	31 ± 5^{a}
10	12 ± 1^{a}	8 ± 4^{ab}	10 ± 1^{b}	1 ± 0^{ab}	61 ± 23^{b}	92 ± 25^{b}
H	23.81**	33.15**	34.20**	25.05**	19.29*	28.51**

Table A2: Present-day average earthworm species biomass (g FW m^{-2}) ± 1 standard error (SE). Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = *H*) with nonparametric multiple comparisons (** $P < 0.01$, * $P < 0.05$).

Plot	EW Biomass ± SE					
	L. rubellus	*L. terrestris*	*Aporrectodea spp.*	*D. octaedra*	juveniles	Total
1	1.37 ± 0.83^{a}	1.88 ± 1.19^{a}	0.15 ± 0.15^{ab}	0.04 ± 0.02^{a}	2.28 ± 0.66^{ab}	5.72 ± 2.17^{a}
2	3.46 ± 1.08^{ab}	3.83 ± 0.74^{a}	1.38 ± 0.3^{a}	0.51 ± 0.13^{ab}	2.16 ± 0.38^{a}	11.35 ± 1.08^{a}
3	9.13 ± 1.81^{b}	27.18 ± 3.46^{b}	1.59 ± 0.38^{a}	0.56 ± 0.16^{b}	4.94 ± 1.56^{ab}	43.41 ± 5.38^{c}
4	1.74 ± 0.12^{ab}	0 ± 0^{c}	0 ± 0^{b}	0 ± 0^{ab}	0.54 ± 0.08^{a}	2.28 ± 0.04^{a}
5	3.98 ± 1.29^{ab}	10.75 ± 3.06^{ab}	0 ± 0^{b}	0.47 ± 0.19^{ab}	3.98 ± 1.15^{ab}	19.18 ± 4.07^{ab}
6	7.58 ± 3.23^{ab}	13.08 ± 2.57^{ab}	0.4 ± 0.4^{a}	0.04 ± 0.04^{a}	4.35 ± 1.27^{ab}	25.45 ± 4.39^{ab}
7	0.62 ± 0.21^{a}	14.32 ± 3.2^{ab}	0.15 ± 0.15^{ab}	0.11 ± 0.03^{ab}	2.25 ± 0.65^{ab}	17.46 ± 3.27^{a}
8	2.01 ± 0.47^{a}	3.83 ± 1.16^{a}	0.66 ± 0.41^{ab}	0.27 ± 0.09^{ab}	1.35 ± 0.09^{a}	8.13 ± 0.91^{a}
9	3.87 ± 0.91^{ab}	10.94 ± 2.32^{ab}	0 ± 0^{b}	0.32 ± 0.06^{ab}	2.47 ± 0.51^{ab}	17.61 ± 2.98^{a}
10	7.58 ± 0.89^{ab}	23.27 ± 12.04^{ab}	7.22 ± 1.08^{c}	0.06 ± 0.04^{a}	8.52 ± 3.17^{b}	46.65 ± 14.3^{c}
H	24.21**	33.15**	34.20**	25.05**	19.29*	34.26**

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Chapter 3

Community–specific impacts of exotic earthworm invasions on soil carbon dynamics in a sandy temperate forest [3]

Abstract

Exotic earthworm introductions can alter above– and below–ground 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 mono-specific 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 mono-specific 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 (*A. rubrum* > *P. grandidentata* > *F. grandifolia* > *Q. rubra* ≥ *P. 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) sub-surface 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.

3.1 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

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recolonize 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. 2004c, 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. 2004c, Marhan and Scheu 2006, Eisenhauer et al. 2007), soil C redistribution (Burtelow et al. 1998, Bohlen et al. 2004b, Wironen and Moore 2006, Straube et al. 2009), and increased soil CO_2 emissions (Marhan and Scheu 2006).
Although endemic earthworms have been slow to recolonize 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. 2004c, 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. 2004c, Marhan and Scheu 2006, Eisenhauer et al. 2007), soil C redistribution (Burtelow et al. 1998, Bohlen et al. 2004b, 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. 2004a, Fisk et al. 2004b, 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). Inter-specific 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. 2005b, 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. We report the results of a mesocosm experiment in which earthworm species of three functional groups: *Lumbricus terrestris* (Linneus), *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 sub-surface 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.

3.2 Materials and Methods

3.2.1 Experimental Design

We conducted a mesocosm experiment from August 2009 – August 2010 in a belowground laboratory (Lussenhop et al. 1991) at the University of Michigan Biological Station (UMBS; see Appendix A for study area description), using 7 combinations of 3 exotic earthworm species present in forest soils as treatments and no-earthworm controls in uniform leaf litter and soil profiles. Adults of earthworm species representing different functional groups included: *L. terrestris* [Anecic = litter feeding, vertical burrowing], *A. trapezoides* [Endogeic = mineral soil feeding and dwelling], and *E. fetida* [Epigeic = litter feeding, 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 m^{-2} fresh weight), 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 weight) 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 weight) of sieved and homogenized B-horizon material packed to a bulk density of 2.5 g cm^{-3}, and 5 kg (fresh weight) of sieved and homogenized A-horizon material packed to a bulk density of 1.3 g cm^{-3}. Leaf litter additions were scaled from area-normalized leaf litter data of the UMBS AmeriFlux site in 2008 (Vogel, unpublished data). Leaf litter additions from overstory tree species summed to 16.5 g: 41% *P. grandidentata*, 32% *A. rubrum*, 21% *Q. rubra*, 4% *F. grandifolia*, and 2% *P. strobus* (Table 1).

3.2.2 Mesocosm C loss measurements (CO_2 and DOC)

Soil CO_2 efflux was measured from August 2010 – 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)(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) 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), and a capillary tube was inserted for air pressure equilibration. Soil CO_2 efflux rates (F_c) were determined by measuring 10 µmol mol^{-1} change in CO_2 concentration (ΔCO_2) over a 20-second measurement period, from which CO_2-C loss rate per unit soil surface area was calculated as:

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

where F_c is corrected for headspace volume (V) and surface area (S) (µmol CO_2 m^{-2} sec^{-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 de-ionized 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 N HCl, and stored at −20°C until analyzed for DOC concentration using an Aurora (Model 1030) OI Analytical TOC analyzer. DOC loss values were integrated to derive cumulative curves for each mesocosm.

3.2.3 3D reconstruction and quantification of burrow systems

Soils containing earthworm treatments were imaged using X-ray computed tomography, X-ray CT (General Electric Discovery CT 750 HD scanner, 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. The sequential analysis of 2-D binarized images enables 3-D tracking of earthworm burrows and subsequent 3-D 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).

3.2.4 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 weight corrections, and pulverized for C and N analyses using a CN elemental analyzer (Costech Elemental Analyzer 1030). 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.

3.2.5 Soil C mass storage

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

$$\Delta C = (L_c + E) - (\int_0^{320} F_c(t)dt + \int_0^{320} DOC_{EX}(t)dt) \qquad (2)$$

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-day 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.

3.2.6 Statistical analyses

We used Kruskal-Wallis H tests (H, df = 7, $n = 32$, $\alpha = 0.05$) with non-parametric 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 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 (www.rstudio.com), using the packages ade4 (Dray and Dufour 2007), Hmisc (Harrell 2012), lattice (Sarkar 2008), and pgirmess (Giraudoux 2012).

3.3 Results

3.3.1 Cumulative CO_2 and DOC losses

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 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.

3.3.2 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, $P > 0.05$). 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).

3.3.3 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 > 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 sub-surface 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 less than 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 positively correlated with leaf litter mass losses (with the exception of *P. strobus*). CO_2 and DOC losses were not correlated with burrow system properties.

3.3.4 Soil C mass storage

Inputs of C to soils from litter in control mesocosms (25.0 ± 4.59 g C m^{-2}) were less than C outputs as CO_2 and DOC (153 ± 6.73 g C m^{-2}). 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 m^{-2}; 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 ΔC occurred across earthworm treatments (Kruskal-Wallis *H* test, $P > 0.05$).

3.4 Discussion

Our results suggest earthworm communities have important non–additive 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 multi-species 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 1996b, Callaham and Hendrix 1997, Uvarov et al. 2011)(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 1996b, 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 1996b), and constrained by leaf litter chemistry and earthworm food preference (Reich et al. 2005, Suárez et al. 2006, Hobbie 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. 2004b). Past disturbances of logging and wildfires constrain soil carbon (C) storage rates in these forests (Gough et al. 2008a), 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)Earthworm-mediated litter decomposition is determined by rates of litter comminution, consumption, and translocation into soils (Shipitalo and Protz 1989, Edwards and Bohlen 1996b), and constrained by leaf litter chemistry and earthworm food preference (Reich et al. 2005, Suárez et al. 2006, Hobbie 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. 2004b). Past disturbances of logging and wildfires constrain soil carbon (C) storage rates in these forests (Gough et al. 2008a), 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, sub–surface burrow systems were associated with vertical redistribution of litter–derived organic material into the A-horizon, as indicated by strong correlations between (1) sub-surface 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)(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.

Acknowledgements

We thank M. Grant for analytical services; C. Vogel for AmeriFlux field data; B. Carson, P. Rink, R. Spray, T. Sutterly, and S. Webster, for preparatory support. The NSF Doctoral Dissertation Improvement Grant 1110494, the NSF-IGERT Biosphere Atmosphere Research and Training Program (NSF-IGERT-0504552), and the University of Michigan Rackham Graduate School and Department of Ecology and Evolutionary Biology funded this research.

Tables

Table 3.1: Initial leaf litter, A-horizon, and B-horizon C and N properties. Values represent means (± 1 SE), *n* = 6.

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)
A. rubrum	39.68 (0.06)	46.5 (0.4)	0.5 (0.04)	98.8 (<0.01)
P. strobus	2.01 (0.01)	50.3 (0.03)	0.4 (0.01)	137.6 (<0.01)
P. grandidentata	53.65 (0.24)	49.2 (0.6)	0.8 (0.1)	62.7 (<0.01)
Q. rubra	27.25 (0.07)	47.9 (0.4)	0.6 (0.03)	74.2 (<0.01)
F. 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)

Figures

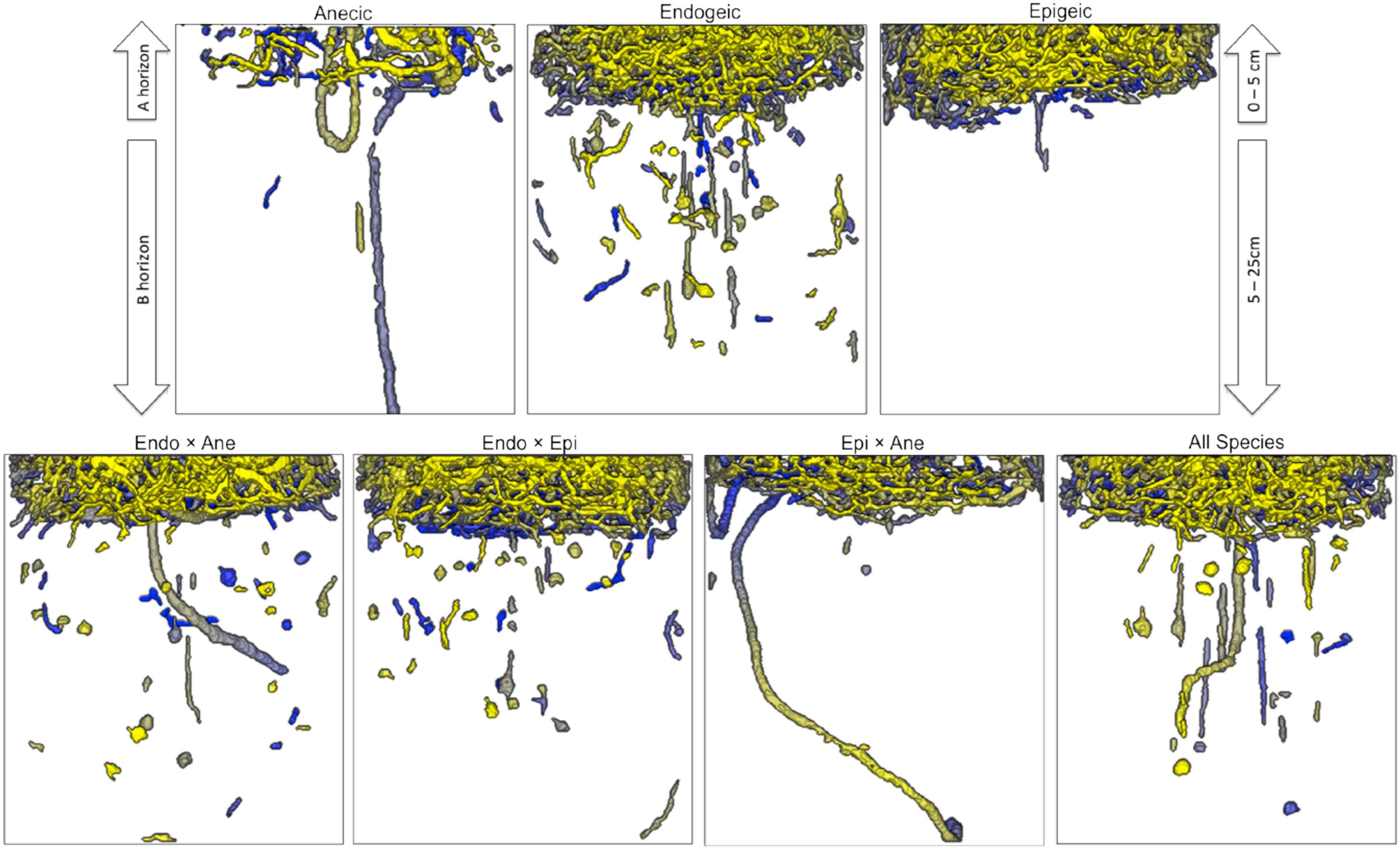

Figure 3.1: Examples of 3-D reconstructions of earthworm community burrow systems imaged by X-ray CT. Color gradations represent the distance of burrows relative to the viewer's perspective (maize for the foreground to blue for the background). Earthworm species of different functional groups included: *L. terrestris* [Anecic], *A. trapezoides* [Endogeic], and *E. fetida* [Epigeic].

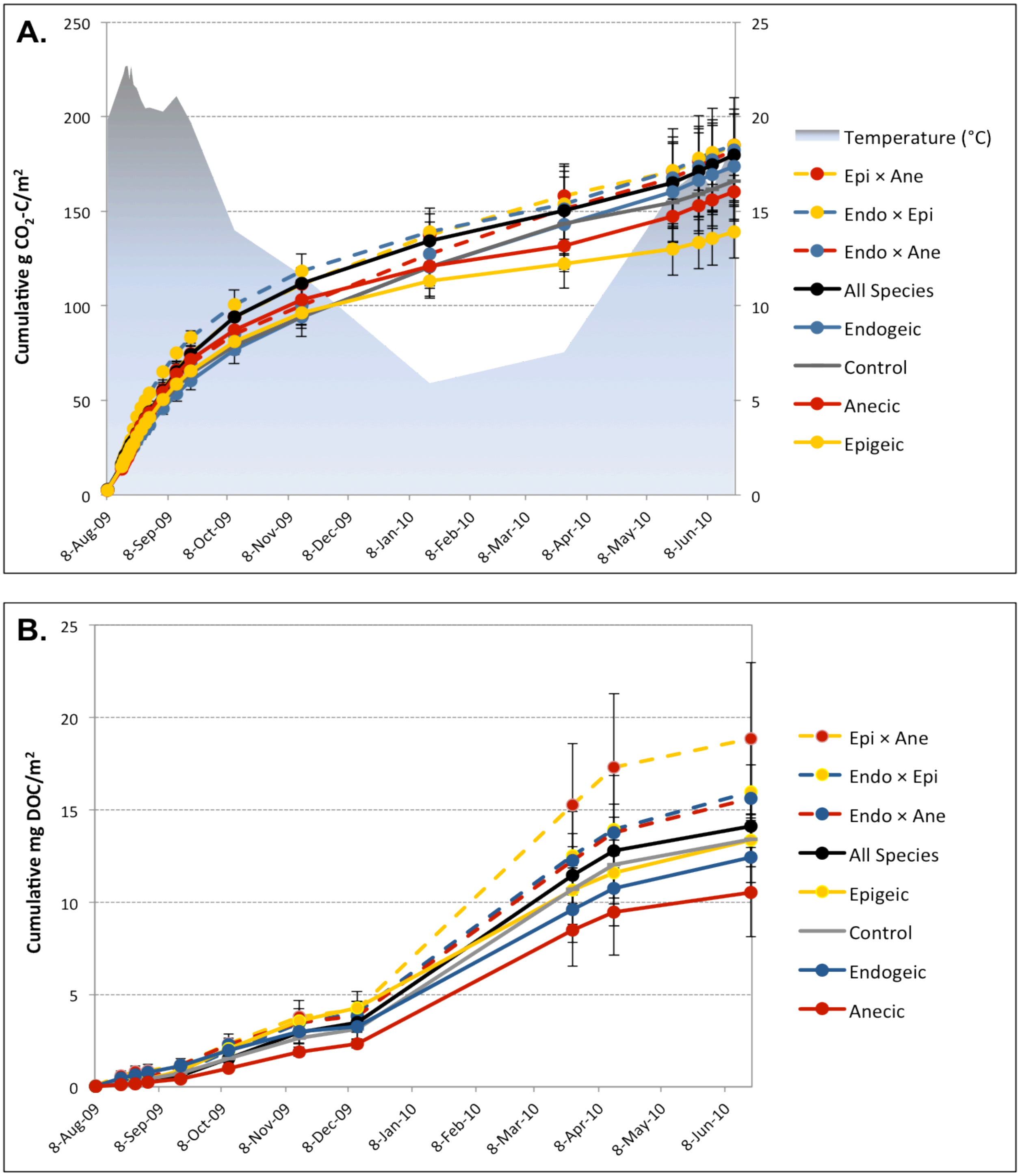

Figure 3.2: [A] Cumulative soil CO_2–C efflux (g CO_2–C m^{-2}) and [B] Cumulative DOC efflux (mg DOC m^{-2}) across earthworm treatments. Values represent means and vertical bars are ± 1 SE. Temperature (°C) is shown in blue shades behind soil CO_2–C efflux curves.

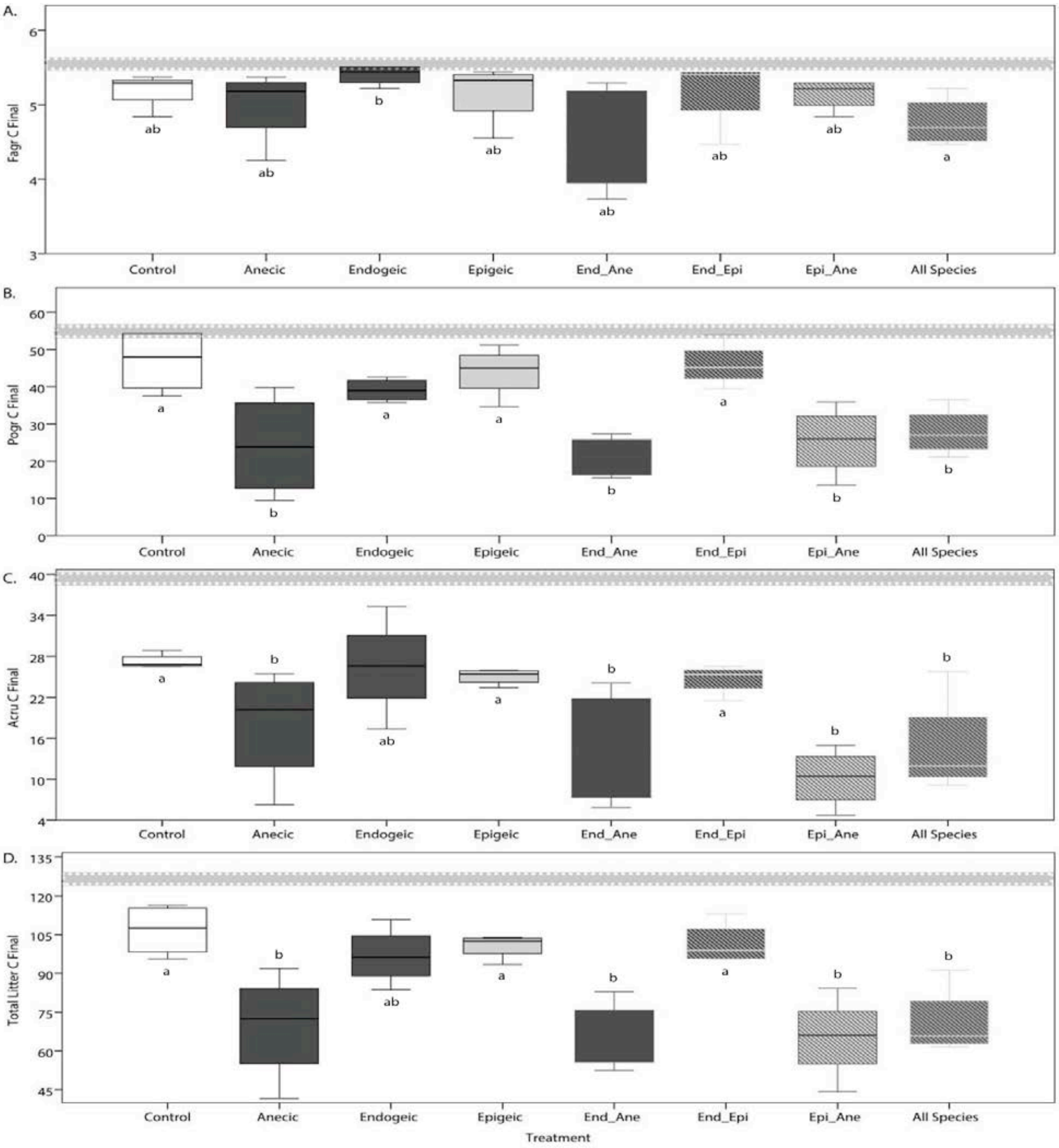

Figure 3.3: Final *F. grandifolia* (Fagr), *P. grandidentata* (Pogr), *A. rubrum* (Acru), and total leaf litter C mass (g C m^{-2}) across control (white), Anecic, Endogeic, and Epigeic (solid), and multi-species earthworm treatments (striped). Grey bars show initial leaf litter C mass. Horizontal lines within boxes indicate median mass values for each leaf litter type; the first and third quartiles of the data (the inter-quartile 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. Lowercase letters represent significant differences determined by Kruskal-Wallis *H* tests with nonparametric multiple comparisons ($P < 0.05$). *P. strobus* and *Q. rubra* losses are not shown.

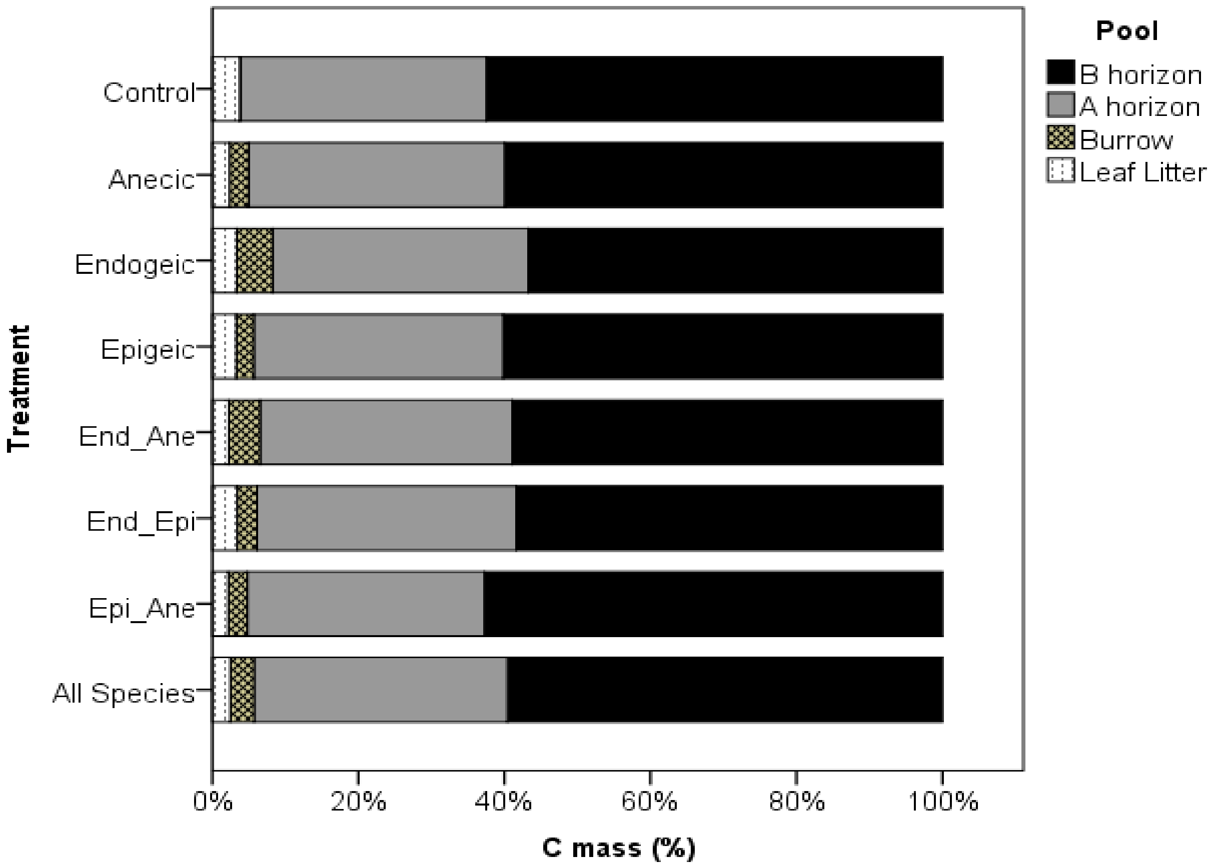

Figure 3.4: Leaf litter and soil C pools expressed as % of total C across control and treatments

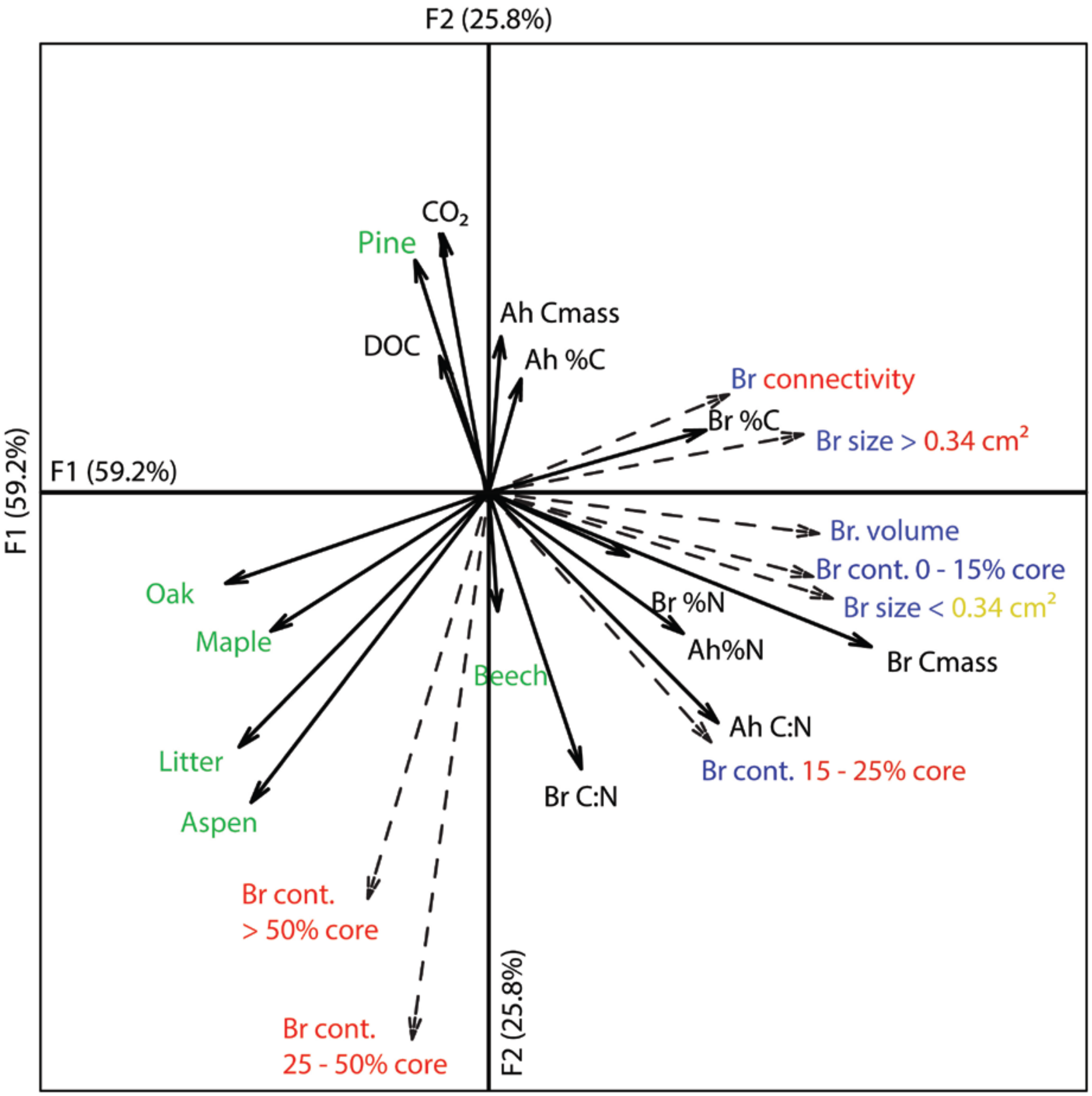

Figure 3.5: Relationships between burrow system (dashed arrows) and C budget measures (solid arrows) according to relative positions on the Fl × F2 co-inertia plane. Colored text indicates the earthworm ecological group(s) associated with the highest values of each respective burrow system property (Red = Anecic, Blue = Endogeic, Yellow = Epigeic; Table C1). Burrow system structure measures: macroporosity (MR), surface connectivity (SC), size class (BS: 2 = 0.17 to < 0.34 cm^2, 3 = > 0.34 cm^2), continuity class (BC 1 = 0 – 15%, 2 = 15 – 25%, 3 = 25 – 50%, 4 = > 50%). C budget components: *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; CO$_2$ and DOC loss. Co-inertia axis eigenvalues: F1 = 4.27; F2 = 1.99.

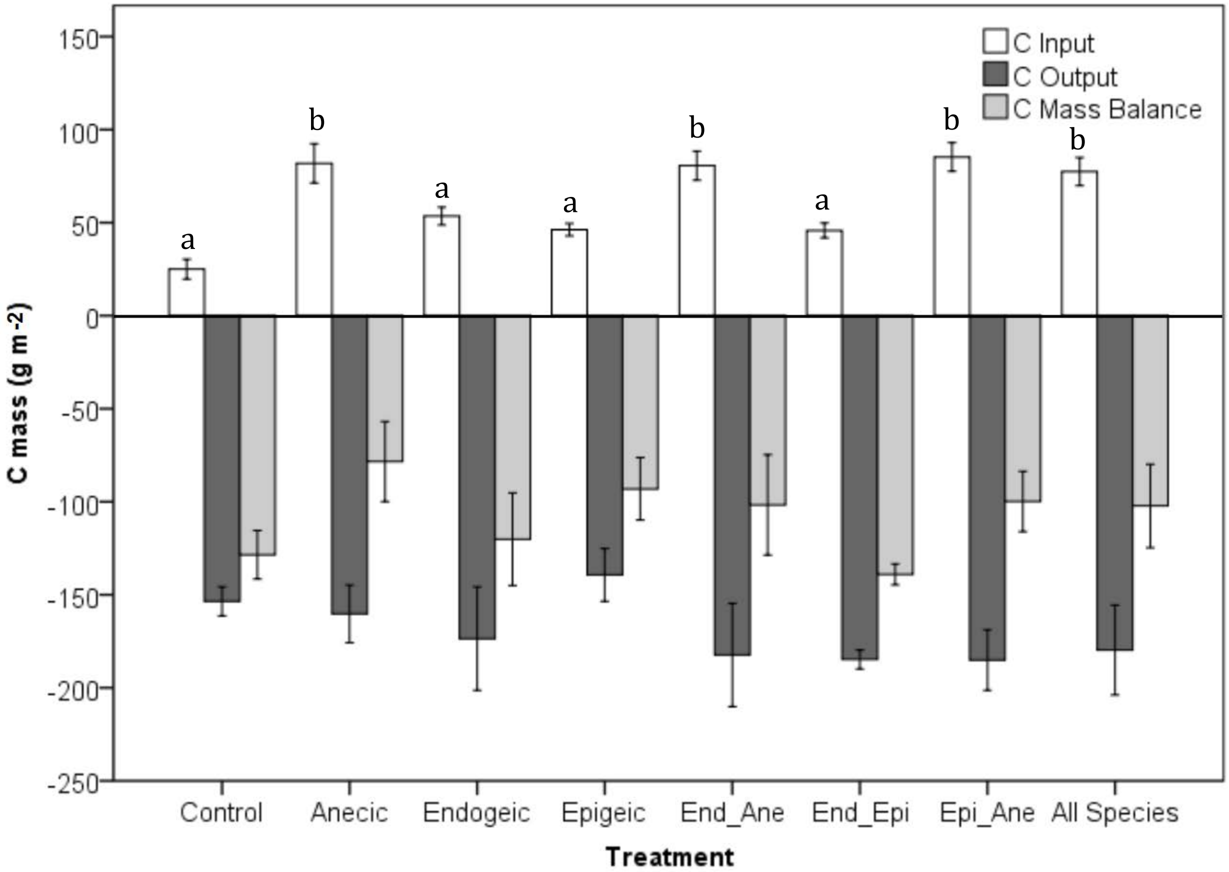

Figure 3.6: C inputs, C outputs, and net C mass storage (ΔC; Eq. 2) across control and treatments. Lowercase letters represent significant differences determined by Kruskal-Wallis H tests with nonparametric multiple comparisons ($P < 0.05$). C output and ΔC are similar across treatments.

Chapter 3 Appendices

APPENDIX A: Description of the study area

This study was conducted at the University of Michigan Biological Station (UMBS) in northern Michigan, US (45^o35.5’N, 84^o43’W), where secondary successional forests are dominated by bigtooth aspen (*Populus grandidentata*), with northern red oak (*Quercus rubra*), red maple (*Acer rubrum*), paper birch (*Betula papyrifera*), and eastern white pine (*Pinus strobus*) occurring as co–dominants (Curtis et al. 2005)(Curtis et al. 2005). UMBS forests lie on outwash plains and moraines with well-drained soils (92.9% sand, 6.5% silt, 0.6% clay, pH 4.8) classified as mixed, frigid Entic Haplorthods of the Rubicon series (National Resources Conservation Service 1991). Earthworm communities are dominated by five species of European origin including, *Dendrobaena octaedra, Aporrectodea caliginosa*, *Aporrectodea trapezoides, Lumbricus rubellus,* and *Lumbricus terrestris*. Average earthworm biomass is 21± 2.66 g m^{-2} (fresh weight), while average earthworm abundance is 39 ± 5 individuals m^{-2} (Crumsey, unpublished data).

Forest stands function as C sinks, with annual photosynthetic C gains greater than that of heterotrophic soil respiration. Annual photosynthetic C gains average 6.54 ± 0.76 Mg C ha^{-1} yr^{-1}, while average heterotrophic soil respiration is 5.02 ± 0.86 Mg C ha^{-1} yr^{-1} (Gough et al. 2008a), and accounts for up to 71% of annual ecosystem respiration losses (Curtis et al. 2005). Forest stands thus have an annual C storage rate of 1.53 ± 1.15 Mg C ha^{-1} yr^{-1}, and contain 180.5 ±12.8 Mg C ha^{-1}, with 44% (80 ± 12.4 Mg C ha^{-1}) stored in soil organic matter (Gough et al. 2008b).

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 Spearman rank correlations.

Table B1: Percentages of initial leaf litter C (± 1 SE, Standard Error) remaining after one year. Values represent means (± 1 SE). Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = *H*) with nonparametric multiple comparisons (** P < 0.01, * P < 0.05).

Treatment	Leaf litter C (% remaining after one year)					
	A. rubrum	*P. strobus*	*P. grandidentata*	*Q. rubra*	*F. grandifolia*	Total litter C
Control	68.8 ^{a} (1.42)	82.0 ^{a} (6.00)	86.8 ^{a} (7.77)	93.5 ^{a} (2.66)	96.6 ^{a} (2.3)	83.0 ^{a} (3.84)
Epigeic	63.4 ^{b} (1.73)	87.5 ^{a} (1.78)	82.7 ^{a} (6.15)	98.0 ^{a} (4.28)	94.2 ^{a} (3.19)	80.5 ^{a} (2.65)
Endogeic	66.6 ^{a} (8.93)	76.6 ^{a} (5.91)	72.2 ^{a} (2.64)	89.3 ^{a} (3.12)	99.3 ^{a} (0.38)	75.3 ^{a} (4.14)
Anecic	45.2 ^{b} (10.6)	88.9 ^{a} (7.56)	44.8 ^{b} (12.94)	76.7 ^{a} (2.28)	91.3 ^{a} (5.28)	54.3 ^{b} (8.27)
End×Epi	62.9 ^{a} (2.63)	91.0 ^{a} (9.00)	85.5 ^{a} (5.27)	87.1 ^{a} (3.92)	95.1 ^{a} (3.66)	79.4 ^{a} (2.97)
Epi×Ane	25.6 ^{b} (5.47)	66.9 ^{a} (14.08)	46.8 ^{b} (8.39)	86.1 ^{a} (4.21)	94.9 ^{a} (1.99)	50.8 ^{b} (6.2)
End×Ane	36.8 ^{b} (10.81)	80.7 ^{a} (8.87)	41.5 ^{b} (6.87)	87.1 ^{a} (5.66)	83.6 ^{a} (6.84)	52.4 ^{b} (6.04)
All Species	37.2 ^{b} (9.51)	72.8 ^{a} (3.62)	52.3 ^{b} (5.92)	80.9 ^{a} (4.22)	89.9 ^{a} (3.82)	55.7 ^{b} (5.4)
H	15.68*	6.87	19.06**	9.27	8.15	19.23**

Table B2: A-horizon, B-horizon, and burrow soils C and N properties, *n*=4. Values represent means (± 1 SE). Different lower case letters within columns represent significant differences determined by Kruskal-Wallis tests (statistic = *H*) with nonparametric multiple comparisons (** P < 0.01, * P < 0.05).

	A-horizon				B-horizon				Burrow				Total C (g m^{-2})
Treatment	C mass (g m^{-2})	% C	%N	C:N	C mass (g m^{-2})	% C	%N	C:N	C mass (g m^{-2})	% C	%N	C:N	
Control	962.9 ^{a} (24.6)	1.64 ^{a} (0.12)	0.07 ^{a} (0.01)	22.3 ^{a} (0.3)	1779.4 ^{a} (46.8)	0.58 ^{a} (0.02)	0.03 ^{a} (0.01)	22.3 ^{a} (1.78)	——	——	——	——	2829.6 ^{a} (39.6)
Epigeic	1051.5 ^{a} (74.1)	1.67 ^{a} (0.14)	0.08 ^{a} (0.01)	21.8 ^{a} (0.98)	1846.7 ^{a} (73.1)	0.60 ^{a} (0.03)	0.03 ^{a} (0.01)	22.9 ^{a} (1.84)	73.2 ^{a} (7.9)	1.81 ^{ac} (0.42)	0.10 ^{c} (0.02)	22.4 ^{a} (1.06)	3060.6 ^{a} (109.7)
Endogeic	1027.6 ^{a} (81.3)	1.68 ^{a} (0.1)	0.08 ^{a} (0.01)	21.6 ^{a} (0.6)	1653.6 ^{a} (62.5)	0.56 ^{a} (0.02)	0.03 ^{a} (0.01)	22.8 ^{a} (1.95)	143.0 ^{b} (13.4)	1.38 ^{b} (0.26)	0.07 ^{a} (0.01)	20.1 ^{a} (1.24)	2890.9 ^{a} (112.6)
Anecic	1044.7 ^{a} (94.3)	1.72 ^{a} (0.12)	0.08 ^{a} (0.01)	21.0 ^{a} (0.31)	1791.1 ^{a} (146.1)	0.60 ^{a} (0.02)	0.03 ^{a} (0.01)	21.7 ^{a} (1.39)	74.6 ^{a} (10.6)	1.45 ^{bc} (0.29)	0.08 ^{abc} (0.02)	20.3 ^{a} (1.62)	2993.3 ^{a} (191.1)
End×Epi	1075.4 ^{a} (142.2)	1.64 ^{a} (0.2)	0.07 ^{a} (0.01)	22.9 ^{a} (0.47)	1737.2 ^{a} (60.7)	0.57 ^{a} (0.02)	0.03 ^{a} (0.01)	21.9 ^{a} (1.65)	82.5 ^{ab} (15.8)	1.83 ^{abc} (0.57)	0.08 ^{ac} (0.02)	22.0 ^{a} (1.67)	2986.9 ^{a} (135.5)
Epi×Ane	975.3 ^{a} (125.7)	1.52 ^{a} (0.18)	0.06 ^{a} (0.01)	21.2 ^{a} (0.41)	1852.6 ^{a} (73.1)	0.58 ^{a} (0.02)	0.03 ^{a} (0.01)	21.6 ^{a} (1.69)	77.3 ^{ab} (19.1)	1.93 ^{abc} (0.28)	0.10 ^{bc} (0.02)	21.3 ^{a} (1.24)	2991.7 ^{a} (185.7)
End×Ane	1034.8 ^{a} (99.6)	1.65 ^{a} (0.15)	0.07 ^{a} (0.01)	22.2 ^{a} (0.74)	1746.2 ^{a} (71.8)	0.57 ^{a} (0.02)	0.03 ^{a} (0.01)	21.2 ^{a} (1.94)	127.7 ^{ab} (16.1)	1.57 ^{b} (0.28)	0.08 ^{ac} (0.01)	21.8 ^{a} (0.96)	2986.4 ^{a} (181.0)
All Species	1009.9 ^{a} (78.2)	1.68 ^{a} (0.08)	0.08 ^{a} (0.01)	22.4 ^{a} (0.26)	1724.8 ^{a} (57.4)	0.57 ^{a} (0.02)	0.03 ^{a} (0.01)	23.5 ^{a} (2.19)	94.9 ^{ab} (12.8)	1.89 ^{a} (0.47)	0.08 ^{abc} (0.02)	23.2 ^{a} (1.89)	2916.2 ^{a} (128.3)
H	1.03	1.80	4.72	9.91	4.50	5.15	9.96	2.73	11.16*	11.72*	12.87*	4.83	0.64

Table B3: Correlation matrix of leaf litter (L), A-horizon (A), burrow (Br), and B-horizon (B) C and N properties (%C, %N, C content, N content); CO_2-C and DOC losses. Values represent Spearman rank correlation coefficients (ρ). Significance levels of correlation are indicated as ** $P < 0.01$, * $P < 0.05$.

	B-horizon				A-horizon				Burrow				Leaf litter			
	B%N (1)	B%C (2)	B.N (3)	B.C (4)	A%N (5)	A%C (6)	A.N (7)	A.C (8)	Br%N (9)	Br%C (10)	Br.N (11)	Br.C (12)	L%N (13)	L%C (14)	L.N (15)	L.C (16)
(1)																
(2)	0.66**															
(3)	0.91**	0.69**														
(4)	0.56**	0.86**	0.75**													
(5)	-0.05	0.05	0.01	0.01												
(6)	-0.03	0.07	0.1	0.16	0.79**											
(7)	-0.02	0.12	0.12	0.18	0.89**	0.81**										
(8)	-0.01	0.10	0.18	0.28	0.72**	0.95**	0.86**									
(9)	0.39*	0.50**	0.42*	0.54**	-0.08	0.03	-0.04	0.04								
(10)	-0.02	0.07	0.09	0.26	-0.01	0.26	0.15	0.32*	0.29*							
(11)	-0.13	-0.15	-0.20	-0.24	0.28	0.28	0.17	0.19	0.15	-0.22						
(12)	-0.27	-0.22	-0.28	-0.24	0.27	0.36*	0.26	0.33*	0.10	-0.16	0.91**					
(13)	-0.30	-0.06	-0.16	0.12	0.08	0.33*	0.22	0.36*	0.16	0.58**	-0.06	0.04				
(14)	-0.28	-0.13	-0.10	0.09	0.22	0.50**	0.38*	0.53**	-0.03	0.58**	-0.20	-0.06	0.88**			
(15)	0.08	0.14	0.05	0.19	-0.14	-0.10	-0.26	-0.18	0.41*	-0.09	0.27	0.16	0.07	-0.2		
(16)	0.01	0.12	0.02	0.18	-0.07	-0.04	-0.18	-0.11	0.40*	-0.01	0.26	0.16	0.19	-0.09	0.98**	
CO_2 (17)	0.36*	0.39*	0.37*	0.26	-0.32*	-0.29*	-0.27	-0.29*	0.14	-0.03	0.10	0.04	-0.05	-0.13	0.08	0.04
DOC (18)	0.27	-0.02	0.06	-0.23	-0.09	-0.17	-0.19	-0.26	0.16	-0.06	0.14	0.02	-0.32	-0.25	-0.09	-0.16

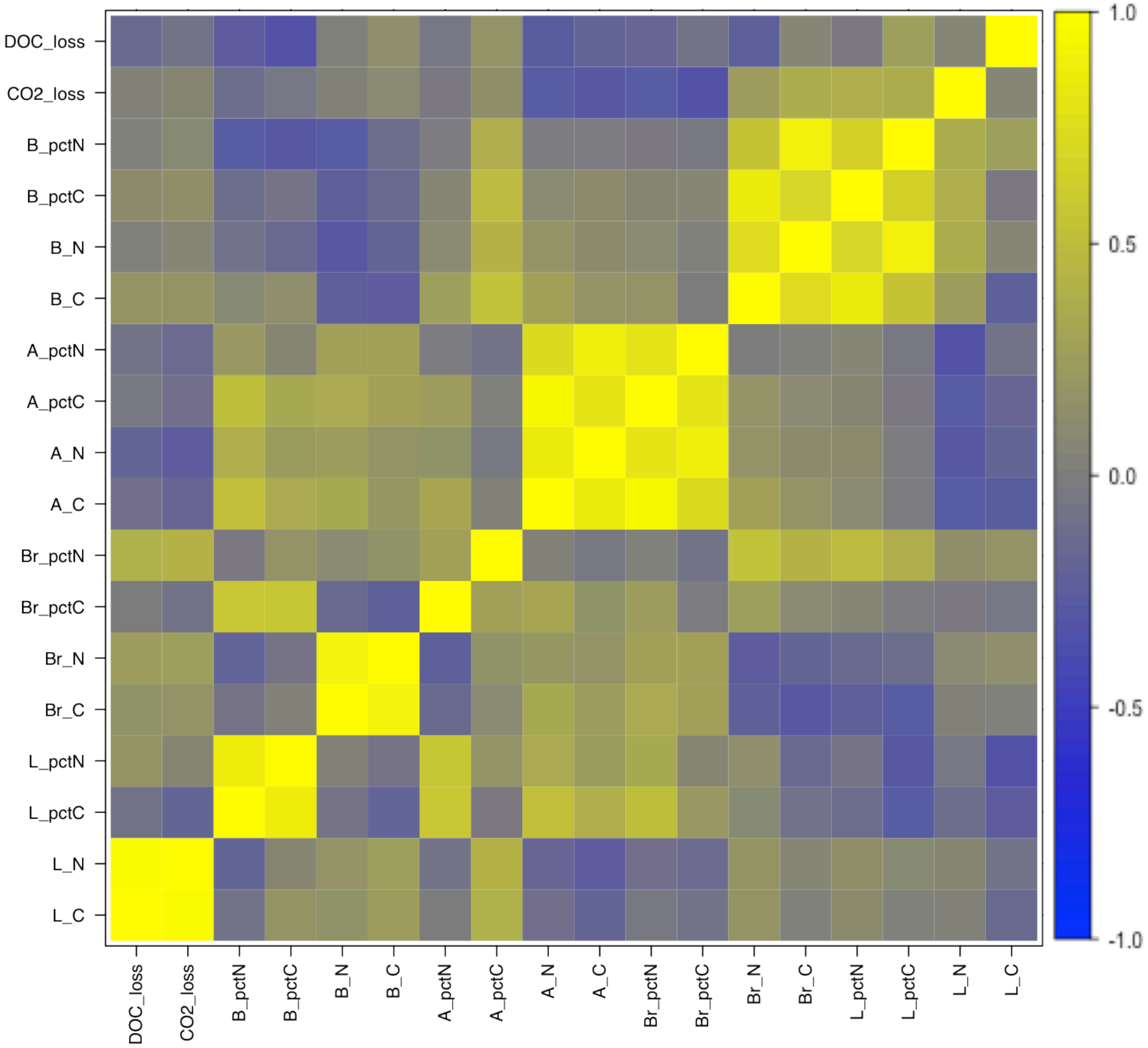

Figure B1: Correlation matrix of leaf litter (L), A-horizon (A), burrow (Br), and B-horizon (B) C and N properties (%C, %N, C content, and N content), CO_2 loss, and DOC loss. Shading intensities represent Spearman rank correlation coefficients (ρ) between leaf litter and soil C and N variables (numerical values and significance levels are given in Appendix B: Table 3).

APPENDIX C: Tables showing numerical values of burrow system structure properties (macrostructure, continuity, size distribution) across treatments, and Spearman rank correlations.

Table C1: Burrow system structure variables across earthworm treatments. Surface connectivity represents burrow volume connected to the soil surface. Burrow continuity is measured as the number of burrows whose length is greater than 0 to > 50% of core length (CL). Values represent means (± 1 SE). Different lower case letters within columns represent significant differences determined by Kruskal-Wallis tests (statistic = *H*) with nonparametric multiple comparisons (** P < 0.01, * P < 0.05).

	Burrow system macrostructure (cm^3)			Burrow Continuity (# burrows with length > X% CL)				Burrow Size Distribution (# burrows within range)		
Treatment	Macroporosity	A-horizon burrow vol.	Surface connectivity	0 – 15%	15 –25%	25 – 50%	> 50%	0.1 to < 0.17 cm^2	0.17 to < 0.34 cm^2	> 0.34 cm^2
Endogeic	664 ^{ac} (34)	564 ^{b} (30)	161 ^{a} (24)	506 ^{b} (7)	158 ^{a} (17)	22 ^{a} (10)	0 ^{a} (0)	1757 ^{c} (15)	696 ^{b} (25)	239 ^{ab} (23)
Anecic	191 ^{d} (27)	151 ^{c} (31)	66 ^{a} (26)	82 ^{c} (21)	45 ^{a} (8)	20 ^{a} (12)	8 ^{a} (5)	241 ^{d} (37)	135 ^{c} (17)	69 ^{c} (8)
End×Epi	563 ^{abc} (75)	492 ^{ab} (70)	150 ^{a} (33)	239 ^{ac} (44)	38 ^{a} (14)	0 ^{a} (0)	0 ^{a} (0)	1229 ^{b} (135)	473 ^{d} (58)	158 ^{abc} (39)
Epi×Ane	382 ^{b} (20)	354 ^{a} (26)	146 ^{a} (10)	147 ^{c} (13)	65 ^{a} (0)	16 ^{a} (0)	0 ^{a} (0)	767 ^{b} (107)	302 ^{d} (30)	114 ^{b} (7)
End×Ane	620 ^{ac} (51)	530 ^{b} (54)	175 ^{a} (24)	294 ^{a} (60)	102 ^{a} (32)	33 ^{a} (9)	8 ^{a} (5)	1305 ^{a} (85)	559 ^{a} (20)	196 ^{a} (35)
All Species	683 ^{a} (54)	561 ^{b} (61)	195 ^{a} (33)	302 ^{a} (41)	94 ^{a} (26)	12 ^{a} (8)	8 ^{a} (5)	1481 ^{ab} (62)	579 ^{ab} (48)	204 ^{a} (22)
H	18.87**	16.96**	9.13	19.43**	11.93	5.99	9.82	23.04**	20.92**	14.78*

Table C2: Correlation matrix of burrow system structure variables (SC = surface connectivity, MR = macroporosity, ABr_vol = burrow volume in the A-horizon, BS = burrow size class [1 = 0.1 to < 0.17 cm^2, 2 = 0.17 to < 0.34 cm^2, 3 = > 0.34 cm^2], BC = burrow continuity class [1 = 0 – 15%, 2 = 15 – 25%, 3 = 25 – 50%, 4 = > 50% of core length]). Values represent Spearman rank correlation coefficients (ρ). Significance levels of correlation are indicated as ** P < 0.01, * P < 0.05.

	Burrow system macrostructure (cm^3)			Burrow continuity (# burrows with length > X% CL)				Burrow size distribution (# burrows within range)		
	MR (1)	ABr_vol (2)	SC (3)	BC_1 (4)	BC_2 (5)	BC_3 (6)	BC_4 (7)	BS_1 (8)	BS_2 (9)	BS_3 (10)
(1)										
(2)	0.97**									
(3)	0.74**	0.76**								
(4)	0.82**	0.79**	0.45*							
(5)	0.64**	0.63**	0.43*	0.79**						
(6)	-0.08	-0.12	-0.17	0.21	0.48*					
(7)	-0.10	-0.19	-0.14	-0.20	-0.03	0.47*				
(8)	0.84**	0.81**	0.41*	0.94**	0.72**	0.17	-0.12			
(9)	0.81**	0.78**	0.40*	0.90**	0.73**	0.24	0.03	0.94**		
(10)	0.82**	0.81**	0.55*	0.82**	0.63**	-0.03	-0.02	0.77**	0.84**	

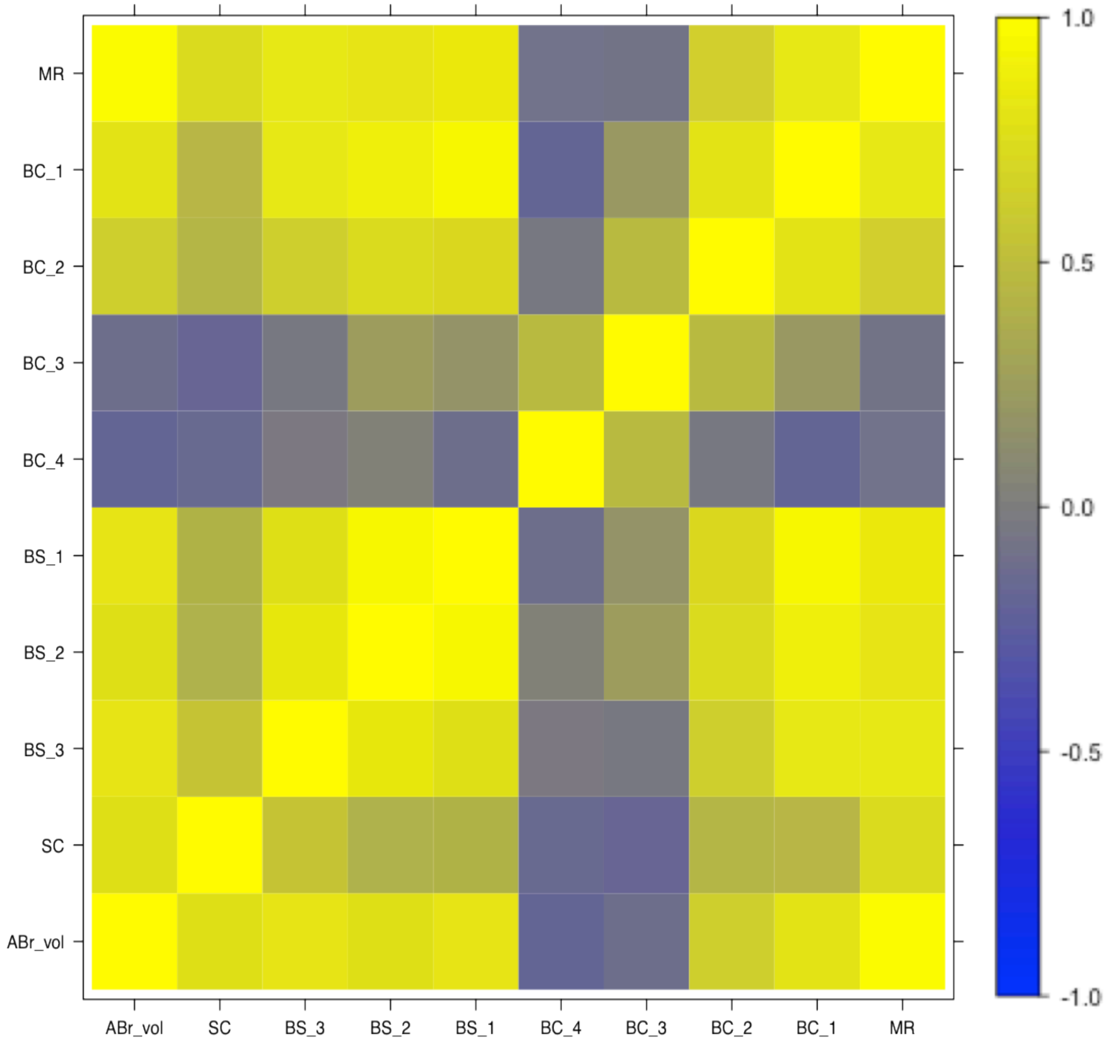

Figure C1: Correlation matrix of burrow system structure variables: SC = surface connectivity, MR = macroporosity, ABr_vol = burrow volume in the A-horizon, BS = burrow size class [1 = 0.1 to < 0.17 cm^2, 2 = 0.17 to < 0.34 cm^2, 3 = > 0.34 cm^2], BC = burrow continuity class [1 = 0 – 15%, 2 = 15 – 25%, 3 = 25 – 50%, 4 = > 50% core length]. Shading intensities represent Spearman rank correlation coefficients (ρ) between leaf litter and soil C and N variables (numerical values and significance levels are given in Appendix C: Table 2).

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Chapter 4

Exotic earthworm community and soil texture controls on soil carbon dynamics in a sandy temperate forest [4]

Abstract

We examined how exotic earthworm communities modify C budgets and the coupled transport of carbon and nitrogen between leaf litter and soils in sandy and sandy loam Spodosols of forests in a northern Michigan landscape. Using ^{13}C- and ^{15}N-enriched leaf litter additions to soil mesocosms, we combined earthworm species of three functional groups (*Lumbricus terrestris* [anecic], *Aporrectodea trapezoides* [endogeic], and *Eisenia fetida* [epigeic]) in a factorial design. To test for the influence of soil texture on earthworm community impacts, selected treatments were replicated in sandy and sandy loam Spodosols. We measured all major components of the soil carbon budget, quantified leaf litter ^{13}C and ^{15}N redistribution using isotopic mass balance, and assessed sub-surface burrow system structures using X-ray computed tomography across a 150-day incubation study. We observed a difference in the onset of earthworm community-enhanced CO_2 release, with sandy loam soils showing a longer temporal lag prior to maximum respiration than sandy soils. Isotopic tracers revealed that A-horizons were dominant sinks for leaf litter C and N, with ^{13}C and ^{15}N transport significantly higher in sandy loam than in sandy soil, and in sandy soil containing both endogeic and anecic species relative to sandy soils in which these groups were absent. Burrow systems of communities that included mineral soil-dwelling endogeic species were also larger sinks for leaf litter C and N. Earthworm biomass was a minor sink for leaf litter ^{13}C and ^{15}N, with levels of enrichment associated with the degree of surface leaf-litter feeding (*L. terrestris* > *E. fetida* > *A. trapezoides*. Our results show distinct functions of surface-soil burrow system properties most associated with endogeic and epigeic species, and sub-soil burrow systems associated with anecic species. Sub-soil burrow systems with high levels of continuity and large burrow size produced by vertical-burrowing and litter-feeding of anecic species are associated with greater leaf litter transport and redistribution into sub-soils, in addition to greater leachate losses of organic carbon from these soils. Burrow systems with the highest volume, greatest surface connectivity, and densest burrow networks in the A-horizon were a product of interspecific interactions between surface-dwelling epigeic species and mineral-soil dwelling endogeic species. These properties were, in turn, associated with greater CO_2 losses and greater transport of leaf litter-derived C and N into A-horizon and burrow soils. Leaf litter degradation and transport are key processes by which earthworm communities influence soil carbon budget components. Leaf litter degradation is primarily driven by the foraging activity of one ecological group, while leaf litter redistribution in the soil profile

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is determined by the volume and connectivity of burrow systems created by the composite earthworm community. The fates of litter-derived C and N (gaseous and leachate loss, retention in the soil profile) in earthworm-invaded soils are strongly influenced by soil properties.

4.1 Introduction

Soil carbon (C) budgets of northern temperate forests are of interest because soil organic matter in these systems functions as a long-term C sink (Gaudinski et al. 2000, Gough et al. 2008a). In the last decade, shifts in individual components of soil C budgets (i.e., C losses as CO_2 and DOC, stocks, turnover time, and depth distribution) have been linked to introductions of exotic earthworm species into previously earthworm-free temperate forests in North America (Bohlen et al. 2004a, 2004c). These shifts include changes in soil C stocks (Alban and Berry 1994, Burtelow et al. 1998, Bohlen et al. 2004c, Wironen and Moore 2006, Fahey et al. 2012), soil C depth distribution (Burtelow et al. 1998, Bohlen et al. 2004b, Wironen and Moore 2006, Straube et al. 2009, Fahey et al. 2013a), CO_2 losses (McLean and Parkinson 1997, Li et al. 2002, Fisk et al. 2004, Eisenhauer et al. 2007, Aira et al. 2009), and dissolved organic C losses (Haimi and Huhta 1990, Scheu and Parkinson 1994, McInerney and Bolger 2000, Bohlen et al. 2004b). Variation in the magnitude and direction of these shifts is likely related to variation in earthworm species composition (Hale et al. 2005, Straube et al. 2009, Crumsey et al. 2013b), land use history (Bohlen et al. 2004b, Crow et al. 2009, Ma et al. 2013), leaf litter chemistry (e.g., C:N ratios) (Hobbie et al. 2006a, Filley et al. 2008, Melvin and Goodale 2013, Fahey et al. 2013b), and soil properties. However, the relative importance of each of these components is unknown, in large part because controlled experiments that measure complete C budgets where earthworm species composition is manipulated are few in number (e.g., Crumsey et al. 2013b).

It is also recognized that forest biogeochemical cycles of C and N are linked by multiple processes , such that aboveground C uptake rates and belowground soil C budgets are generally constrained by N availability (Zak et al. 1989, Nadelhoffer et al. 1999a, Waldrop et al. 2004, Nave et al. 2011). A number of studies have shown that exotic earthworm activity in northern temperate forests can increase N pool size, N mineralization rates (NO_3^- and NH_4^+), and organic N leaching losses (Alban and Berry 1994, Burtelow et al. 1998, Bohlen et al. 2004b, Wironen and Moore 2006, Costello and Lamberti 2009, Fahey et al. 2013b). In order to assess the implications of exotic earthworm activity on soil C budgets, it is thereby necessary to determine how C and N inputs are distributed among forest soil components following exotic earthworm introductions. For example, earthworm activity could increase long-term soil C storage if C redistribution from surface to sub-soils is greater than that of N redistribution. Alternatively, long-term soil C storage could decrease if C redistribution from surface to sub-soils is less than that of N redistribution. Furthermore, because the species composition of earthworm communities in northern temperate forests is spatially and temporally variable, and earthworm species can vary in foraging and burrowing behaviors, it is important to determine whether C and N retention and redistribution varies with earthworm community composition. Finally, soil physical and chemical properties (e.g., texture, mineralogy, moisture, pH) function as factors constraining both earthworm species distributions and soil C storage capacity (Sollins et al. 1996, Torn et al. 1997, Swanston et al. 2005, Suárez et al. 2006c, Crumsey et al. 2013a). Once again, the relative importance of earthworm species, burrow structures, and soil properties on C and N inputs and distribution in northern temperate forest soils is unknown.

In this study, we measured C budgets and used ^{13}C and ^{15}N tracer additions to laboratory mesocosms to examine how monospecific and multi–species earthworm communities control C and N transport between leaf litter and forest soils of two different textures. We focused on interspecific interactions involving mineral soil-dwelling endogeic species that influence soil macroporosity and organic matter stabilization through subsurface cast and horizontal burrow production (Jégou et al. 1997, 2000, Marhan and Scheu 2006, Crumsey et al. 2013b). Results are presented from a 5-month mesocosm experiment in which earthworm species of three functional groups were combined in a partial factorial design, and community treatments were replicated in sandy and sandy loam Spodosols. We measured all major soil C budget components, CO_2 and DOC losses, imaged and quantified the structure of subsurface burrow systems, and used isotopic mass balance to quantify sinks for isotopically enriched leaf litter C and N inputs.

4.2 Materials and Methods

4.2.1 Experimental Design

We conducted a mesocosm experiment from May – October 2011 in a belowground laboratory (Lussenhop et al. 1991) at the University of Michigan Biological Station (UMBS; see appendix for study area description), using 5 combinations of 3 earthworm species as treatments, and no-earthworm controls in uniform reconstructed soil profiles. We used two dominant forest soil types from the site classified as Spodosols (1) a sandy, mixed Entic Haplorthod of the

Rubicon series (92.9% sand, 6.5% silt, 0.6% clay), and (2) a coarse sandy loam, mixed Alfic Haplorthod of the Alcona series (69.2% sand, 14.2% silt, 16.6% clay). Mesocosms were contained in 20-L plastic buckets, and were 20 cm in diameter and 35 cm in depth with a port in the bottom for draining. Soil profiles were constructed by adding 25 kg (fresh weight) of sieved (2mm) and homogenized B-horizon material packed to a bulk density of 2.5 g cm^{-3}, 25cm in depth; 5 kg (fresh weight) of sieved and homogenized A-horizon material packed to a bulk density of 1.3 g cm^{-3}, 5cm in depth.

Earthworm species of different ecological groups included: *L. terrestris* [Anecic = litter feeding, deep vertical burrowing], *E. fetida* [Epigeic = litter feeding, surface–dwelling], and *A. trapezoides* [Endogeic = mineral soil feeding and dwelling]. To focus on interspecific interactions involving mineral soil-dwelling endogeic species, we implemented a partial factorial experimental design where treatments, hereafter capitalized, included (a) species monocultures: Epigeic and Anecic; and (b) mixed treatments: Epigeic×Endogeic, Anecic×Endogeic, and All Species. To evaluate the interactions of soil texture with earthworm community impacts, the All Species treatment was replicated in the finer-textured sandy loam. Total earthworm biomass was held constant at 20 ± 0.5 g (fresh weight) per mesocosm. Thus, earthworm biomass was 10 ± 0.5g of each species in two-species treatments and 6.5 ± 0.5 g for each species in the All Species treatments.

4.2.2 Acer rubrum ^{13}C and ^{15}N enrichment and mixed leaf litter additions

In our previous study using the sandy Spodosol of this study, *A. rubrum* leaf litter was consumed at higher rates than litter from other tree species dominating this forest site (Crumsey et al. 2013b). Therefore, we use isotopically labeled *A. rubrum* leaf litter as an isotopic tracer to quantify C and N transport from leaf litter to soil horizons. We obtained this material from *A. rubrum* seedlings grown in a temperature-controlled chamber at Queens College, City University of New York (Flushing, NY), in which seedlings were labeled weekly with ^{13}C-enriched CO_2 for 18 weeks, and with^{15}N-enriched NH_4Cl and KNO_3 fertilizer (liquid) weekly for 21 weeks. The C and N chemistry of dual-labeled *A. rubrum* leaf litter was as follows: 41.9 % C, 1.06 % N, $\delta^{13}C$ = 3170‰, and $\delta^{15}N$ = 15918‰. Senesced leaves were dried at 60 °C and were included in mixed leaf litter additions scaled in composition and mass from area-normalized leaf litter data of the UMBS AmeriFlux site in 2008 (C.S. Vogel, unpublished data). In total, 16.8 g mixed leaf litter from dominant canopy tree species was added to each mesocosm at the start of incubation as follows: 26.8% dual-labeled *A. rubrum*, 39.8% *Populus grandidentata*, 20.8% *Quercus rubra*, 6.8% *Betula papyrifera*, 4.3% *Fagus grandifolia*, and 1.5% *Pinus strobus*.

4.2.3 Mesocosm C loss measurements (CO_2 and DOC)

Soil CO_2 efflux was measured from May – September (15 times over a 150-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. 1998, 2000, Capowiez et al. 2011), and weekly thereafter. CO_2 efflux was measured using an infrared gas analyzer (IRGA, LICOR-6400) connected to an air-tight lid placed on each mesocosm. Air flowed through a 4.67-L headspace over each mesocosm in a closed loop to the LI–6400, temperature was measured with a type E thermocouple (Omega), and a capillary tube was inserted for air pressure equilibration. Soil CO_2 efflux rates (F_c) were determined by measuring the rate of 10 μmol mol^{-1} change in CO_2 concentration (ΔCO_2), from which CO_2-C flux rate per unit soil surface area was calculated as:

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

where F_c is C flux corrected for headspace volume (V) and surface area (S) (μmol CO_2 m^{-2} sec^{-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 over measurements to derive cumulative area-normalized curves for each mesocosm. We then divided total CO_2 and DOC losses by initial g soil C to express soil-C-normalized CO_2 and DOC losses across treatments.

Soil moisture was maintained at field capacity (~20% v/v) with 500 mL de-ionized water additions. Soil leachates were collected weekly from zero-tension lysimeters installed below each mesocosm. Collected leachates were weighed, filtered using glass-fiber filters (Whatman, GF/F), acidified with 6 N HCl, and stored at −20°C until analyzed for DOC concentration using an Aurora (Model 1030W) OI Analytical TOC analyzer. DOC loss values were integrated to derive cumulative leaching losses for each mesocosm. 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.

4.2.4 3D reconstruction and quantification of burrow systems

Following the 5-month incubation period, soils containing earthworm treatments were imaged using X-ray computed tomography (Discovery CT 750 HD scanner, 120 kV, 110 mA, 9.04s, 0.969:1 pitch, 1.25mm slice interval, 1.25mm slice thickness, 40 cm field of view, Bone reconstruction filter) at the School of Radiology, University of Michigan Hospital. Two-D images (horizontal sections of soil every 1.25mm, 0.78mm X and Y resolution) show the attenuation of X-rays through soils. The sequential analysis of 2-D binarized images enabled 3-D tracking of each earthworm burrow in the core, and subsequent 3-D volumetric reconstructions of earthworm community burrow systems. Image preparation and the quantification of burrow continuity, volume, and size distribution was conducted at the French National Institute for Agricultural Research following methods previously described (Capowiez et al. 1998, Pierret et al. 2002, Bastardie et al. 2005).

4.2.5 Litter and soil sampling, C and N content, isotopic analysis

Mesocosms were destructively harvested by first collecting intact leaf litter remaining on the soil surface. Soils were then excavated by removing the A-horizon (5 cm depth), followed by removal of B-horizon in 5-cm to 10-cm depth increments (i.e., 0−5 cm, 5−10 cm, 10−15 cm, and 15−25 cm below the A-horizon) 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 high-density burrow networks created across all earthworm treatments (Figure 1). Litter and soil pool subsamples were weighed fresh, dried at 60°C, weighed again to obtain dry weight correction factors, and pulverized for C and N analyses. Type-specific leaf litter mass loss was used in calculating a weighted average of composite leaf litter C and N expressed at the end of the experiment. Litter, soil, and earthworm %C, %N, and stable isotope ratios (expressed as δ^{13}C and δ^{15}N) were measured by continuous flow isotope ratio mass spectrometry (Thermo Finnigan Delta Plus XL) after sample combustion to CO_2 and N_2 at 1000°C by an on-line elemental analyzer (Costech Elemental Analyzer 4010. Instrument error determined by repeated internal standards was ± 0.16‰ for δ^{15}N, and ± 0.19‰ for δ^{13}C. The C and N properties of starting materials (soil pools, earthworm species, and leaf litter types) are shown in Table B1.

4.2.6 ^{13}C and ^{15}N tracer recoveries in soils and earthworm biomass

We calculated *A. rubrum* litter ^{13}C and ^{15}N movements into soil biomass components (i.e. A-horizon, B-horizon, burrow soil) and earthworm species biomass using elemental pool size estimates, changes in ^{13}C and ^{15}N content of pools following leaf litter additions, and elemental mass balances (Nadelhoffer and Fry 1994, Fahey et al. 2011). *A. rubrum* litter ^{13}C and ^{15}N pools were calculated at time zero and at the time of soil harvesting as the product of dry weight, C and N concentration, and isotopic atom % enrichments of ^{13}C and ^{15}N. The differences between initial and final isotopic pool estimates were used to calculate percent recoveries of isotopes derived from dual-labeled *A. rubrum* litter, and the amount of isotopic enrichment expressed on a per m^2 basis. *A. rubrum* ^{13}C recovery in each pool was calculated using the following equation:

$$^{13}C_{rec} = \frac{m_{pool}(atom\,\%^{13}C_{pool} - atom\,\%^{13}C_{ref})}{(m_{Acru} \times atom\,\%^{13}C_{Acru})_i - (m_{Acru} \times atom\,\%^{13}C_{Acru})_f} \tag{2}$$

where $^{13}C_{rec}$ = *A. rubrum* litter ^{13}C mass recovered in the labeled earthworm or soil pool (mg ^{13}C m^{-2}); m_{pool} = C mass of the labeled pool (mg C/m^2); $atom\,\%^{13}C_{pool}$ = atom percent ^{13}C in the labeled C pool; $atom\,\%^{13}C_{ref}$ = atom percent ^{13}C in the reference C pool; m_{Acru} = mass of the dual-labeled *A. rubrum* litter at the beginning (*i*) and end (*f*) of the incubations; and $atom\,\%^{13}C_{Acru}$ = atom percent ^{13}C of the dual-labeled *A. rubrum* litter at the beginning (*i*) and end (*f*) of the incubations. *A. rubrum* ^{15}N recovery in each pool was calculated using the following equation:

$$^{15}N_{rec} = \frac{m_{pool}(atom\,\%^{15}N_{pool} - atom\,\%^{15}N_{ref})}{(m_{Acru} \times atom\,\%^{15}N_{Acru})_i - (m_{Acru} \times atom\,\%^{15}N_{Acru})_f} \tag{3}$$

where $^{15}N_{rec}$ = *A. rubrum* litter ^{15}N mass recovered in the labeled earthworm or soil pool (mg ^{15}N m^{-2}); m_{pool} = N mass of the labeled pool (mg N m^{-2}); $atom\,\%^{15}N_{pool}$ = atom percent ^{15}N in the labeled N pool; $atom\,\%^{15}N_{ref}$ = atom percent ^{15}N in the reference N pool; m_{Acru} = mass of the dual-labeled *A. rubrum* litter at the beginning (*i*) and end (*f*) of the incubations; and $atom\,\%^{15}N_{Acru}$ = atom percent ^{15}N of the dual-labeled *A. rubrum* litter at the beginning (*i*) and end (*f*) of the incubations. Finally, an *A. rubrum* ^{13}C to ^{15}N recovery ratio (^{13}C$_{rec}$:^{15}N$_{rec}$) was calculated for each soil and earthworm pool calculating the quotient of percent ^{13}C recovery and percent ^{15}N recovery in each respective pool. This approach assumes no changes over time in the natural abundance of ^{13}C and ^{15}N in the soil pools considered. As all other leaf litter types were not isotopically enriched and show decompose at different rates from that of *A. rubrum*

(Pregitzer et al. 2006), all isotopic enrichment of soil and earthworm pools is attributed to the incorporation of dual-labeled *A. rubrum* litter into each respective pool.

4.2.7 Soil C mass storage

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

$$\Delta C = (L_c + E) - (\int_0^{150} F_c(t)dt + \int_0^{150} DOC_{EX}(t)dt) \qquad (4)$$

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 150-day 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.

4.2.8 Statistical analyses

We used Kruskal-Wallis *H* tests (H, df = 7, $n = 48$, $\alpha = 0.05$) with non-parametric multiple comparisons to assess treatment effects on leaf litter mass loss, cumulative CO_2 and DOC losses, elemental pool sizes and chemistry (i.e., C and N content and isotopic values), % recoveries, and burrow system variables. To assess treatment effects on CO_2 and DOC losses over time, we used a General Linear Model with repeated measures, followed by Bonferroni-corrected pairwise comparisons of cumulative curves. We used Spearman rank correlations (ρ, $n = 48$, $\alpha = 0.05$) to characterize relationships among pool chemistry variables and burrow system variables using $\rho > 0.80$ as a criterion for the exclusion of one of two highly correlated C and N variables, and burrow system variables. Carbon variables, total macroporosity, a subset of burrow size classes were retained for the co-inertia analysis. Associations between the remaining soil and burrow system variables 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; $P < 0.05$). Control values (i.e., no earthworm additions) were excluded from the co-inertia analysis. Statistics were done in R v2.15.2 (R Development Core Team 2012) on RStudio v0.96.331 (www.rstudio.org/), using the packages ade4 (Dray and Dufour 2007), Hmisc (Harrell 2012), lattice (Sarkar 2008), and pgirmess (Giraudoux 2012). Where appropriate, results are presented first as comparisons between earthworm community effects in sandy and sandy loam soils (i.e., comparisons among soil texture controls and corresponding All Species treatments), and then as comparisons among mono-specific and multi-species earthworm treatments in sandy soils.

4.3 Results

4.3.1 C losses: Cumulative CO_2 and DOC losses

Soil texture comparisons. Area-normalized CO_2 losses were significantly higher in sandy loam soils than in sandy soils (GLM repeated measures, $P = 0.060$; Figure 2A). The cumulative CO_2 flux between the All Species treatment and sandy loam control were similar over the first 75 days but diverged in the second half of the experiment (GLM repeated measures, $P = 0.045$), resulting in 10% greater CO_2 losses from the All Species treatment by the end of the experiment (Mann-Whitney U tests, $P < 0.01$; Figure 2A). Area-normalized DOC losses were significantly higher in sandy loam soils, and increased with earthworm community additions over time (GLM repeated measures, $P < 0.01$; Figure 2B), while DOC losses were similar in control and the All Species treatment of sandy soils (GLM repeated measures, $P > 0.05$). Mass-normalized CO_2 losses at the end of the experiment showed significantly higher CO_2 losses in sandy soils than marginally higher CO_2 losses in the All Species treatment of sandy loam soils, (Kruskal-Wallis H tests, $P < 0.05$; Figure 3A). Mass-normalized DOC losses, however, did not differ between soil textures (Figure 3B).

Earthworm community comparisons. In sandy soils, more CO_2 evolved from the earthworm treatments than from the control initially, but earthworm community effects lessened over time (GLM repeated measures, $P = 0.034$; Figure 2A). Cumulative CO_2 flux from sandy controls and the Epigeic×Endogeic treatment were lower than that of the Anecic Alone and All Species treatments (Kruskal-Wallis H tests, $P < 0.05$). Area-normalized DOC losses were lowest in mono-specific earthworm treatments, and increased significantly in mixed-species treatments and the control over time (GLM repeated measures, $P < 0.05$; Figure 2B). Mass-normalized CO_2 losses showed no significant responses to earthworm community treatments (Kruskal-Wallis H tests, $P > 0.05$; Figure 3A). Mass-normalized DOC losses showed significant responses to earthworm community treatments (Figure 3B): DOC losses from the Anecic×Endogeic and

Anecic treatments were significantly higher than losses from the Epigeic and Epigeic×Endogeic treatments (Kruskal-Wallis H tests, $P > 0.05$).

4.3.2 *C inputs: Litter mass loss and chemistry*

Leaf litter selection and consumption by earthworm communities resulted in the differential mass loss of leaf litter species and tissue types, such that leaf litter remaining following earthworm community activity was comprised of petioles and mid-veins of *A. rubrum* and *P. grandidentata*, largely intact *F. grandifolia* and *Q. rubra* litter (i.e., most soft tissue, mid-veins, and petioles remained), and intact *P. strobus* litter. Differences in litter mass losses (Table B2) and isotopic losses of ^{13}C and ^{15}N derived from *A. rubrum* litter (Table B3) across soil textures and earthworm community treatments are discussed below.

Soil texture comparisons. Leaf litter C mass loss (32 ± 3 g C m^{-2}) in the control (no earthworm) sandy soil, was 30% higher in the sandy loam (Kruskal-Wallis H tests, $P < 0.05$; Figure 4A). In the sandy soil, the addition of all three earthworm species increased leaf litter C mass losses by 80% (Kruskal-Wallis H tests, $P < 0.05$). In the sandy loam, however, earthworm communities only increased litter C mass losses by 30% (Kruskal-Wallis H tests, $P < 0.05$). Final leaf litter C mass losses were thereby similar in across soil textures following earthworm community additions (Kruskal-Wallis tests, $P > 0.05$).

Earthworm community comparisons. In sandy soils, significant increases in leaf litter C mass losses relative to the control (by 58 - 80%) occurred in earthworm communities containing anecic species (Anecic Alone, Anecic×Endogeic, and All Species; Kruskal-Wallis tests, $P < 0.05$; Figure 4B). Epigeic and endogeic species had no significant effect on litter C mass losses relative to sandy soil controls (Kruskal-Wallis tests, $P > 0.05$).

4.3.3 *Soil C mass storage (C inputs – C losses)*

Soil C mass storage was calculated as the difference between C inputs to soils from litter and unrecovered earthworm biomass, and C losses as CO_2 and DOC (Figure 4). C inputs in sandy control mesocosms (33.0 $\pm$ 2 g C m^{-2}) were less than C outputs as CO_2 and DOC (293 $\pm$ 9 g C m^{-2}). As a result, soil C storage (ΔC) in controls was negative, representing a baseline net loss from the soil system (−260 $\pm$ 9 g C m $^{-2}$; Figure 7). Similar patterns were observed in sandy loam soils, where C inputs (38 $\pm$ 3 g C m^{-2}) were less than C outputs (342 $\pm$ 6 g C m^{-2}); C storage

(ΔC) was thereby negative, representing a baseline net loss (−304 ± 9 g C m^{-2}) that was similar to that observed in sandy soils (Kruskal-Wallis *H* test, $P > 0.05$). C inputs to soils were higher in all treatments containing anecic species (Kruskal-Wallis tests, $P < 0.05$). In contrast, C outputs only increased significantly with earthworm community activity in sandy loam soils (Kruskal-Wallis *H* test, $P < 0.05$). Significant shifts in ΔC were not detected, though trends of greater ΔC in sandy loam soils and multi-species earthworm treatments relative to sandy soils were observed (Kruskal-Wallis *H* test, $P > 0.05$). Cumulative C inputs and outputs were small fluxes to and from relatively large soil pools (burrow, A-horizon, B-horizon) that did not change significantly over the 150-day incubations.

4.3.4 Natural ^{13}C and ^{15}N abundances of soil and earthworm pools

Prior to mesocosm incubations, individual soil and earthworm biomass pools were easily separated with respect to their initial isotopic compositions (Table B1). Sandy loam A-horizon soil δ^{13}C values were highly enriched relative to sandy soils (δ^{13}C = -19.8 ± 0.3 vs. -26.5 ± 0‰; Mann-Whitney *U* tests, $P < 0.05$), likely indicative of extensive *in situ* processing by soil fauna and microbes prior to soil field collections. Sandy B-horizons were isotopically enriched relative to A-horizons; δ^{13}C values were similar across soil types (δ^{13}C = -26.1 to -25.9‰). The opposite patterns were observed for A-horizon δ^{15}N values, which were higher in sandy than sandy loam soil (δ^{15}N = 1.14 ± 0.3 vs. - 0.11 ± 0.1‰; Mann-Whitney *U* tests, $P < 0.05$). B-horizon natural abundance δ^{15}N values were higher in sandy loam than sandy soil (δ^{15}N = 11.0 ± 1.5 vs. 3.6 ± 0.1‰; Mann-Whitney *U* tests, $P < 0.05$). Earthworm species isotopic values generally fell between those of soil pools and non-labeled leaf litter. Earthworm δ^{13}C values were similar across species (δ^{13}C = -24.5 to -26.1‰). *A. trapezoides* δ^{15}N values were enriched (δ^{15}N = 2.21 ± 0.2‰) relative to *L. terrestris* and *E. fetida* values (δ^{15}N = - 0.51 to -0.91‰; Kruskal-Wallis *H* tests, $P < 0.05$).

4.3.5 ^{13}C and ^{15}N tracer recoveries in soil and earthworm pools

The 150-day incubations resulted in significant increases (i.e., enrichment) in the δ^{13}C and δ^{15}N values of soil and earthworm biomass pools (Tables 1 and 2). The isotopic enrichment of these pools was due to enriched *A. rubrum* litter redistribution, the decomposition of this material, and subsequent assimilation into soil organic matter and earthworm biomass. The difference between initial and final isotopic values for each respective pool was thereby used to estimate isotopic recoveries of ^{13}C and ^{15}N derived from the enriched *A. rubrum* litter (expressed as mg ^{13}C m^{-2} and mg ^{15}N m^{-2}; Eq. 2 and 3). Total tracer recoveries in measured individual soil and earthworm biomass pools ranged from 5 – 47% of ^{13}C additions and 13 – 102% of ^{15}N additions in sandy soils, and from 77 – 95% of ^{13}C additions and 49 – 108% of ^{15}N additions in sandy loam soils (Tables B4 and B6). C tracer recoveries less than 100% are expected as considerable fractions of fresh litter C are likely respired early on in decomposition, whereas most litter N is recycled among soil inorganic and organic pools with minor N losses through denitrification and leaching in well-drained oxic soils such as these (Groffman and Tiedje 1989, Gaudinski et al. 2000, Fahey et al. 2011, Nave et al. 2011).

Soil texture comparisons. Earthworm community additions to sandy soils significantly increased the amount of leaf litter transported to the A-horizon, but did not affect the amount transferred deeper to B-horizons (Figure 5). In control (no earthworm) sandy soils, we recovered 5% of ^{13}C (28 mg ^{13}C m^{-2}) and 13% of ^{15}N (2 mg ^{15}N m^{-2}) derived from labeled *A. rubrum* litter. Earthworm community additions to sandy soils significantly increased total ^{13}C recovery to 35% and total ^{15}N recovery to 74% (Kruskal-Wallis H tests, $P < 0.05$). Similar patterns of increased leaf litter transported to the A-horizon following earthworm additions were observed in sandy loam soils, though the amount of leaf litter ^{13}C transferred deeper to B-horizons was significantly greater than that observed in sandy soils. In control (no earthworm) sandy loam soils, we recovered 77% of ^{13}C (315 mg ^{13}C m^{-2}) and 51% of ^{15}N (16 mg ^{15}N m^{-2}) derived from *A. rubrum* litter. Earthworm community additions to sandy loam soils significantly increased total ^{13}C recovery to 94% and total ^{15}N recovery to 105% (Kruskal-Wallis H tests, $P < 0.05$). Differences in ^{15}N recoveries between soil textures corresponded to significant differences in *A. rubrum*-derived $^{13}C_{rec}$:$^{15}N_{rec}$ recovery ratios ($^{13}C_{rec}$:$^{15}N_{rec}$ values <1) across all soil pools: relatively lower $^{13}C_{rec}$:$^{15}N_{rec}$ values in sandy burrow and B-horizon soils reflect lower ^{15}N recoveries compared to the same soil pools in B-horizon soils, $^{13}C_{rec}$:$^{15}N_{rec}$ increased significantly with earthworm additions in both soil textures (Kruskal-Wallis *H* tests, $P < 0.05$; Table 3).

Earthworm community comparisons. Similar patterns were observed for dual-labeled *A. rubrum* ^{13}C and ^{15}N losses across earthworm community treatments in sandy soils (Figure 6). In earthworm communities containing anecic species, *A. rubrum* ^{13}C and ^{15}N losses increased by 60

– 76%, and 36 – 44% respectively. *A. rubrum* ^{13}C and ^{15}N losses in the Epigeic Alone and Epigeic×Endogeic treatments were similar to those observed in sandy controls with no earthworm additions (Kruskal-Wallis *H* tests, $P > 0.05$). The inclusion of endogeic species in earthworm community treatments of sandy soils significantly increased the amount of leaf litter transported to the A-horizon and found in burrow soils, but did not affect the amount transferred deeper to B-horizons (Figure 6). Across Epigeic×Endogeic, Anecic×Endogeic, we recovered up to 44% of ^{13}C (200 - 370 mg ^{13}C m^{-2}) and up to 74% of ^{15}N (10 - 20 mg ^{15}N m^{-2}) derived from labeled *A. rubrum* litter (Tables B4 and B6). A-horizon and burrow soil pools show the highest $^{13}C_{rec}$:$^{15}N_{rec}$ values in mixed earthworm community treatments (Table 3): *A. rubrum*-derived $^{13}C_{rec}$:$^{15}N_{rec}$ recovery ratios in A-horizon soils increased by 22-32% across Epigeic×Endogeic, Anecic×Endogeic, and the sandy All Species treatment, but remained constant in B-horizon soils (Kruskal-Wallis *H* tests, $P < 0.05$).

Earthworm species biomass comparisons. Dual-labeled *A. rubrum* ^{13}C and ^{15}N enrichments in earthworm species biomass also differed with earthworm community composition (Table B5). In earthworm biomass, ^{13}C and ^{15}N percent recoveries were calculated from final elemental and isotopic pool estimates of live earthworms remaining at the end of the incubation. Overall, earthworm biomass functioned as a small pool for tracer recovery, with differences in ^{13}C and ^{15}N assimilation among species associated with the degree of surface leaf-litter feeding and interspecific interactions (Table B6). *A. trapezoides* (endogeic) δ^{13}C and δ^{15}N values were most enriched in the Anecic×Endogeic and the All Species treatment of sandy and sandy loam soils (Kruskal-Wallis *H* tests, $P < 0.05$). Tracer recoveries in *L. terrestris* biomass were similar across treatments, and ranged from 0.8 – 1.5% of applied ^{13}C and 1.8 – 3.0% of applied ^{15}N (Kruskal-Wallis *H* tests, $P > 0.05$). In *E. fetida* biomass, isotope recovery decreased significantly in the All Species treatments (Kruskal-Wallis *H* tests, $P < 0.05$). Tracer recoveries in *A. trapezoides* biomass were similar across all treatments by the end of the incubations (Kruskal-Wallis *H* tests, $P > 0.05$). Earthworm species differences in isotopic tracer recoveries, and thereby $^{13}C_{rec}$:$^{15}N_{rec}$ values, varied as follows (largest to smallest isotopic recovery): *L. terrestris* > *E. fetida* > *A. trapezoides* (Kruskal-Wallis *H* tests, $P < 0.05$; Table 3).

4.3.6 Earthworm Burrow System Structure

Soil texture comparisons. Burrow system structures were statistically similar between the All Species treatments of sandy and sandy loam soils (Kruskal-Wallis tests, $P > 0.05$), though mean values of burrow system macrostructure (total macroporosity, A-horizon burrow volume, and surface connectivity) were consistently higher in sandy loam soils (Table C1).

Earthworm species comparisons. Burrow system structures differed as a function of earthworm community composition in sandy soils (Table C1). The All Species and Anecic×Endogeic treatment significantly increased burrow system macroporosity, A-horizon burrow volume, and the smallest burrow size class relative to mono-specific earthworm treatments (Kruskal-Wallis H tests, $P < 0.05$). Burrow systems created in within the Epigeic and Epigeic×Endogeic treatments were statistically similar though mean values of burrow system macrostructure, burrow continuity, and burrow size classes were consistently higher in the Epigeic×Endogeic treatment. Burrow systems having the smallest volume and continuity were observed in the mono-specific Anecic treatment (Kruskal-Wallis H tests, $P < 0.05$).

4.3.7 Relationships between Burrow Systems and Soil C and N Properties

To characterize co-relationships between soil chemistry and burrow system data matrices, we evaluated correlations among chemistry and burrow system variables (Tables C2 and C3) and retained soil carbon variables, total macroporosity, and burrow size classes with correlation coefficients (ρ) less than 0.80. We then evaluated variable associations along the first two co-inertia axes (eigenvalues: F1 = 4.22; F2 = 1.76). The first two axes of the co-inertia analysis explained 90.5% of the total variability in the chemistry and burrow system data co-structure (Monte Carlo permutation tests, $P = 0.002$; Figure 7). Along F1 (76.6% of total inertia), sub-soil burrow systems having high levels of continuity (25 to > 50% of core length) and large burrow size (> 0.34 cm^2) were (1) associated with anecic species, and (2) strongly related to the C mass of burrow and B-horizon soils, as well as *A. rubrum* ^{13}C and ^{15}N recovery in these pools. Along F2 (13.9% of total inertia), surface-soil burrow system properties were most associated with endogeic and epigeic species, and sub-soil burrow systems were most associated with anecic species. First, burrow systems with the highest volume, greatest surface connectivity, and dense networks of small burrows (< 0.34 cm^2) in the A-horizon were (1) most associated with endogeic and epigeic species, and (2) strongly related to CO_2 loss, *A. rubrum* ^{13}C recovery in the A-horizon, and A-horizon C mass. Second, burrow systems of low to intermediate continuity (0 – 25% of core length) were (1) most associated with anecic species, and (2) strongly related to the magnitude of *A. rubrum* ^{13}C and ^{15}N loss, and DOC losses over the 150-day incubations.

4.4 Discussion

Cumulative CO_2 and DOC losses

Over the course of mesocosm incubations, we observed differences in the patterns of C losses as CO_2 and DOC across soil textures and earthworm community treatments. For example, results showed a temporal lag between the onset of earthworm community-induced CO_2 losses from sandy loam versus sandy soils, and higher DOC losses from the sandy loam soil, which represented minor components of C losses from these reconstructed soil systems. Soil texture can influence soil C loss rates through its effects on moisture content, temperature, preferential flow path stability, and nutrient availability (Pastor and Post 1986, Saxton et al. 1986, Schimel et al. 1994, Bowden et al. 1998, Davidson et al. 1998, Fang and Moncrieff 2001, Lal 2005). Soil texture also determines the degree to which C compounds are protected from biodegradation through two general processes: (1) physical protection from microbial and faunal communities in soil aggregates and (2) the formation of intimate associations with soil mineral surfaces and metal ions in sub-soils (Tisdall and Oades 1982, Sollins et al. 1996, 2006, Kaiser et al. 2002, Swanston et al. 2005, Lützow et al. 2006, Bird et al. 2008).

Previous studies show soil CO_2 losses increasing from 7 to 58% following earthworm invasions in forest soils, and negligible or decreased DOC losses from earthworm-invaded forest soils (Borken et al. 2000, Bohlen et al. 2004a, Speratti et al. 2007, Crumsey et al. 2013b). Processes associated with these losses include 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). The temporal lag in earthworm community-induced CO_2 losses from our finer textured sandy loam soils relative to that observed for sandy soils is likely attributable to a greater degree of physical protection of labile carbon. Among earthworm treatments applied to sandy soils, species monocultures had the lowest CO_2 efflux rates, and significant increases in CO_2 efflux rates in multi-species treatments suggest greater retention of C or enhanced access to C resources due to interactions among earthworm functional groups. Low DOC losses across both soil types could be due to the lack of live roots in our mesocosms, which precluded root exudates and decay as sources of DOC, or to adsorption of DOC transported from A-horizon to B-horizon soils (Currie et al. 1996, Kaiser and Zech 1998, Kalbitz et al. 2000). These findings support our hypothesis that initial CO_2 and DOC losses would be greater in mixed earthworm species treatments, but that enhanced carbon protection would offset increases in CO_2 losses from the finer textured sandy loam soils.

Litter mass loss and chemistry

We observed differences in total leaf litter mass loss rates (resulting from losses as CO_2 or DOC and transport from the surface litter layer) as a function of soil type, and differential mass losses among leaf litter types, determined by leaf litter chemistry and earthworm food preference. Higher rates of leaf litter mass loss from our sandy loam mesocosm surfaces are consistent with previous studies characterizing increased leaf litter decomposition rates in soils of higher organic matter content and nutrient cycling rates (Aber and Melillo 1980, Melillo et al. 1989, Reich et al. 2005). 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, Suárez et al. 2006a, Hobbie et al. 2006b, Holdsworth et al. 2008). Results showed that leaf litter C losses increased when anecic species were added to soils, and this increase was 30% in the sandy loam soil and 80% in the sandy soil. Differential mass loss and morphology of decayed leaf litter types was as follows: *A. rubrum* > *P. grandidentata* > *F. grandifolia* ≥ *Q. rubra* > *P. strobus*, and is consistent with previous work characterizing differential leaf litter mass loss as a function of earthworm community composition. Enhanced leaf litter processing with earthworm invasions has been widely observed in temperate forests (Scheu and Wolters 1991, Suarez 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, Suarez 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 leaf litter translocation by enhanced access by the broader soil invertebrate community.

Soil C mass storage

In agreement with a previous investigation of earthworm community effects on soil C budgets in sandy Spodosols after one year (Crumsey et al. 2013b), significant changes in overall C storage were not linked to earthworm community composition. Additions of all three earthworm species to sandy and sandy loam soils resulted in higher C losses as CO_2 and DOC (in sandy loam soils only) and increased transport of leaf litter C into soil pools, which corresponded to slightly larger soil C storage than that observed in no-earthworm controls. In sandy loam soils, larger C losses and net soil C storage than that observed in sandy soils, suggest a larger labile C pool (i.e., humified, low density organic material) that is subject to increased rates of microbial degradation in the presence of earthworm communities (Gaudinski et al. 2000). Field-based studies assessing earthworm impacts on soil C stocks of sandy loam soils generally show significant declines in soil C stocks within 1 – 5 years (e.g., Nielsen and Hole 1964, Alban and Berry 1994, Bohlen et al. 2004b, Fahey et al. 2013b). In contrast, significant changes in soil C stocks have not been observed in sandy soils, although earthworm community effects on leaf litter processing and organic matter redistribution (Crumsey et al. 2013a, 2013b) are comparable to those observed in sandy loam soils (Bohlen et al. 2004b, Suárez et al. 2006b, Holdsworth et al. 2008, Fahey et al. 2011, 2013b). Differences in soil-texture controls on carbon storage and feedbacks with earthworm-community controls on soil C processes will likely propagate to differences in long-term trajectories of soil carbon dynamics across dominant soil types of previously earthworm-free northern temperate forests.

^{13}C and ^{15}N natural abundances of soil and earthworm biomass pools

The experiment results suggest exotic earthworm community composition, the soil matrix, and their multiple interactions control the coupled transport of leaf litter C and N into these and similar forest soils in the north temperate zone, as indicated by the differential isotopic enrichment of pools across earthworm community and soil texture treatments. The narrow ranges of δ^{13}C and δ^{15}N values in non-labeled soil and earthworm biomass pools facilitated detection of excess ^{13}C and ^{15}N incorporated into these pools via dual-labeled *A. rubrum* leaf litter decomposition and redistribution. We observed slight ^{13}C and ^{15}N difference in *A. rubrum* litter at the end of the experiment (Tables 1 and 2), which was likely due to differential preservation of petioles and veins with different isotopic values than the consumed parts of litter. Overall, the large differences between the δ^{13}C and δ^{15}N values between non-labeled pools and dual-labeled *A. rubrum* leaf litter minimized the effects of isotopic fractionation on our estimates of *A. rubrum* ^{13}C and ^{15}N recoveries in soil system pools. Possible mechanisms for heavy isotope (i.e., ^{13}C and ^{15}N) enrichment of soil materials include (Nadelhoffer and Fry 1988, Jégou et al. 2000, Garten et al. 2008, Fahey et al. 2011, Capowiez et al. 2011): (1) the physical redistribution of isotopically enriched organic matter by earthworm feeding and burrowing behaviors, which is likely the dominant process explaining isotopic enrichment of soil and earthworm pools here, (2) overall discrimination against ^{13}C and ^{15}N during leaf litter decomposition, (3) differential preservation of isotopically enriched leaf litter structural components, and (4) eluviation of ^{13}C and ^{15}N enriched dissolved organic matter into soil layers.

^{13}C and ^{15}N tracer recoveries in soil and earthworm biomass pools

Significant differences in isotopic recoveries among ecosystem pools indicate differences in the roles of soils and earthworm species as sinks for leaf litter C and N. Tracer recoveries show that transport of leaf litter was greatest into A-horizon and burrow soils, with ^{13}C and ^{15}N recovery significantly higher in sandy loam soil containing the composite earthworm community, and in sandy soil containing both endogeic and anecic earthworm species. Accelerated rates of leaf litter processing in the presence of anecic species and bioturbation of mineral soils by endogeic species has been widely observed (Hendriksen 1990, Araujo et al. 2004, Suárez et al. 2006b, Holdsworth et al. 2008, Fahey et al. 2013a), and is likely responsible for greater transport of leaf litter into A-horizon and burrow soils. Total isotope recoveries showed that patterns of leaf litter loss and transport into surface soils were similar in magnitude to those previously reported for transfers of C and N from leaf litter into soil pools of northern temperate forests by various processes (Nadelhoffer et al. 1999b, Zeller et al. 2001, Fahey et al. 2011, 2013a), and support the idea that the interactions between earthworms and organic materials of different quality influence rates and patterns of C and N redistribution in soils (Bohlen et al. 1999). Previous work suggests C and N dynamics in earthworm-invaded soils shift over time, and that levels of enrichment vary within physical fractions of forest soils. For example, two years following the application of dual-labeled leaf litter to field plots in a maple-dominated temperate forest, earthworm-invaded soils showed lower total ^{13}C and ^{15}N recovery than earthworm-free soils, but higher isotopic enrichment in macroaggregates and microaggregates (Fahey et al. 2013a); these trends were attributed to earthworm over-wintering activity, subsequent losses in forest floor mass, and accelerated leaf litter processing in earthworm-invaded soils. Together, results of past studies and this study suggest short-term increases in leaf litter loss by incorporation into soils and possible protection of labile organic matter in earthworm burrow soils and casts, which contrast with reported longer-term dynamics in which the retention of labile organic carbon derived from leaf litter decreases with sustained earthworm activity.

Earthworm biomass was a minor sink for leaf litter labeled with ^{13}C and ^{15}N, and levels of enrichment were associated with the degree of surface leaf litter feeding (*A. trapezoides* < *E. fetida* < *L. terrestris*). Variation in $^{13}C_{rec}$:$^{15}N_{rec}$ ratios in earthworm species biomass, where mean $^{13}C_{rec}$:$^{15}N_{rec}$ values increased as follows: *L. terrestris* < *E. fetida* < *A. trapezoides,* also suggests differential processing of leaf litter and soil organic matter among ecological groups. Low tracer recoveries and the observed patterns in *A. rubrum*-derived $^{13}C_{rec}$:$^{15}N_{rec}$ ratios in earthworm tissues are likely due to biomass loss (death of earthworms over the course of the experiment) and low C and N assimilation efficiencies. Earthworm assimilation efficiencies vary greatly with the quality of organic matter ingested, but are generally higher in litter-feeding anecic species than endogeic species which primarily consume organic-rich fractions of mineral soils (Curry and Schmidt 2006). The general pattern of increased isotopic enrichment with the degree of leaf litter feeding, and facilitation of nutrient assimilation through intraspecific interactions with anecic species and those of other ecological groups, appears to be robust among previous tracer studies measuring C and N assimilation across ecologically-diverse earthworm communities (Cortez et al. 1989, Zhang and Hendrix 1995, Fahey et al. 2013b).

Carbon and nitrogen interactions in ecosystem pools

Variation in *A. rubrum*-derived $^{13}C_{rec}$:$^{15}N_{rec}$ recovery ratios across soil and earthworm pools (Table 3) is a product of differential supply (*A. rubrum* decomposition and redistribution) and removal processes (gaseous and leachate losses of C and N), and reflects the C:N of organic matter and biomass assimilated following the application of dual-labeled *A. rubrum* leaf litter (Martin et al. 1992, Nadelhoffer et al. 1999b, Butenschoen et al. 2009, Fahey et al. 2011, 2013a). High $^{13}C_{rec}$:$^{15}N_{rec}$ recovery ratios observed in A-horizon and burrow soils of mixed earthworm communities and the lower recoveries in B-horizon soils, indicate differences in the turnover time and quality of organic matter remaining after earthworm processing. This finding is important as C:N ratios are directly relevant to the long-term fate of organic matter in temperate forest soils. For example, light fractions of organic matter in temperate forest soils (humified, low-density plant and microbial residues not associated with minerals) typically have the highest C:N ratios, the lowest mineral contents, and the fastest C turnover time of all soil organic matter fractions; while large reservoirs of C and N can accumulate in heavy fraction material (Sollins et al. 1996, Gaudinski et al. 2000, Bird et al. 2008).

Burrow system structure and soil chemistry associations

Burrow system structures differed significantly across earthworm treatments, and patterns of continuity, size distribution, and volume were in agreement with the known behavior of the different ecological groups (Bastardie et al. 2005). Experiment results show that surface-soil burrow system properties are most associated with endogeic and epigeic species, and sub-soil burrow system properties are most associated with anecic species. Sub-soil burrow systems with high levels of continuity and large burrow size produced by vertical-burrowing and litter-feeding of anecic species are associated with leaf litter translocation and redistribution into sub-soils, in addition to leachate losses of organic carbon from the temperate forest soils studied here. Burrow systems with the highest volume, greatest surface connectivity, and dense burrow networks in the A-horizon were a product of interspecific interactions between surface-dwelling epigeic species and mineral-soil dwelling endogeic species. These properties were, in turn, associated with greater gaseous CO_2 losses and greater transport of leaf litter-derived C and N into the A-horizon. Increased soil C losses with greater soil porosity observed in this study, contrasts with results from a previous experiment in which burrow systems of earthworm communities and C losses were assessed over one year and no correlations between soil porosity shifts due to earthworm activity and C losses were detected (Crumsey et al. *in press*). The results from the present study demonstrate the short-term biotic control of exotic earthworm communities on soil C losses driven by burrow system production, whereas our previous study indicates that long-term C losses may be more strongly controlled by production rather than by gas diffusion or dissolve C infiltration rates in temperate forest soils.

Conclusions

These results demonstrate how earthworm community effects on C and N dynamics and soil structure are influenced by soil texture. The fates of litter-derived C and N in this study likely reflect those of short-term responses of forest soils to earthworm introductions, following the initial production of sub-surface burrow systems, enhanced leaf litter processing, and organic matter redistribution. Long-term field studies that can account for both earthworm community dynamics and other influences on soil C and N dynamics (e.g., above- and belowground effects of tree species, leaf litter loading, and the broader soil faunal community) will help determine the consequences of different earthworm-altered soil carbon storage trajectories in northern temperate forests.

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Tables

Table 4.1: Carbon (C) and nitrogen (N) pool size and isotopic values (δ^{13}C and δ^{15}N) of soil pools (A-horizon [AH], B-horizon [BH], and burrow soil [BR]) after 150-day incubations. Values represent means (± 1 SE), *n* = 4. Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = *H*) with nonparametric multiple comparisons (** *P* < 0.01, * *P* < 0.05).

Treatment		Carbon (g C m^{-2})			Nitrogen (g N m^{-2})			δ^{13}C (‰)			δ^{15}N (‰)		
Soil	Earthworm (EW)	AH	BH	BR	AH	BH	BR	AH	BH	BR	AH	BH	BR
Entic Haplorthod (sand)	Control (no EW)	1030^{ab} (50.1)	386^{a} (22.6)	-----	41.8^{a} (1.68)	14.9^{ab} (0.88)	-----	-23.7^{a} (0.17)	-25.5^{a} (0.10)	-----	16.5^{a} (1.39)	8.21^{a} (0.36)	-----
	Epigeic	964^{ab} (38.1)	447^{b} (3.27)	37.1^{ab} (13.3)	43.0^{a} (1.71)	17.8^{a} (0.31)	1.72^{a} (0.62)	-18.3^{ab} (1.34)	-25.8^{a} (0.09)	-6.74^{a} (4.66)	41.7^{b} (4.79)	9.32^{a} (0.42)	98.1^{a} (22.3)
	Epi×End	1181^{b} (133.3)	362^{a} (19.2)	59.6^{c} (17.0)	44.4^{a} (9.27)	14.8^{bc} (0.64)	2.8^{b} (0.86)	-10.3^{b} (4.40)	-25.5^{a} (0.18)	4.44^{a} (8.88)	49.1^{b} (16.4)	14.5^{b} (0.9)	112.3^{a} (31.8)
	Anecic	1042^{ab} (38.8)	477^{b} (16.9)	14.2^{a} (5.05)	47.4^{a} (2.05)	18.8^{a} (0.86)	0.65^{a} (0.24)	-11.8^{b} (3.42)	-25.3^{a} (0.18)	174.1^{c} (26.4)	60.9^{b} (9.52)	22.3^{b} (1.13)	616.1^{c} (80.3)
	Ane×End	922^{a} (45.3)	502^{b} (83.7)	26.9^{ab} (5.87)	41.3^{a} (2.02)	21.0^{a} (3.26)	1.2^{ab} (0.25)	9.10^{c} (5.23)	-25.6^{a} (0.23)	75.6^{b} (17.5)	145.1^{c} (23.0)	23.03^{b} (1.66)	370.3^{b} (67.3)
	All Species	1069^{ab} (32.6)	392^{a} (21.0)	19.9^{a} (5.51)	45.5^{a} (1.54)	16.5^{b} (0.87)	0.9^{a} (0.24)	2.92^{c} (2.32)	-25.5^{a} (0.17)	93.9^{bc} (30.9)	119.0^{c} (5.21)	19.1^{b} (1.25)	449.5^{bc} (117.4)
Alfic Haplorthod (sandy loam)	Control (no EW)	1827^{c} (41.3)	445^{b} (19.6)	-----	86.1^{b} (2.36)	9.32^{c} (0.49)	-----	-10.2^{b} (0.91)	-0.98^{b} (0.07)	-----	51.2^{b} (4.27)	9.4^{a} (2.00)	-----
	All Species	1761^{c} (33.7)	489^{b} (2.49)	44.3^{bc} (4.56)	79.82^{b} (1.18)	10.83^{c} (0.13)	2.02^{bc} (0.2)	7.43^{c} (0.38)	-0.65^{b} (0.09)	48.1^{b} (11.3)	105.4^{c} (3.89)	22.6^{b} (4.10)	330.6^{b} (59.3)
	H	31.87 **	35.38 **	18.07 *	29.79 **	42.44 **	17.85 *	33.82 **	64.09 **	32.54 **	32.39 **	63.62 **	26.23 **

Table 4.2: Carbon (C) and nitrogen (N) pool size and isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of earthworm biomass pools (i.e., *L. terrestris* [Lterr], *E. fetida* [Efoet], and *A. trapezoides* [Atrap]) after 150-day incubations. Values represent means (± 1 SE), *n* = 4. Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = *H*) with nonparametric multiple comparisons (** $P < 0.01$, * $P < 0.05$).

Treatment		Carbon (g C m^{-2})			Nitrogen (g N m^{-2})			$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
Soil	Earthworm	Lterr	Efoet	Atrap	Lterr	Efoet	Atrap	Lterr	Efoet	Atrap	Lterr	Efoet	Atrap
Entic Haplorthod (sand)	Epigeic	-----	-----	-----	-----	0.44^{a} (0.03)	-----	-----	8.51^{a} (5.1)	-----	-----	83.0^{ab} (23.66)	-----
	Epi×End	-----	-----	-----	-----	0.15^{b} (0.02)	0.83^{a} (0.29)	-----	78.9^{b} (32.1)	-13.8^{a} (2.14)	-----	292.6^{b} (106.0)	22.7^{a} (6.16)
	Anecic	8.69^{a} (0.59)	0.69^{a} (0.05)	0.69^{a} (0.05)	0.69^{a} (0.05)	-----	-----	74.6^{a} (16.3)	-----	-----	266.0^{a} (41.0)	-----	-----
	Ane×End	5.49^{ab} (0.56)	0.44^{ab} (0.04)	0.44^{ab} (0.04)	0.44^{ab} (0.04)	-----	0.63^{a} (0.07)	126.7^{ab} (15.5)	-----	-1.02^{b} (4.42)	504.8^{ab} (61.5)	-----	44.4^{b} (8.04)
	All Species	6.02^{ab} (0.26)	0.48^{ab} (0.02)	0.48^{ab} (0.02)	0.48^{ab} (0.02)	0.2^{b} (0.04)	0.44^{a} (0.03)	163.6^{b} (14.0)	20.1^{b} (7.65)	-10.4^{a} (2.18)	735.4^{b} (70.5)	111.2^{b} (26.4)	29.3^{ac} (5.5)
Alfic Haplorthod (sandy loam)	All Species	5.19^{b} (0.35)	0.41^{b} (0.03)	0.41^{b} (0.03)	0.41^{b} (0.03)	0.17^{b} (0.04)	0.48^{a} (0.03)	171.2^{b} (7.78)	-3.59^{a} (5.04)	-4.45^{ab} (3.61)	738.6^{b} (77.1)	41.9^{a} (7.04)	47.9^{bc} (9.13)
	H	12.18 **	12.18 **	12.18 **	12.18 **	13.49 **	5.49	12.08 **	11.01*	19.45 **	15.66 **	10.30*	7.92*

Table 4.3: C:N and $^{13}C_{rec}$:$^{15}N_{rec}$ values of soil pools (A-horizon [AH], B-horizon [BH], and burrow soil [BR]) and earthworm biomass pools (*L. terrestris* [Lterr], *E. fetida* [Efoet], and *A. trapezoides* [Atrap]) after 150-day incubations. Values represent means (± 1 SE), *n* = 4. Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = *H*) with nonparametric multiple comparisons (** *P* < 0.01, * *P* < 0.05).

Treatment		Soil Pools						Earthworm Species					
		C:N			$^{13}C_{rec}$:$^{15}N_{rec}$			C:N			$^{13}C_{rec}$:$^{15}N_{rec}$		
Soil	Earthworm (EW)	AH	BH	BR	AH	BH	BR	Lterr	Efoet	Atrap	Lterr	Efoet	Atrap
Entic Haplorthod (sand)	Control (no EW)	24.64^{a} (0.39)	26.07^{a} (0.31)	-----	0.4^{ab} (0.04)	0.25^{a} (0.04)	-----	-----	-----	-----	-----	-----	-----
	Epigeic	22.44^{ab} (0.18)	24.94^{a} (0.66)	22.09^{a} (0.45)	0.46^{ab} (0.05)	0.09^{a} (0.03)	0.46^{a} (0.02)	-----	4.17^{a} (0.06)	-----	-----	0.21^{a} (0.03)	-----
	Epi×End	22.85^{ab} (0.39)	24.46^{a} (0.68)	22.63^{a} (0.38)	0.53^{c} (0.03)	0.08^{a} (0.02)	0.47^{a} (0.05)	-----	4.03^{a} (0.15)	3.7^{a} (0.08)	-----	0.16^{a} (0.02)	0.21^{a} (0.02)
	Anecic	22.01^{ab} (0.31)	25.37^{a} (0.51)	22.51^{a} (0.68)	0.49^{bc} (0.04)	0.08^{a} (0.02)	0.66^{b} (0.04)	4.32^{a} (0.08)	-----	-----	0.16^{a} (0.01)	-----	-----
	Ane×End	22.32^{ab} (0.44)	23.93^{a} (0.48)	22.87^{a} (0.35)	0.56^{c} (0.03)	0.04^{a} (0.01)	0.62^{b} (0.11)	4.35^{a} (0.12)	-----	3.83^{ab} (0.05)	0.12^{ab} (0)	-----	0.2^{a} (0.01)
	All Species	23.49^{a} (0.12)	24.05^{a} (0.58)	23.9^{a} (0.69)	0.53^{c} (0.03)	0.06^{a} (0.02)	0.53^{a} (0.04)	4.33^{a} (0.03)	4.3^{a} (0.2)	3.97^{ab} (0.03)	0.1^{b} (0.01)	0.17^{a} (0.01)	0.2^{a} (0.02)
Alfic Haplorthod (sandy loam)	Control (no EW)	21.24^{b} (0.15)	48.67^{b} (0.99)	-----	0.41^{b} (0.02)	24.75^{b} (3.86)	-----	-----	-----	-----	-----	-----	-----
	All Species	22.06^{ab} (0.12)	45.83^{b} (1.87)	30.38^{b} (1.46)	0.52^{c} (0.02)	53.36^{b} (23.13)	0.75^{b} (0.11)	4.4^{a} (0.07)	4.08^{a} (0.07)	4.18^{b} (0.08)	0.11^{b} (0.01)	0.18^{a} (0.02)	0.17^{a} (0.01)
	H	30.40 **	66.02 **	22.20 **	15.04 *	74.33 **	15.02 **	1.29	1.36	13.90 *	12.99 *	2.15	1.30

Figures:

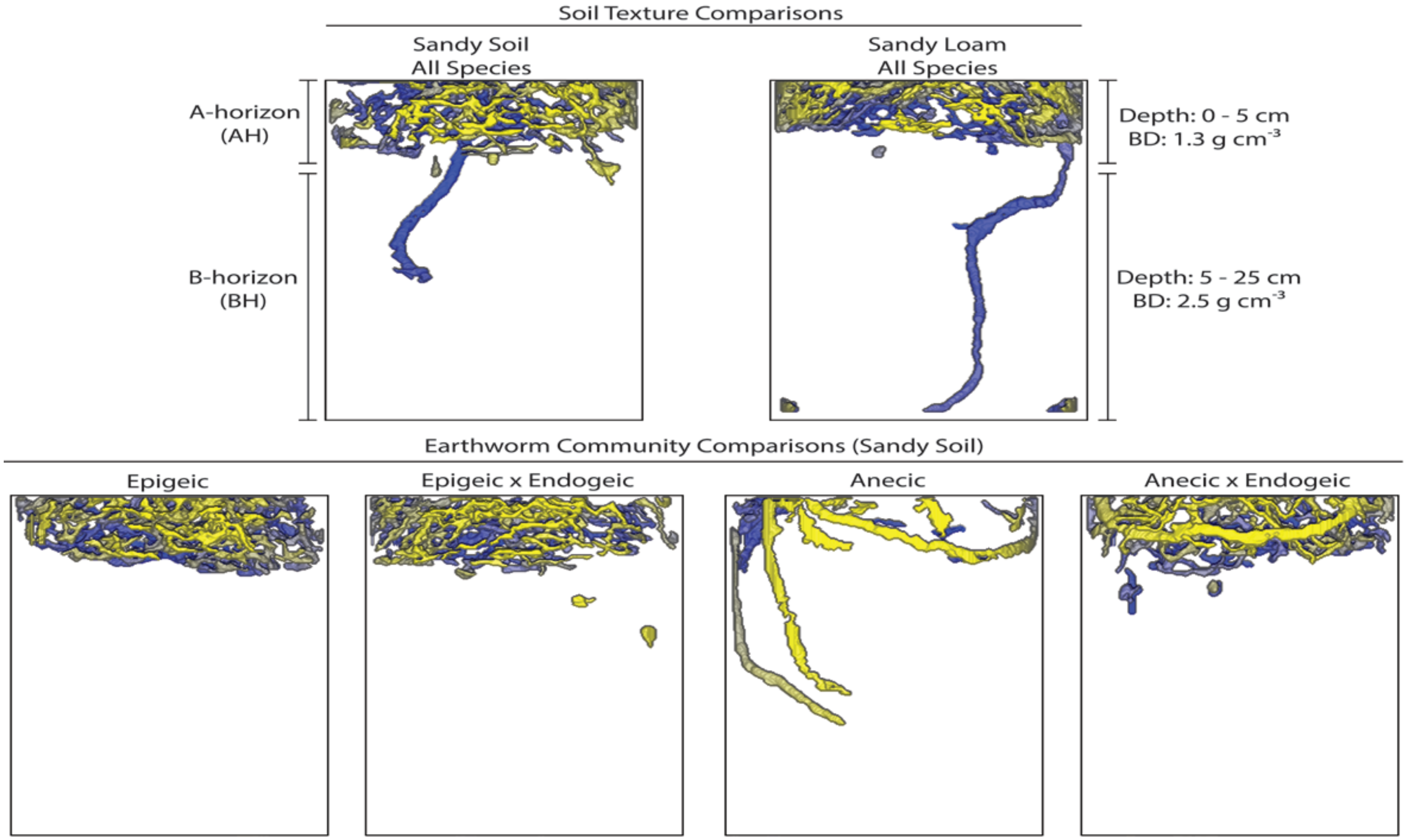

Figure 4.1: Example of 3-D burrow reconstructions. Color gradations represent distance of burrow relative to the viewer's perspective. Examples of three-dimensional reconstructions of earthworm community burrow systems, imaged by X-ray CT. Color gradations used for 3-D rendering, yellow for the foreground to blue for the background, represent distance of burrows relative to the viewer's perspective. Earthworm species of different functional groups included: *L. terrestris* [Anecic = litter feeding, vertical burrowing], *A. trapezoides* [Endogeic = mineral soil feeding and dwelling], and *E. fetida* [Epigeic = litter feeding, surface–dwelling]. Soil depth (cm) and bulk density (BD) of the A-horizon and B-horizon are uniform across treatments. The experimental design permits comparisons between earthworm community effects across soil types, comparisons among mono-specific and multi-species earthworm treatments in sandy Spodosols, and the assessment of relationships between burrow system structure and changes in soil C and N redistribution.

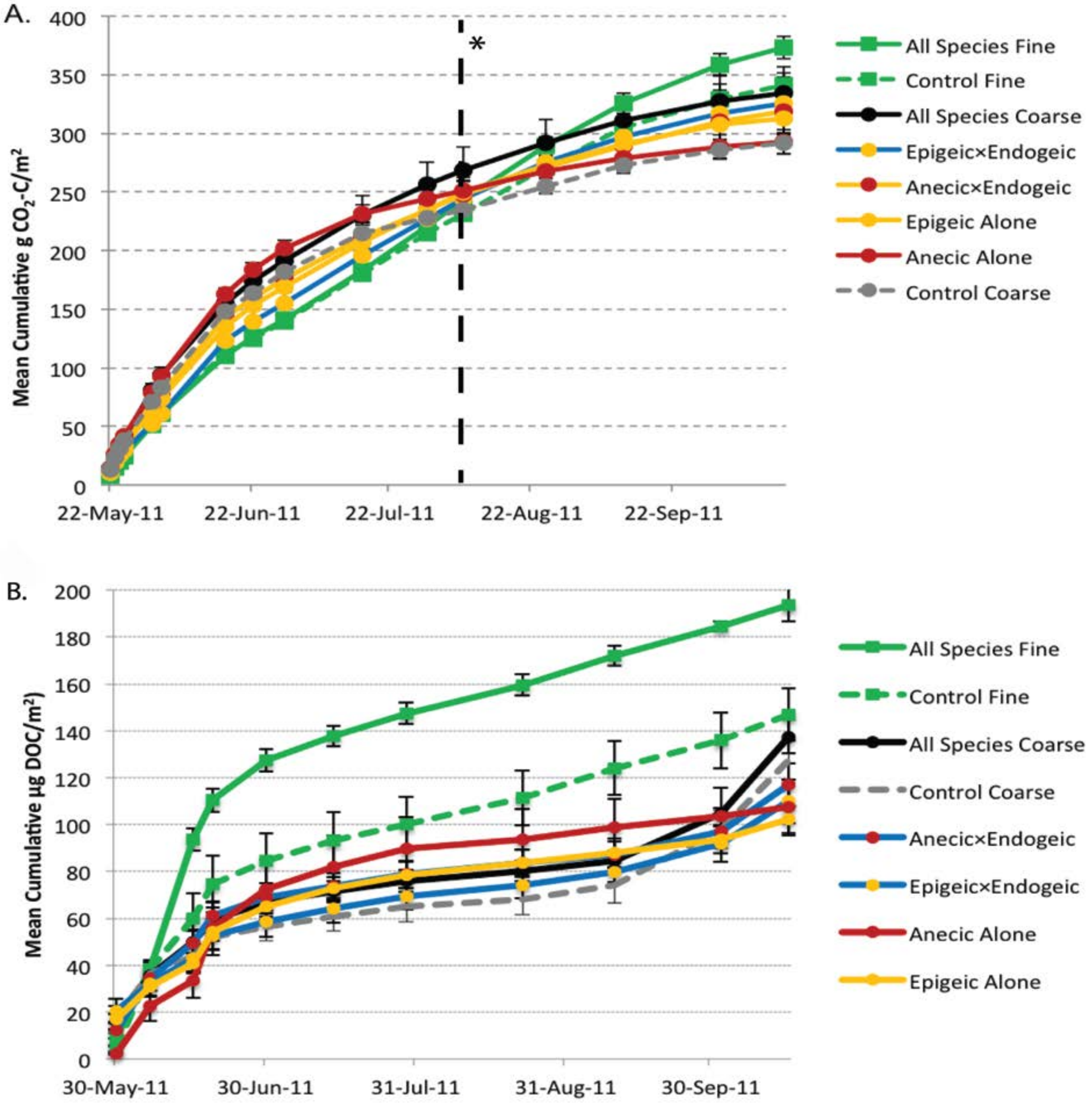

Figure 4.2: Area-normalized cumulative [A] soil CO_2–C efflux (g CO_2-C m^{-2}) and [B] DOC efflux (µg DOC m^{-2}) over 150-day incubations. Soil texture treatments are indicated as Fine = sandy loam Spodosol and Coarse = sandy Spodosol. Values represent means and vertical bars are ± 1 SE. A general linear model with repeated measures was used to evaluate differences in cumulative CO_2-C losses between earthworm community treatments of sandy soils; differences between cumulative CO_2-C losses in sandy loam soil with and without earthworm community additions were evaluated at two time points (divided by vertical dashed line and asterisk) Differences in cumulative DOC efflux were evaluated over the entire measurement period for sandy and sandy loam soils.

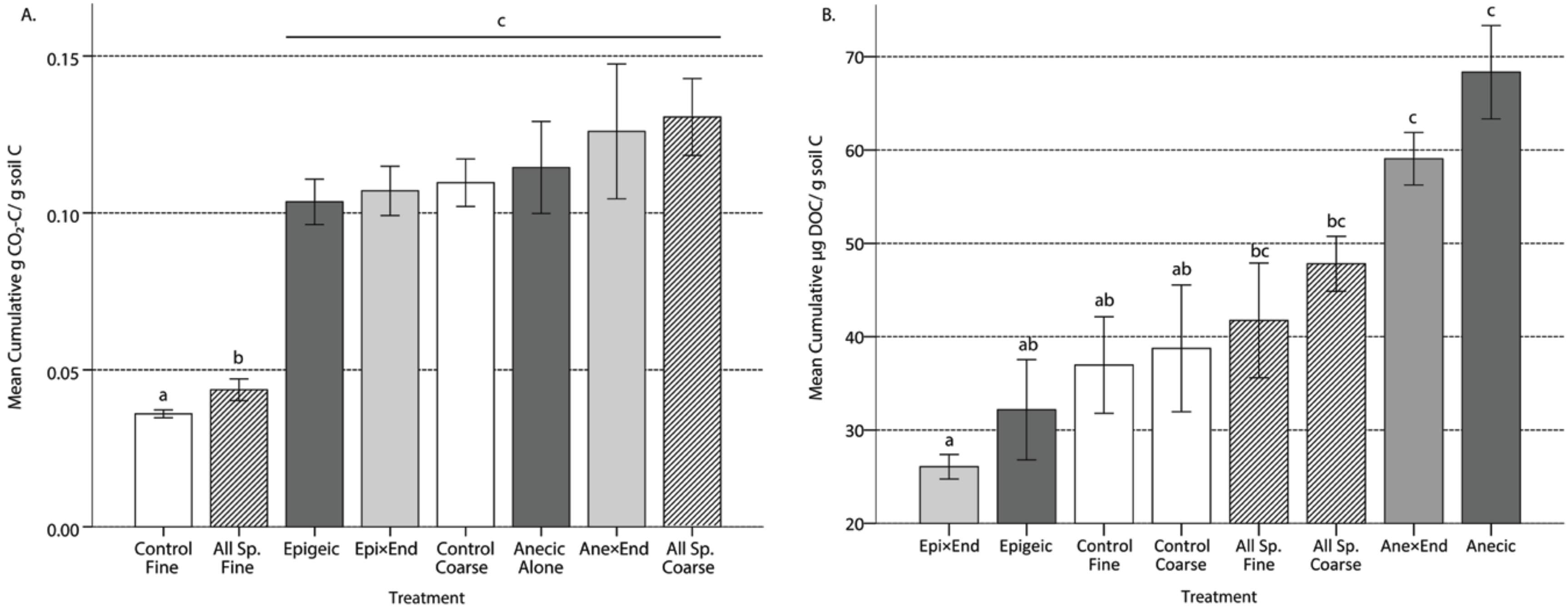

Figure 4.3: Mass-normalized cumulative [A] soil CO_2–C efflux (g CO_2-C per g soil C) and [B] DOC efflux (µg DOC per g soil C) across treatments. Bars show mean cumulative C losses from control soils (white), mono-specific (dark grey), paired (light grey), and all species (striped) earthworm community treatments. Soil texture treatments are indicated as Fine = sandy loam Spodosol and Coarse = sandy Spodosol. Values represent means and vertical bars are ± 1 SE. Lowercase letters represent significant differences determined by Kruskal-Wallis tests with nonparametric multiple comparisons at $P < 0.10$ for comparisons of CO_2-C efflux and $P < 0.05$ for comparisons of DOC efflux.

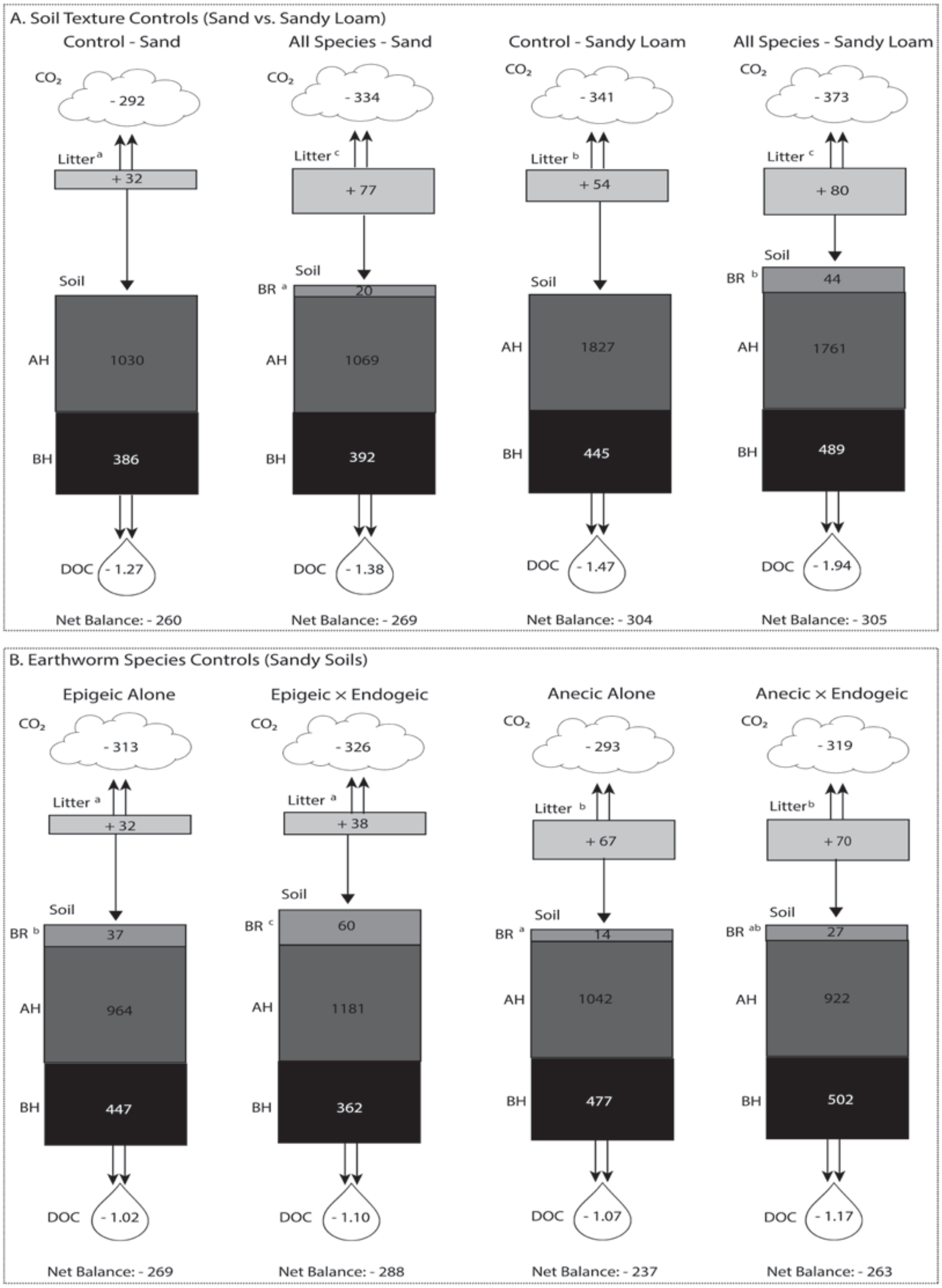

Figure 4.4: Carbon mass balance (g C m^{-2}) of the soil-litter matrix across A) soil texture treatments and B) earthworm community treatments after 150-day incubations. Pools are indicated as: litter, soil pools: AH = A-horizon, BH = B-horizon, BR = Burrow). Minus (-) signs represent C losses from the soil-litter matrix as CO_2 and DOC, indicated with double arrows. Plus (+) signs represent C gains to the soil system from leaf litter removed from the soil surface by earthworm communities. Differences in box sizes represent significant differences in pool sizes for each respective, and are indicated by lower case letters, Kruskal-Wallis tests, *P* < 0.05. The net C balance is calculated as the difference between C losses and C gains.

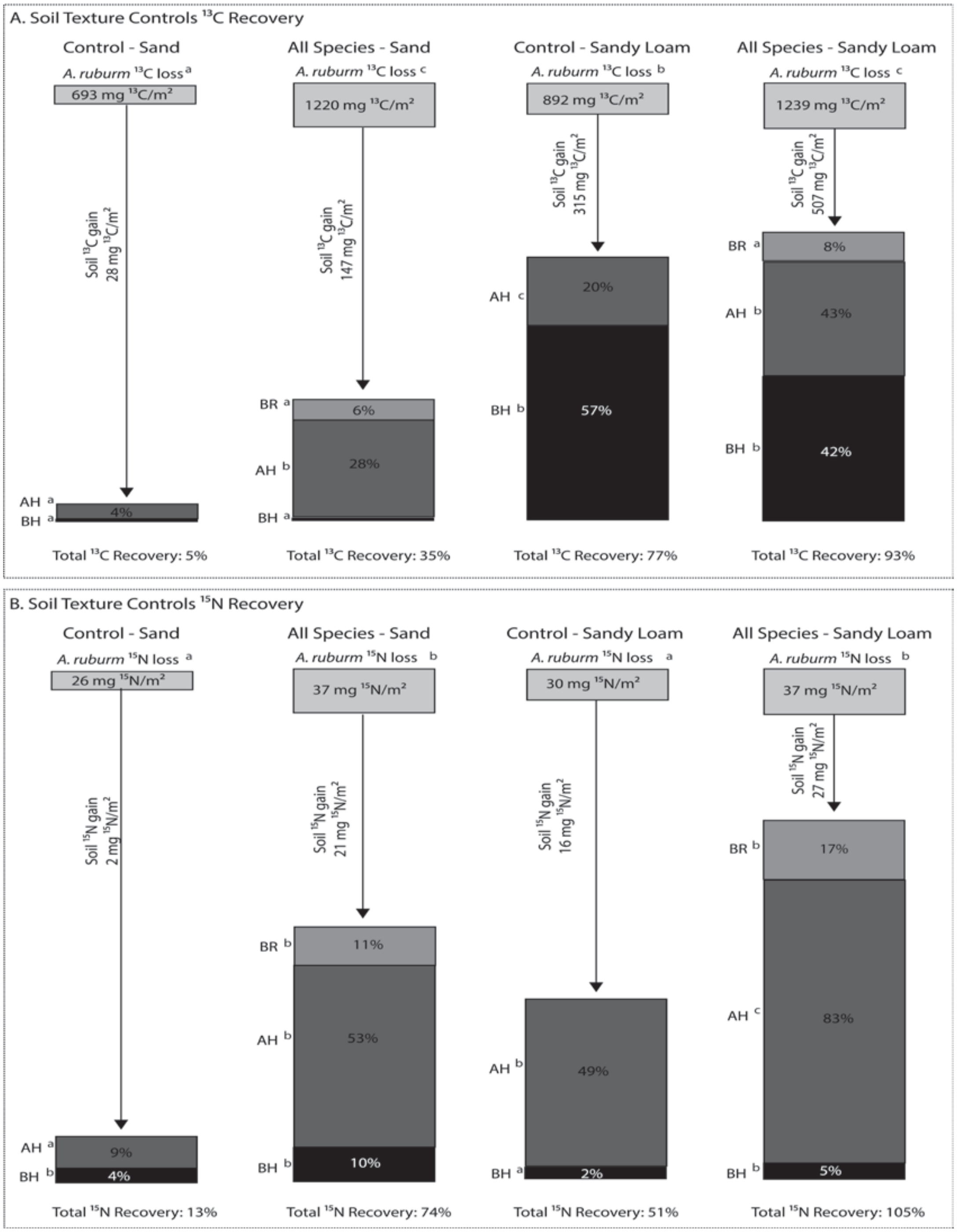

Figure 4.5: Percent recoveries of (A.) *A. rubrum*-derived ^{13}C (mg ^{13}C m^{-2}) and (B.) *A. rubrum*-derived ^{15}N (mg ^{15}N m^{-2}) across two soil textures with and without earthworm community additions, after 150-day incubations. *A. rubrum* ^{13}C losses and ^{15}N losses and total ^{13}C and ^{15}N enrichment in soils are shown as mean values. Tracer recoveries in soil pools (AH = A-horizon, BH = B-horizon, BR = Burrow) are shown as the percentage of total *A. rubrum*-derived ^{13}C and ^{15}N enrichment. Lower case letters indicate significant differences in tracer isotopic losses and tracer recovery in each respective pool across treatments (Kruskal-Wallis tests with nonparametric multiple comparisons). Mean values (± 1 S.E.) are provided in Table B4.

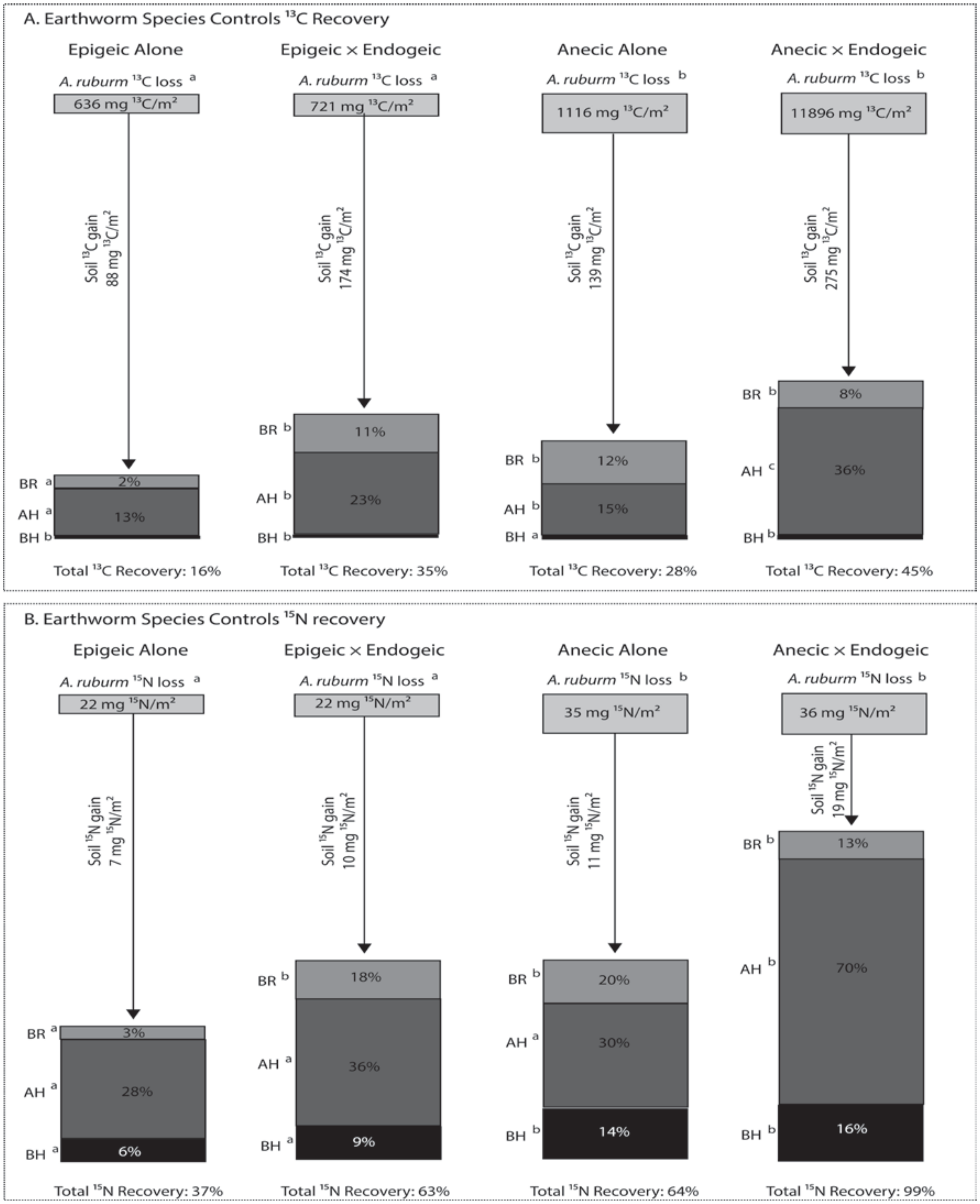

Figure 4.6: Percent recoveries of (A.) *A. rubrum*-derived ^{13}C (mg ^{13}C/m^2) and (B.) *A. rubrum*-derived ^{15}N (mg ^{15}N/m^2) across earthworm community treatments in sandy soils, after 150-day incubations. *A. rubrum* ^{13}C losses and ^{15}N losses and total ^{13}C and ^{15}N enrichment in soils are shown as mean values. Tracer recoveries in soil pools (AH = A-horizon, BH = B-horizon, BR = Burrow) are shown as the percentage of total *A. rubrum*-derived ^{13}C and ^{15}N enrichment. Lower case letters indicate significant differences in tracer isotopic losses and tracer recovery in each respective pool across treatments (Kruskal-Wallis tests with nonparametric multiple comparisons). Mean values (± 1 S.E.) are provided in Table B4.

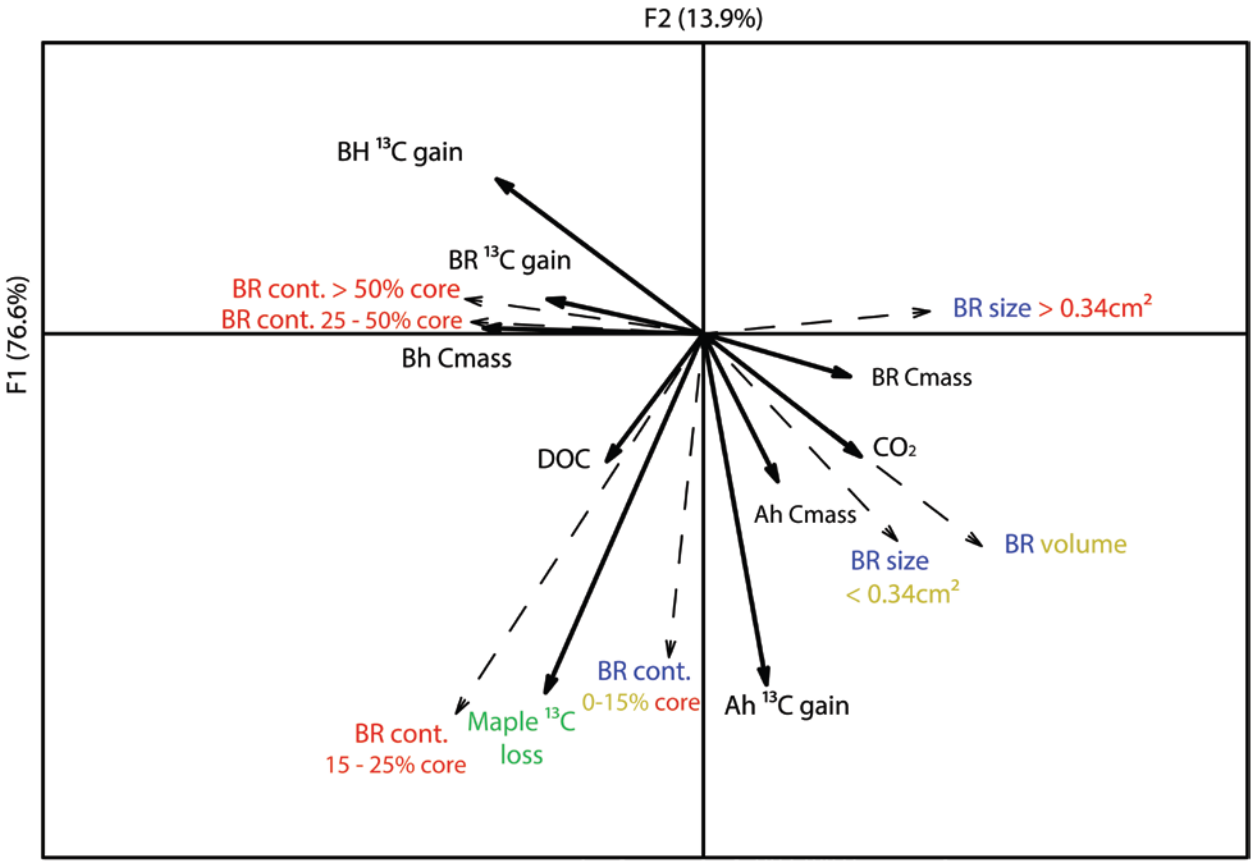

Figure 4.7: Relationships between burrow system properties (dashed arrows) and C budget measures (solid arrows) according to relative positions on the Fl × F2 co-inertia plane. Colored text indicates the earthworm ecological group(s) associated with the highest values of each respective burrow system property (Red = Anecic, Blue = Endogeic, Yellow = Epigeic; Table C1). Burrow system structure measures: macroporosity (MR), surface connectivity (SC), size class (BS: 2 = 0.17 to < 0.34 cm^2, 3 = > 0.34 cm^2), continuity class (BC 1 = 0 – 15%, 2 = 15 – 25%, 3 = 25 – 50%, 4 = > 50%). C budget components: *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; CO_2 and DOC loss. Co-inertia axis eigenvalues: F1 = 4.22; F2 = 1.76. Data used include earthworm community treatments in the sandy Spodosol only.

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Chapter 4 Appendices

Appendix A: Description of the study area.

Site description

This study was conducted at the University of Michigan Biological Station (UMBS) in northern Michigan, US (45^{o}35.5'N, 84^{o}43'W), where secondary successional forests are dominated by bigtooth aspen (*Populus grandidentata*), with northern red oak (*Quercus rubra*), red maple (*Acer rubrum*), paper birch (*Betula papyrifera*), and eastern white pine (*Pinus strobus*) occurring as co–dominants (Curtis et al. 2005). Earthworm communities are dominated by five species of European origin including *Dendrobaena octaedra, Aporrectodea caliginosa, Aporrectodea trapezoides, Lumbricus rubellus,* and *L. terrestris*. Average earthworm community biomass is 21± 2.66 g m^{-2} (fresh weight), while average abundance is 39 ± 5 individuals m^{-2} (Crumsey, unpublished data). Forest stands function as C sinks, with annual photosynthetic C gains greater than that of heterotrophic soil respiration. Annual photosynthetic C gains average 6.54 ± 0.76 Mg C ha^{-1} yr^{-1}, while average heterotrophic soil respiration is 5.02 ± 0.86 Mg C ha^{-1} yr^{-1} (Gough et al. 2008a), representing 71% of annual ecosystem respiration losses (Curtis et al. 2005). Forest stands thus have an annual C storage rate of 1.53 ± 1.15 Mg C ha^{-1} yr^{-1}, and contain 180.5 ±12.8 Mg C ha^{-1}, with 44% (80 ± 12.4 Mg C ha^{-1}) stored in soil organic matter (Gough et al. 2008b). Soils contain 2000 kg N ha^{-1}, and show an average in situ net N-mineralization rate of 42 kg N ha^{-1} yr^{-1} and < 2% net nitrification (Nave et al. 2009).

Appendix B: Tables of elemental and isotopic C and N properties of soil, leaf litter, and earthworm biomass pools.

Table B1: Initial C and N properties of leaf litter types, soil pools, and earthworm species biomass. Values represent means (± 1 SE), *n* = 6. C and N properties for earthworm species are given for the All Species treatment replicated in sandy and sandy loam Spodosols. Isotopic values (expressed as δ^{13}C and δ^{15}N) are given for bulk soil, whole-body earthworm biomass, and leaf litter types.

Pool		% C	% N	C:N	δ^{13}C (‰)	δ^{15}N (‰)
Leaf Litter	*A. rubrum* (red maple)	41.86 (0.66)	1.06 (0.02)	39.5 (0.21)	3170.1 (25.74)	15917.51 (2084.03)
	F. grandifolia (American beech)	45.67 (0.5)	0.73 (0.06)	64.44 (4.94)	-30.36 (0.26)	-2.41 (0.64)
	P. grandidentata (Bigtooth aspen)	49.23 (0.58)	0.78 (0.12)	70.19 (11.12)	-28.89 (0.24)	-3.27 (0.62)
	P. strobus (White pine)	50.26 (0.03)	0.37 (0.01)	137.63 (2.17)	-28.96 (0.01)	-0.78 (0.93)
	Q. rubra (Red oak)	47.95 (0.36)	0.65 (0.03)	74.95 (2.85)	-28.89 (0.2)	-3.24 (0.6)
Earthworm	*A. trapezoides* (Endogeic)	41.83 (0.74)	9.74 (0.27)	4.31 (0.06)	-24.51 (0.09)	2.21 (0.2)
	L. terrestris (Anecic)	39.98 (1.17)	7.97 (0.4)	5.11 (0.16)	-26.14 (0.2)	-0.51 (0.22)
	E. fetida (Epigeic)	42.31 (0.45)	9.39 (0.15)	4.56 (0.05)	-24.99 (0.1)	-0.91 (0.15)
Entic Haplorthod (sand)	A-horizon	1.56 (0.07)	0.07 (0)	21.39 (0.4)	-26.46 (0.03)	1.14 (0.29)
	B-horizon	0.58 (0.01)	0.03 (0)	22.36 (0.28)	-25.93 (0.03)	3.59 (0.11)
Alfic Haplorthod (sandy loam)	A-horizon	3.35 (0.16)	0.15 (0.01)	22.45 (0.31)	-19.78 (0.28)	-0.11 (0.05)
	B-horizon	1.5 (0.94)	0.06 (0.04)	23.94 (1.58)	-26.1 (0.12)	10.97 (1.53)

Table B2: Leaf litter C and N mass loss (g m^{-2}; treated as leaf litter additions to soil): *A. rubrum* (Acru), *P. strobus* (Pist), *P. grandidentata* (Pogr), *Q. rubra* (Quru), *F. grandifolia* (Fagr), *B. papyrifera* (Bepa), and total losses. Values represent means (± 1 SE), n = 6. Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = H) with nonparametric multiple comparisons (** P < 0.01, * P < 0.05).

	Treatment	Acru	Pist	Pogr	Quru	Fagr	Bepa	Total C loss
Leaf litter C mass loss (g C/m^{2})	Control (sand)	14.28^{a} (1.16)	0.77^{a} (0.12)	15.41^{a} (1.1)	7.13^{ab} (1.2)	0.77^{a} (0.08)	5.12^{a} (0.32)	32.36^{a} (2.9)
	Epigeic	13.75^{a} (1.27)	0.77^{a} (0.2)	13.32^{a} (1.72)	3.5^{a} (0.57)	0.41^{a} (0.09)	4.34^{a} (0.43)	31.75^{a} (3.16)
	Epi-End	17.36^{a} (1.75)	0.7^{a} (0.15)	15.00^{a} (0.85)	3.95^{b} (1.45)	0.66^{a} (0.16)	4.02^{a} (0.58)	37.67^{a} (2.35)
	Anecic	26.5^{bc} (2.2)	0.81^{a} (0.21)	31.34^{bc} (6.17)	7.66^{b} (1.42)	0.70^{a} (0.27)	7.2^{b} (0.89)	67^{bc} (8.48)
	Ane_End	28.61^{b} (1.7)	0.66^{a} (0.15)	34.54^{b} (4.15)	5.49^{ab} (1.62)	1.16^{a} (0.29)	7.02^{b} (1.12)	70.46^{bc} (6.27)
	All Sp. (sand)	29.3^{b} (1.06)	0.49^{a} (0.07)	35.73^{b} (3.67)	10.2^{b} (1.66)	0.91^{a} (0.4)	8.12^{b} (0.58)	76.63^{c} (4.18)
	Control (sandy loam)	20.57^{ac} (0.65)	0.59^{a} 0.07)	23.89^{ac} (1.29)	7.86^{b} (1.43)	1.44^{a} (0.29)	4.6^{a} (0.71)	53.96^{b} (3.25)
	All Sp. (sandy loam)	29.59^{b} (1.02)	0.66^{a} (0.11)	34.4^{b} (3.42)	14.32^{b} (1.12)	1.53^{a} (0.32)	8.25^{b} (0.37)	80.49^{c} (3.37)
	H	35.08**	12.10	29.52**	24.42**	15.17	25.38**	34.37**
Leaf litter N mass loss (g N/m^{2})	Treatment	Acru	Pist	Pogr	Quru	Fagr	Bepa	Total N loss
	Control (sand)	0.36^{a} (0.03)	0.01^{a} (0)	0.31a (0.02)	0.1^{a} (0.02)	0.01^{a} (0)	0.06^{a} (0)	0.79^{a} (0.05)
	Epigeic	0.35^{a} (0.03)	0.01^{a} (0)	0.21a (0.03)	0.05^{a} (0.01)	0.01^{a} (0)	0.05^{a} (0.01)	0.62^{a} (0.06)
	Epi-End	0.44^{a} (0.04)	0.01^{a} (0)	0.24a (0.01)	0.05^{a} (0.02)	0.01^{a} (0)	0.05^{a} (0.01)	0.75^{a} (0.05)
	Anecic	0.67^{bc} (0.06)	0.01^{a} (0)	0.5bc (0.1)	0.1^{a} (0.02)	0.01^{a} (0)	0.09^{b} (0.01)	1.29^{bc} (0.15)
	Ane_End	0.72^{b} (0.04)	0^{a} (0)	0.55b (0.07)	0.07^{ab} (0.02)	0.02^{a} (0)	0.09^{b} (0.01)	1.37^{bc} (0.11)
	All Sp. (sand)	0.74^{b} (0.03)	0^{a} (0)	0.57b (0.06)	0.14^{c} (0.02)	0.01^{a} (0.01)	0.1^{b} (0.01)	1.47^{c} (0.07)
	Control (sandy loam)	0.52^{ac} (0.02)	0^{a} (0)	0.38ac (0.02)	0.11^{bc} (0.02)	0.02^{a} (0)	0.06^{a} (0.01)	1.03^{b} (0.05)
	All Sp. (sandy loam)	0.75^{b} (0.03)	0^{a} (0)	0.55b (0.05)	0.19^{c} (0.02)	0.02^{a} (0.01)	0.1^{b} (0)	1.52^{c} (0.06)
	H	36.66**	10.19	29.56**	24.31**	13.17	26.23**	34.27**

Table B3: Dual-labeled *A. rubrum* litter C and N properties of isotopically enriched (^{13}C and ^{15}N) after 150-day incubations. Values represent means (± 1 SE), $n = 6$ for pre–treatment soils, and $n = 4$ for post–treatment soils. Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = *H*) with nonparametric multiple comparisons (** $P < 0.01$, * $P < 0.05$).

Treatment		Dual-labeled *A. rubrum* C and N properties					
Soil	Earthworm (EW)	% C	% N	δ^{13}C (‰)	δ^{15}N (‰)	^{13}C loss (mg ^{13}C/ m^2)	^{15}N loss (mg ^{15}N/ m^2)
	[INITIAL]	41.9 (0.66)	1.06 (0.02)	3170 (26)	15918 (2084.03)	-----	-----
Entic Haplorthod (sand)	Control (no EW)	43.8^{a} (0.53)	1.36^{ab} (0.18)	2624^{a} (484)	9544^{a} (558)	693^{a} (31.0)	25.5^{a} (0.63)
	Epigeic	42.5^{a} (0.2)	1.26^{ab} (0.08)	2581^{a} (329)	11045^{a} (1219)	636^{a} (48.6)	22.5^{a} (1.13)
	Epi×End	42.1^{a} (0.75)	1.35^{b} (0.07)	2965^{a} (67)	14676^{a} (809)	721^{a} (73.4)	22.3^{a} (1.98)
	Anecic	42.6^{a} (0.23)	1.12^{ab} (0.12)	2712^{a} (126)	7635^{ab} (724)	1116^{bc} (86.7)	34.8^{bc} (1.4)
	Ane×End	42.6^{a} (0.89)	0.99^{a} (0.09)	3213^{a} (116)	6705^{ab} (829)	1189^{bc} (75.5)	36.2^{bc} (0.97)
	All Species	42.2^{a} (0.17)	1.05^{ab} (0.16)	3121^{a} (290)	5579^{b} (803)	1220^{c} (46.1)	36.8^{c} (0.51)
Alfic Haplorthod (sandy loam)	Control (no EW)	38.0^{b} (0.46)	0.84^{ac} (0.11)	2020^{a} (373)	5294^{b} (309)	955^{b} (20.9)	32.7^{b} (0.31)
	All Species	36.6^{b} (0.15)	0.64^{c} (0.1)	2402^{a} (224)	3095^{b} (445)	1242^{c} (37.2)	37.1^{c} (0.31)
	H	13.07*	14.44*	11.14	19.23*	36.25**	36.21**

Table B4: A-horizon (AH), B-horizon (BH), and burrow soil (BR) percent C, percent N, and isotopic enrichment (^{13}C and ^{15}N) after 150-day incubations. Values represent means (± 1 SE), $n = 6$ for pre–treatment soils, and $n = 4$ for post–treatment soils. Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = *H*) with nonparametric multiple comparisons (** $P < 0.01$, * $P < 0.05$).

Treatment		Soil pool chemistry after 150-day incubations											
		% Carbon			% Nitrogen			^{13}C enrichment (mg ^{13}C m^{-2})			^{15}N enrichment (mg ^{15}N m^{-2})		
Soil	Earthworm (EW)	AH	BH	BR	AH	BH	BR	AH	BH	BR	AH	BH	BR
Entic Haplorthod (sand)	Control (no EW)	1.58^{a} (0.08)	0.54^{a} (0.01)	-----	0.06^{a} (0)	0.02^{a} (0)	-----	25.8^{a} (5.26)	2.27^{a} (0.75)	-----	2.03^{a} (0.45)	0.27^{b} (0.02)	-----
	Epigeic	1.55^{a} (0.03)	0.56^{ac} (0.02)	3.56^{b} (0.88)	0.07^{a} (0)	0.02^{a} (0)	0.16^{b} (0.04)	87.4^{ab} (16.19)	0.26^{b} (0.15)	9.16^{a} (3.36)	6.41^{a} (0.76)	0.18^{ab} (0.08)	0.71^{a} (0.26)
	Epi×End	2.01^{a} (0.3)	0.43^{b} (0.03)	2.38^{a} (0.34)	0.07^{a} (0.02)	0.02^{a} (0)	0.11^{a} (0.02)	173.6^{b} (34.1)	0.76^{b} (0.34)	33.26^{b} (7.14)	8.36^{a} (1.9)	0.29^{b} (0.13)	1.71^{b} (0.38)
	Anecic	1.62^{a} (0.05)	0.6^{c} (0.02)	1.71^{ac} (0.18)	0.07^{a} (0)	0.02^{a} (0)	0.08^{ab} (0.01)	137.8^{b} (41.39)	1.59^{a} (1)	40.51^{b} (15.54)	8.62^{a} (2.16)	0.66^{c} (0.3)	2.08^{b} (0.83)
	Ane×End	1.44^{a} (0.09)	0.55^{b} (0.02)	1.99^{ac} (0.29)	0.06^{a} (0)	0.02^{a} (0)	0.09^{a} (0.01)	274.7^{c} (89.54)	0.63^{b} (0.36)	32.65^{b} (8.18)	16.77^{b} (5.55)	0.74^{bc} (0.34)	1.66^{b} (0.35)
	All Sp. (sand)	1.69^{a} (0.06)	0.51^{b} (0.02)	2.12^{ac} (0.23)	0.07^{a} (0)	0.02^{a} (0)	0.09^{ab} (0.01)	345.89^{c} (30.74)	0.89^{ab} (0.43)	25.58^{b} (9.56)	19.62^{b} (0.92)	0.47^{bc} (0.21)	1.39^{ab} (0.52)
Alfic Haplorthod (sandy loam)	Control (no EW)	3.11^{b} (0.08)	0.52^{b} (0)	-----	0.15^{b} (0)	0.01^{b} (0)	-----	192.03^{b} (16.79)	122.64^{c} (5.42)	-----	16.04^{b} (1.23)	0.01^{a} (0.07)	-----
	All Sp. (sandy loam)	3.12^{b} (0.05)	0.53^{b} (0.01)	3.09^{b} (0.11)	0.14^{b} (0)	0.01^{b} (0)	0.11^{b} (0.01)	439.01^{c} (88.49)	68.17^{c} (30.49)	26.26^{b} (5.71)	25.6^{c} (5.2)	0.21^{b} (0.1)	1.61^{b} (0.27)
	H	27.80 **	29.97 **	31.28 **	25.70 **	71.90 **	13.04*	41.99 **	37.26 **	16.41*	42.61 **	45.88 **	16.94*

Table B5: Earthworm species biomass (*L. terrestris* [Lterr], *E. fetida* [Efoet], and *A. trapezoides* [Atrap]) percent C, percent N, and isotopic enrichment (^{13}C and ^{15}N) after 150-day incubations. Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = *H*) with nonparametric multiple comparisons (** $P < 0.01$, * $P < 0.05$).

	Earthworm species biomass C and N chemistry after 150-day incubations											
	% Carbon			% Nitrogen			^{13}C enrichment (mg ^{13}C m^{-2})			^{15}N enrichment (mg ^{15}N m^{-2})		
Treatment	Lterr	Efoet	Atrap	Lterr	Efoet	Atrap	Lterr	Efoet	Atrap	Lterr	Efoet	Atrap
Epigeic	-----	32.03^{a} (5.2)	-----	-----	7.76^{a} (1.34)	-----	-----	1.77^{a} (0.33)	-----	-----	0.14^{a} (0.04)	-----
Epi×End	-----	33.67^{a} (4.55)	53.19^{a} (0.5)	-----	8.6^{a} (1.46)	14.4^{a} (0.27)	-----	1.66^{a} (0.52)	1.00^{a} (0.42)	-----	0.15^{a} (0.05)	0.06^{a} (0.03)
Anecic	24.55^{a} (0.8)	-----	-----	5.69^{a} (0.19)	-----	-----	9.21^{a} (1.18)	-----	-----	0.66^{a} (0.1)	-----	-----
Ane×End	27.07^{ab} (0.97)	-----	52.4^{a} (0.55)	6.22^{ab} (0.12)	-----	13.69^{a} (0.21)	8.85^{a} (0.77)	-----	1.72^{a} (0.41)	0.77^{a} (0.09)	-----	0.10^{a} (0.02)
All Species (sand)	24.23^{a} (1.59)	34.03^{a} (4.39)	45.09^{b} (2.01)	5.59^{a} (0.34)	8.19^{a} (1.32)	11.35^{a} (0.49)	10.8^{a} (2.14)	0.87^{ab} (0.12)	0.67^{a} (0.07)	1.08^{a} (0.25)	0.06^{ab} (0.01)	0.04^{a} (0.01)
All Species (sandy loam)	32.89^{b} (1.16)	39.79^{a} (1.24)	33.53^{c} (3.59)	7.49^{b} (0.31)	9.78^{a} (0.44)	8.04^{a} (0.92)	11.25^{a} (0.87)	0.5^{b} (0.22)	1.06^{a} (0.17)	1.11^{a} (0.12)	0.03^{b} (0.01)	0.08^{a} (0.01)
H	14.52**	1.21	18.75**	13.13**	1.61	6.52	3.29	8.61**	6.06	6.63	9.64**	6.23

Table B6: Tracer recoveries (^{13}C and ^{15}N derived from *A. rubrum* litter) in soil and earthworm species biomass pools after 150-day incubations. Tracer recovery values are percent recoveries of *A. rubrum* ^{13}C and ^{15}N mass loss (provided in Table 2) at the end of the 150-day incubations. Values represent means (± 1 SE), n = 6 for pre–treatment soils, and n = 4 for post–treatment soils. Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = H) with nonparametric multiple comparisons (** $P < 0.01$, * $P < 0.05$).

	Soil Pools						Earthworm Pools					
	^{13}C Recovery (% of applied after 150 d)			^{15}N Recovery (% of applied after 150 d)			^{13}C Recovery (% of applied after 150 d)			^{15}N Recovery (% of applied after 150 d)		
Treatment	AH	BH	BR	AH	BH	BR	Lterr	Efoet	Atrap	Lterr	Efoet	Atrap
Control Coarse	4.4^{a} (0.26)	0.78^{a} (0.17)	-----	9.45^{a} (0.83)	4.01^{a} (0.16)	-----	-----	-----	-----	-----	-----	-----
Epigeic	13.18^{ab} (1.73)	0.32^{a} (0.04)	1.59^{a} (0.49)	28.2^{b} (2.38)	6.44^{ab} (0.1)	3.42^{a} (1.09)	-----	1.01^{a} (0.6)	-----	-----	0.38^{a} (0.11)	-----
Epi×End	23.32^{b} (2.73)	0.77^{a} (0.06)	10.91^{b} (2.42)	36.17^{bc} (5.96)	9.43^{ab} (0.36)	17.6^{b} (3.72)	-----	0.23^{ab} (0.07)	0.15^{a} (0.06)	-----	0.4^{a} (0.15)	0.17^{a} (0.08)
Anecic	14.7^{b} (2.14)	1.03^{a} (0.23)	12.36^{b} (4.03)	29.79^{b} (3.33)	14.45^{c} (0.7)	20.08^{b} (7.06)	1.52^{a} (0.64)	-----	-----	1.76^{a} (0.27)	-----	-----
Ane×End	36.43^{c} (1.18)	0.40^{a} (0.07)	7.96^{b} (1.98)	70.43^{d} (3.64)	15.97^{c} (1.1)	13.19^{b} (3)	0.88^{a} (0.23)	-----	0.18^{a} (0.07)	2.08^{a} (0.25)	-----	1.76^{a} (0.27)
All Sp. (sand)	28.49^{c} (2.59)	0.56^{a} (0.05)	6.1^{ab} (3.24)	53.46^{bc} (2.89)	10.07^{bc} (0.2)	11.23^{b} (6.06)	0.99^{a} (0.17)	0.08^{ab} (0.02)	0.06^{a} (0.01)	2.88^{a} (0.68)	0.17^{ab} (0.02)	0.11^{a} (0.02)
Control (sandy loam)	20^{b} (1.49)	56.77^{b} (0.81)	-----	49.04^{bc} (3.58)	2.19^{a} (0.24)	-----	-----	-----	-----	-----	-----	-----
All Sp. (sandy loam)	42.82^{c} (0.93)	42.42^{b} (0.11)	8.34^{b} (1.37)	82.88^{d} (2.11)	4.58^{a} (0.03)	17.48^{b} (2.39)	1.28^{a} (0.39)	0.05^{b} (0.02)	0.12^{a} (0.03)	2.96^{a} (0.33)	0.08^{b} (0.02)	0.21^{a} (0.03)
H	41.81**	36.83**	35.38**	41.91**	45.20**	34.54**	3.01	8.84*	3.39	6.63	10.14*	6.37

Appendix C: Tables of burrow system structure across earthworm community treatments and Spearman rank correlations among burrow system and soil chemistry variables.

Table C1: Burrow system structure variables across treatment, area normalized to m^2. Surface connectivity represents burrow volume connected to the soil surface. Burrow continuity is measured as the number of burrows whose length is greater than 0 to >50% of core length (CL). Values represent means (± 1 SE). Different lower case letters within columns represent significant differences between groups determined by Kruskal-Wallis tests (statistic = *H*) with nonparametric multiple comparisons (** P < 0.01, * P < 0.05).

	Burrow system macrostructure (cm^3)			Burrow continuity class (# burrows with length > X% CL)				Burrow size distribution class (# burrows of area within range)		
Treatment	Macroporosity	A-horizon burrow vol.	Surface connectivity	0 – 15%	15 –25%	25 – 50%	> 50%	0.1 to < 0.17 cm^2	0.17 to < 0.34 cm^2	> 0.34 cm^2
Epigeic	48.75^{a} (20.45)	48^{b} (21)	26^{ab} (15)	6^{a} (2)	1^{a} (1)	0^{a} (0)	0^{a} (0)	33^{b} (21)	16^{ab} (6)	9^{ab} (4)
Epi×End	61.23^{a} (12.1)	59^{b} (12)	33^{ab} (20)	9^{a} (6)	2^{a} (1)	0^{a} (0)	0^{a} (0)	44^{b} (11)	20^{b} (6)	8^{ab} (2)
Anecic	20.57^{b} (4.85)	16^{a} (6)	16^{a} (4)	5^{a} (2)	3^{a} (1)	1^{a} (1)	1^{a} (1)	8^{a} (3)	6^{a} (2)	4^{a} (1)
Ane×End	41.73^{a} (15.63)	41^{b} (17)	26^{ab} (11)	8^{a} (6)	2^{a} (2)	0^{a} (0)	1^{a} (0)	33^{b} (11)	13^{ab} (6)	5^{ab} (2)
All Sp. (sand)	61.01^{a} (9.02)	59^{b} (9)	39^{b} (13)	8^{a} (3)	3^{a} (1)	1^{a} (1)	1^{a} (0)	38^{b} (7)	19^{b} (3)	8^{ab} (3)
All Sp. (sandy loam)	79.69^{ac} (9.74)	70^{b} (9)	56^{b} (9)	8^{a} (4)	3^{a} (1)	1^{a} (1)	1^{a} (0)	36^{b} (9)	17^{ab} (5)	9^{b} (2)
H	23.70**	16.96**	17.43**	5.62	11.39	11.64	7.76	16.84**	17.08**	16.17**

Table C2: Correlation matrix of burrow system structure variables (SC = surface connectivity, MR = macroporosity, ABr_vol = burrow volume in the A-horizon, BS = burrow size class [1 = 0.1 to < 0.17 cm^2, 2 = 0.17 to < 0.34 cm^2, 3 = > 0.34 cm^2], BC = burrow continuity class [1 = 0 – 15%, 2 = 15 – 25%, 3 = 25 – 50%, 4 = > 50% core length]). Values represent Spearman rank correlation coefficients (ρ). Significance levels of correlation are indicated as ** P < 0.01, * P < 0.05.

	A. rubrum mass loss (g/m^2)		% ^{13}C Recovery			% ^{15}N Recovery			C content ($g\ C/m^2$)			N content ($g\ N/m^2$)		
	(1) 13C	(2) 15N	(3) AH	(4) BH	(5) Br	(6) AH	(7) BH	(8) Br	(9) AH	(10) BH	(11) BR	(12) AH	(13) BH	(14) BR
(1)														
(2)	0.94**													
(3)	0.56**	0.50**												
(4)	0.32	0.36*	0.43**											
(5)	0.23	0.16	0.14	0.37*										
(6)	0.61**	0.57**	0.9**	0.30	0.08									
(7)	0.14	0.14	-0.20	-0.26	0.22	-0.16								
(8)	0.23	0.16	0.20	0.39*	0.98**	0.14	0.11							
(9)	0.21	0.22	0.36*	0.71**	0.25	0.24	-0.41*	0.30						
(10)	0.11	0.21	0.30	0.18	0.03	0.37*	0.09	0.06	-0.01					
(11)	-0.03	-0.19	0.16	0.04	0.41*	0.07	-0.36*	0.48**	0.15	-0.08				
(12)	0.21	0.28	0.27	0.61**	0.13	0.33	-0.39*	0.17	0.86**	0.16	-0.05			
(13)	-0.15	-0.10	-0.41*	-0.54**	-0.18	-0.30	0.76**	-0.26	-0.63**	0.36*	-0.44**	-0.48 **		
(14)	-0.04	-0.18	0.18	0.05	0.40*	0.10	-0.37*	0.48**	0.17	-0.05	0.99**	-0.02	-0.45 **	
(15) CO_2	-0.03	0.04	-0.24	-0.20	0.02	0.06	-0.08	-0.11	-0.01	-0.06	-0.34	-0.30	0.33	0.34
(16) DOC	-0.02	0.07	0	0.18	-0.06	-0.06	0.35*	0.49**	0.21	0.32	0.15	0.21	-0.51 **	-0.50 **

Table C3: Correlation matrix of burrow system structure variables (SC = surface connectivity, MR = macroporosity, ABr_vol = burrow volume in the A-horizon, BS = burrow size class [1 = 0.1 to < 0.17 cm^2, 2 = 0.17 to < 0.34 cm^2, 3 = > 0.34 cm^2], BC = burrow continuity class [1 = 0 – 15%, 2 = 15 – 25%, 3 = 25 – 50%, 4 = > 50% core length]). Values represent Spearman rank correlation coefficients (ρ). Significance levels of correlation are indicated as ** P < 0.01, * P < 0.05.

	Burrow system macrostructure (cm^3)			Burrow continuity (# burrows with length > X% CL)				Burrow size distribution (# burrows within range)		
	(1) MR	(2) ABr_vol	(3) SC	(4) BC_1	(5) BC_2	(6) BC_3	(7) BC_4	(8) BS_1	(9) BS_2	(10) BS_3
(1)										
(2)	0.98**									
(3)	0.90**	0.89**								
(4)	0.31	0.30	0.18							
(5)	0.19	0.14	0.31	0.35*						
(6)	-0.08	-0.15	-0.04	0.05	0.43*					
(7)	-0.35*	-0.40*	-0.32	0.01	0.28	0.68**				
(8)	0.50**	0.49**	0.23	0.57**	0.01	-0.34*	-0.39*			
(9)	0.49**	0.47**	0.24	0.71**	0.10	-0.18	-0.26	0.88**		
(10)	0.67**	0.66**	0.40*	0.60**	0.09	-0.21	-0.29	0.79**	0.85**	

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Chapter 5

Detritus Inputs and Removal: Preliminary results of earthworm community responses and long-term effects on soil carbon dynamics in a sandy temperate forest [5]

5.1 Introduction

In 2004, a long-term field experiment was initiated in sandy Spodosols of northern Michigan to determine detrital controls on soil organic matter accumulation and stabilization over decadal time scales (Nadelhoffer et al. 2006, Coleman 2008). The Detritus Inputs and Removal Experiment (DIRT) was first conceptualized by Nielson and Hole (1963), and permits the study of (1) soil faunal community responses to altered resource availability (i.e., detrital inputs) and (2) interactive effects of detrital inputs and soil faunal communities on long-term soil C dynamics. Using the DIRT experiment, we quantified earthworm community responses after seven years of leaf litter manipulations and characterized associated changes in soil C content and chemistry. Findings presented here will be integrated into another paper focusing on the outcomes of long-term leaf litter manipulations across the network of DIRT sites. Here, I provide an overview of the survey and sampling methods, the preliminary results, and the next steps needed to prepare this manuscript for publication.

5.2 Methods

5.2.1 Site Description

The 30-hectare study area functions as an experimental forest in which the University of Michigan Biological Station (UMBS) Forest Ecosystem Study has been established as a long-term site for research on forest succession and ecosystem processes in northern Lower Michigan,

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[5] Manuscript will be published by J.M. Crumsey, S. Atkins, J.M. Le Moine, and K.J. Nadelhoffer in 2014. Preliminary data presented here will be submitted as part of a research proposal to the 2014 Call for Science Theme Proposals by the Pacific Northwest National Laboratory.

USA (45°35.5’N, 84°43’W; Figure 1A). Tree species composition, forest age, and disturbance history of the aspen-dominated forest represents a regionally dominant forest type (USDA Forest Service 2002). The secondary successional forest areas are currently dominated by bigtooth aspen (*Populus grandidentata*), with northern red oak (*Quercus rubra*), red maple (*Acer rubrum*), paper birch (*Betula papyrifera*), American beech (*Fagus grandifolia*), and eastern white pine (*Pinus strobus*) occurring as co–dominants (Curtis et al. 2005). Primary successional forests, dominated by pine and hemlock, were logged around 1880 and disturbed repeatedly by fire until 1923 (Gough et al. 2007). UMBS forests lie on outwash plains with well-drained soils (92.9% sand, 6.5% silt, 0.6% clay) classified as mixed, frigid Entic Haplorthods of the Rubicon series (National Resources Conservation Service 1991). Five earthworm species of European origin representing different ecological groups (Bouché 1977) dominate soil-dwelling earthworm communities across the research area (Crumsey et al. 2013c). The species include *Dendrobaena octaedra* (Savigny), *Aporrectodea caliginosa* (Savigny), *Aporrectodea trapezoides* (Dugès), *Lumbricus rubellus* (Hoffmeister), and *Lumbricus terrestris* (Linneus). Baseline soil C and N properties, along with measures of earthworm species density and biomass, are given in Table 1.

5.2.2 Leaf Litter Manipulation Field Experiment

Leaf litter manipulation plots of the Detritus Inputs and Removal (DIRT) experiment were established in 2004 as an extension of Neilson and Hole (1963) and the UMBS Forest Ecosystem Study (Figure 1A,B). In total, six litter manipulation treatments were replicated across three blocks and randomly assigned to plot locations (Figure 1C). Treatment plots were enclosed in 5m^2 plots beneath an intact forest canopy and included: Control (CTL), No Litter (NL), Double Litter (DL), No Roots (NR), No Input (NI), and DL plots fertilized with 30 kg N.ha^{-1}.yr^{-1}NH$_3$Cl (F). Here, we focus on soil C dynamics in across No Litter, Control, and Double Litter treatments which represents a gradient of leaf litter inputs that spans roughly 0 to 2400 kg C ha^{-1} yr^{-1}. No Litter plots are covered with 91% tan shade cloths (Green-Tek Inc.) to collect annual litterfall, regulate plot albedo, and inhibit moss growth. Within each sampling block, litterfall from the No Litter plots is weighed and transferred to Double Litter plots every two weeks during peak litterfall (late September to mid-November) and monthly otherwise. Ambient leaf litter is permitted in Control plots. Understory plants and mosses are removed periodically from each treatment. Control and leaf litter manipulation plot. Reference plots

(REF) represent background forest conditions in which no manipulations to litterfall or the understory have occurred.

5.2.3 Earthworm Community and Soil Chemical Analyses

In June 2011, we characterized earthworm community diversity in three subplots (0.25 m^2) established at random locations within DIRT treatment plots. We removed and sorted the Oi horizon to collect surface-dwelling earthworms, and used an electroshock extraction method to sample sub-surface earthworms (Satchell et al. 1955, Thielemann 1986, Bohlen et al. 1995, Staddon et al. 2003). Electroshocking probes were sets of eight steel rods (50 cm long × 6 mm diameter), installed in a 4 × 4 array at 6.25 cm apart. Eight-wire delivery cables were connected to a gasoline-powered generator (Honda EU 2000i) at one end, and split at the other end where alligator clips were connected and attached to probes. We applied 120 V A.C. for 20 minutes, and collected all earthworms that surfaced. Adult earthworms were identified to species, and juvenile earthworms were identified to genus according to Schwert (1990). Each specimen was measured field moist, and frozen at -80°C until freeze-dried for archiving. Earthworm freeze-dried weights from previous field collections were used to calculate a fresh-to-dry weight ratio and estimate species-specific earthworm biomass across plots for each sampling period. Soil volumetric water content (%) was recorded at each subplot using a soil moisture sensor (CS620 HydroSense, Campbell Scientific) during earthworm sampling.

In 2004, 2009, and 2011 we sampled soils at three random locations within DIRT treatment plots using a 15cm^2 monolith, removing the A-horizon underneath (to ~ 5 cm depth). We then used a slide hammer soil corer (AMS, 48 mm ID, split spoon sampler) and separated soil samples into A-horizon, 0 – 10cm, and 10 – 20cm depths. Sampling sites were marked with PVC pipes and a 1m^2 buffer around each site was established for subsequent sampling. After removing roots, soils were dried at 60°C, and pulverized (SPEX Certiprep 8000D Ball Mill) for subsequent chemical analyses. Soil samples across all depth fractions (A-horizon, 0-10cm, and 10-20cm) were analyzed for bulk C and N properties. Soil %C, %N, δ^{13}C, and δ^{15}N were measured by continuous flow isotope ratio mass spectrometry (Thermo Finnigan Delta Plus XL) after sample combustion to CO_2 and N_2 at 1000°C by an on-line elemental analyzer (Costech Elemental Analyzer 1030). Instrument error determined by repeated internal standards was ± 0.19‰ for δ^{13}C and ± 0.16‰ for δ^{15}N. Dry soil mass was multiplied by %C and %N to determine soil C and N content.

We analyzed the molecular structure of C compounds present in surface soils (A-horizon) across DIRT treatment plots using solid-state ^{13}C Cross-Polarization Magic Angle Spinning Nuclear Magnetic Resonance (CPMAS-NMR). Solid-state ^{13}C CPMAS-NMR spectrometry is a non-destructive measurement of ^{13}C-nuclei resonance peak shifts as affected by the nature and configuration of adjacent atoms; this technique provides a semi-quantitative evaluation of carbon compound distribution. The resulting spectrum is divided into general classes related to the chemical structure of organic compounds. Three A-horizon-composites from each set of treatment plots were homogenized, dried at 60°C, and sent to Spectral Data Services, Inc. (Champaign, IL) for ^{13}C CPMAS-NMR analysis. NMR spectra were obtained at 91MHz and 5kHz CP-MAS on a 360-1 instrument (H-1 Larmor frequency of 363.335 MHz, 7mm CPMAS Doty probe, 5 mm SuperSonic Doty probe, and 5 and 10 mm solution probes). Spectra were digitally processed using an exponential weighting equation with a line broadening at 100 Hz and a Fourier transformation on MestReC459 software (Universidad de Santiago de Compostela, Spain). The software was used to integrate peak areas under the following five chemical shift regions (and the general C types they represent): carboxyl-C (190–160 ppm), aromatic-C (160–110 ppm), O-alkyl-C (110–60 ppm), methoxyl/N-alkyl-C (60–45 ppm), and alkyl-C (45–0 ppm). The integrated spectral areas were normalized to the total signal intensity for each spectrum, and the relative percentage of each major C-type was calculated by dividing the area of each region by the total spectral area (Smejkalova et al. 2008). Lastly we calculated a humification index (Sequi et al. 1986) as the ratio of alkyl C to O-alkyl-C (i.e., ratio of organic C content of the non-humified to humified fractions) from each spectra.

5.2.4 Data analysis

We analyzed the effects of treatment (control, clipped, induced) and sampling block on the density and biomass of earthworm species present using a multivariate analysis of variance (MANOVA, statistic = Wilk's λ, df = 8, $\alpha = 0.10$). We employed the MANOVA approach because the densities and biomass values for co-occurring earthworm species are potentially not independent response variables. Interaction terms could not be tested due to the low level of replication in the experimental design. Following a significant MANOVA result, we used univariate analyses to examine effects on the different response variables. Pairwise comparisons of earthworm community composition across leaf litter manipulation plots were computed as

Bray–Curtis distances, and visualized using non-metric multidimensional scaling (NMDS). The significance of the NMDS ordination was determined using a Monte Carlo permutation test (999 permutations; $P < 0.05$). The multi-response permutation procedure (MRPP, statistic = δ) (McCune et al. 2002), was used to determine significant differences in earthworm community composition across treatments, followed by a permutation-based multivariate analysis of variance test (PerMANOVA; statistic = F) (Anderson 2001) to determine significant differences in treatment-level earthworm community composition nested by sampling block.

Using separate general linear models, a repeated-measures analysis of variance (ANOVA) was used to test for differences in each of the soil chemical properties (C content, N content, δ^{13}C, and δ^{15}N) attributable to soil depth increment, leaf litter manipulation treatment, time (years 2004, 2009, and 2011), and their interaction. Bonferroni-corrected pairwise comparisons were performed on significant main effects from repeated-measures tests. Finally, we evaluated relationships between earthworm community diversity, leaf litter inputs, and soil chemical properties using observations made in 2011. Earthworm species biomass was used as a proxy for earthworm activity and related to soil chemical properties by co-inertia analysis (CoIA), which identifies co-relationships between transformed species and environmental data matrices (Doledec and Chessel 1994, Dray et al. 2003). CoIA is complementary to canonical correspondence analysis, but is recommended when the number of measured variables is greater than sites sampled (Doledec and Chessel 1994, Borcard et al. 2011). Data matrices of earthworm species biomass and A-horizon chemical properties were each transformed by principal components analysis prior to the CoIA. Statistical significance of the CoIA was assessed by Monte Carlo permutation tests (999 permutations; $P < 0.05$). Statistics were done in R version 2.15.2 (R Development Core Team 2012) on RStudio version 0.96.331 (http://www.rstudio.org/), using the packages: ade4 (Dray and Dufour 2007), pgirmess (Giraudoux 2012), and vegan (Oksanen et al. 2012).

5.3 Results

5.3.1 Earthworm community diversity across leaf litter manipulation treatments

Earthworm species collected across plots included *Dendrobaena octaedra*, *Lumbricus rubellus*, and *Lumbricus terrestris*; *Aporrectodea caliginosa* and *Aporrectodea trapezoides*, were not collected in leaf litter manipulation plots. Earthworm density ranged from 6 ± 4 in No Litter

Plots to 87 ± 11 individuals m^{-2} in Double Litter plot (Table 2), with variation in species density as follows: juveniles > *L. rubellus* > *D. octaedra* > *L. terrestris* (Figure 2A). Earthworm biomass (fresh weight) ranged from 1 ± 0 in No Litter treatment to 40 ± 9 g m^{-2} in Double Litter treatment, with variation in species density as follows: *L. terrestri*s > *L. rubellus* > juveniles > *D. octaedra* (Figure 2B, Table 2). We observed marginally significant main effects of block and leaf litter manipulation treatments on earthworm species density and biomass (MANOVA tests, $P < 0.10$, Table 3). The effect of sampling block was only significant for *L. terrestris* density and biomass, while *L. rubellus* and juvenile density and biomass increased in response to increased leaf litter inputs (ANOVA tests, $P < 0.05$). *D. octaedra* density and biomass was statistically similar across sampling block and leaf litter manipulation treatments (ANOVA tests, $P > 0.05$).

Differences in earthworm community composition were evaluated using earthworm species biomass data, which we considered an indicator of species responses to altered leaf litter (i.e. resource) availability. The NMDS ordination was reliable (Stress = 0.017) and clearly discriminated earthworm communities across leaf litter manipulation treatments (Figure 3). Earthworm community composition in the Control plots were similar to that of Reference (or background) earthworm community composition, while distinct earthworm communities were observed in No Litter and Double Litter plots. The MRPP showed that earthworm community dissimilarity across plots was highly significant, indicating strong responses of earthworm communities to leaf litter manipulations ($\delta = 0.364$, $P = 0.011$; Table 4). Pairwise comparisons of earthworm community composition nested by sampling block also indicated spatial dependence of earthworm communities across the study area (PerMANOVA, $F = 6.325$, $P = 0.15$; Table 4).

5.3.2 *Soil chemical properties across leaf litter manipulation treatments, depth increment, and time*

General linear models indicated that mean soil chemical properties (%C, %N, C:N, δ^{13}C, and δ^{15}N) integrated across depth increments and time were statistically similar across leaf litter manipulation treatments, though trends of increasing C and N content and C:N ratios with increasing litter inputs were observed (Table 5). However, significant differences in soil chemical properties with depth increment (A-horizon, 0-10cm, 10-20cm) and time (2004 – 2011), as well as the interaction between these parameters were observed (Figure 4, Table 5).

Total soil C and N pools decreased over time, but this trend was similar across leaf litter manipulation treatments. Soil $\delta^{13}C$ values were increasingly enriched with soil depth and time, but were also similar across leaf litter manipulation treatments. Interactions between treatment, depth increment, and time were only significant for soil $\delta^{15}N$ values (GLM repeated measures, $P < 0.05$; Table A3).

Visual comparison of ^{13}C CPMAS NMR spectra for composite samples collected across plots of each treatment revealed differences in the relative contribution of C types to total signal intensity from 2004 to 2011 across leaf litter manipulation treatments (Figure 5). Digital processing of NMR spectra showed that spectra representing the Double Litter and Control surface soils had higher contributions from the alkyl-C region (0–45 ppm) and O-alkyl-C (45 – 110 ppm) relative to No Litter spectra (Figure 6, Table 6); peaks within these spectral region are primarily attributed to plant cellulose and long-chain aliphatic compounds (i.e. fatty acids, lipids, cutin acids) (Kögel-Knabner 1997, Baldock et al. 2004). Over the seven-year period, contributions of aromatic-C (110 – 160ppm) and carboxyl-C (160-200ppm) increased in the No Litter plots, while the opposite was observed for Double Litter plots (Figure 6, Table 6); peaks within these spectral region are primarily attributed to lignin and structures derived from black carbon (Kögel-Knabner 1997, Baldock et al. 2004). Another notable difference between leaf litter manipulation plots was the change in the humification index where the greatest gains in the alkyl-C:O-alkyl ratio were as follows Double Litter > Control > No Litter surface soils (Table 3).

5.3.3 Relationships between earthworm communities and soil chemical properties

To characterize co-relationships between earthworm community diversity and soil C and N chemistry, we evaluated variable associations along the first two co-inertia axes (eigenvalues: F1 = 9.72; F2 = 1.60). The co-inertia factorial plane discriminated between variables directly and inversely associated with leaf litter inputs and earthworm species biomass: (1) alkyl-C, O-alkyl-C, and soil C:N; were positively associated with leaf litter inputs and earthworm biomass. (2) Soil isotopic values, aromatic C, and carbonyl-C; these variables were negatively associated leaf litter inputs and earthworm biomass. *L. rubellus* and *L. terrestris* biomass was associated with both co-inertia axes, while litter inputs and remaining measures of earthworm biomass were associated with the first co-inertia axis. The first two axes of the co-inertia analysis explained 98.3% of the total variability in the earthworm community diversity and soil C and N chemistry data co-structure (Monte Carlo permutation tests, $P = 0.002$; Figure 7). Along F1 (84.4% of total inertia), alkyl-C, O-alkyl-C, and soil C:N were correlated with litter inputs, total earthworm biomass, and *D octaedra*, juveniles, *L. rubellus*, and *L. terrestris* biomass. Soil δ^{15}N, aromatic-C, and carbonyl-C were negatively associated with leaf litter inputs and earthworm biomass. Along F2 (13.9% of total inertia), *L. rubellus* was correlated with soil C and N pools, while *L. terrestris* was negatively associated with surface soil δ^{13}C and the humification index (O-alkyl-C: alkyl-C).

5.4 Discussion

Leaf litter manipulations and earthworm community diversity

We observed differences in earthworm species distributions and overall community composition across sampling blocks and leaf litter manipulation treatments, which are likely due to variations in the ecological behaviors and dispersal patterns of species present. Earthworm species and their corresponding ecological groups included: *Lumbricus terrestris* (Epi-anecic = litter feeding, vertical burrowing), *Lumbricus rubellus* (Epi-endogeic litter feeding, mineral soil dwelling), and *Dendrobaena octaedra* (Epigeic = litter feeding, surface–dwelling). Earthworm species generally show low dispersal abilities, with anecic species having the lowest dispersal rates of c.a. 2 m^2 yr^{-1} and epigeic species showing the highest dispersal rates of up to 11 m^2 yr^{-1} (Marinissen and Van den Bosch 1992).

While *L. rubellus* and *D. octaedra* population densities were statistically similar across sampling blocks, *L. terrestris* population densities indicated significant spatial variation with population densities higher in the southern-most block. Similar patterns in earthworm species distributions have been observed and modeled during incipient invasions as ‘invasion fronts’ (i.e., a succession of earthworm species across a visible leading edge due to different patterns of colonization) (Hale et al. 2005a, Suárez et al. 2006a, Cameron et al. 2007, Addison 2009a). Here, the long time over which these organisms have been present across the landscape (> 60 years) (Smith and Green 1916, Murchie 1956), limited forest disturbance (Gough et al. 2007), and the fairly stable temporal dynamics of earthworm communities in demonstrated in forest landscapes (Whalen 2004b, Stoscheck et al. 2012, Crumsey et al. 2013c), suggests dispersal as the primary mechanism limiting *L. terrestris* distributions. *L. rubellus* and juveniles showed the strongest responses to leaf litter removal and doubling, whereas *D. octaedra* population densities were statistically similar across leaf litter manipulation treatments. Species-specific responses to leaf litter manipulation treatments corresponded to differences in community composition with a greater response observed in leaf litter removal plots than plots where leaf litter was doubled. These results agree with previous studies where the number earthworms in a given area declines by 40 – 80% with litter removal over one to five-years (Pearse 1943, Nielsen and Hole 1964, Judas 1990, David et al. 1991), in which stronger responses to leaf litter removal are attributed to food resource losses and shifts in microclimate conditions (i.e. moisture and temperature).

Soil chemical properties across leaf litter manipulation treatment, depth increment, and time

Over a seven-year period of leaf litter manipulations, we did not detect significant shifts in soil C and N pools or isotopic values along the experimental leaf litter gradient. Soil chemical properties differed with depth increment and time, showing a general decline over the seven-year observation period. NMR spectra from composite A-horizons suggest differences in soil carbon chemistry with a higher abundance of recalcitrant C forms (carboxyl C and aromatic C) in No Litter than that observed in Control or Double Litter plots. NMR spectra also suggest an accumulation of labile C forms (alkyl-C and O-alkyl C) in Double Litter treatment with the highest earthworm densities. These shifts are likely a function of increased leaf litter degradation and incorporation into soil organic matter pools associated with earthworm activity. Associations between earthworm species biomass, litter inputs, and soil chemical properties are also consistent with previous research findings and highlight the importance of organic matter redistribution as a mechanism through which earthworms alter litter degradation rates and soil carbon dynamics.

Future Directions

Our inability to detect changes in soil properties within this field-based experiment can be attributed to a number of factors. First, the status of earthworm communities prior the start of leaf litter manipulations and the temporal dynamics of earthworm communities are unknown. Differences in earthworm community composition by sampling block (i.e., lower population densities of *L. terrestris* in northern blocks, and the absence of endogeic species responsible for mixing organic residues throughout mineral soils) may correspond to non-uniform or decreased impact of earthworms on soil carbon processes in the study area, relative to those observed for the broader earthworm community present across the landscape (Crumsey et al. 2013). Leaf litter degradation in sandy Spodosols may also result in CO_2 losses that outweigh the transfer of leaf litter into subsurface soils. Trends in soil carbon properties may continue to diverge with continued leaf litter manipulations and earthworm activity, such that statistically significant differences can be detected. A more rigorous characterization of the molecular composition of soil organic matter across leaf litter manipulation plots is also warranted, as ^{13}C CPMAS NMR is considered a semi-quantitative technique, such that the ability to infer chemical shifts is improved with sample replication and fractionation of soil particles (Golchin et al. 1994, Kögel-Knabner 1997, Marín-Spiotta et al. 2008); this permits calculation of mean values for % contributions across C groups, multivariate statistical techniques relating soil chemical classes to soil processes, larger signals within the NMR spectra, and finer delineation of C molecular structures (Kögel-Knabner 1997, Mahieu et al. 1999, Mathers et al. 2000, Smejkalova et al. 2008). Data needed to evaluate the effects of leaf litter manipulations and earthworm communities on complete soil carbon budgets (i.e. losses as CO_2 and DOC) are necessary to compare these findings to those reported in laboratory studies of this dissertation. Further, comparative studies that characterize earthworm communities shifts in soil C pool size and chemistry are warranted across the network of DIRT sites, particularly DIRT plots established on fine-textured Spodosols where exotic earthworm introductions and impacts were documented by Nielsen and Hole (Nielsen and Hole 1963, 1964).

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Figure 1B: Nadelhoffer, K.J., R.D. Boone, R.D. Bowden, J.D. Canary, J.P. Kaye, P. Micks, A. Ricca, J.A. Aitkenhead, K. Lajtha, and W. H. McDowell. 2006. The DIRT experiment: litter and root influences on forest soil organic matter stocks and function. Pages 300–315 *in* D.R. Foster and J.D. Aber, editors. Forests in time: the environmental consequences of 1,000 years of change in New England. © 2006 by Yale University. Reproduced with permission.

Tables:

Table 5.1: Soil chemical and physical properties and earthworm community composition across the UMBS DIRT site. Bulk soil characteristics represent seven-year averages (2004 – 2011) from Reference (REF) plots in which no manipulations have taken place since the establishment of the DIRT treatment plots in 2004. Background earthworm species density (individuals m^{-2}) and biomass (g fresh weight [FW] m^{-2}) was measured in REF plots in 2011.Values represent means ± 1 SE.

Soil Physical and Chemical Properties								Earthworm (EW) community		
Soil Depth	%C	%N	C:N	δ^{13}C (‰)	δ^{15}N (‰)	Bulk Density (g cm^{-3})	pH	EW Species	Density (individuals m^{-2})	Biomass (g FW m^{-2})
A-horizon	8.98 (1.84)	0.38 (0.09)	25.02 (0.32)	-27.34 (0.06)	-1.12 (0.13)	0.39 (0.05)	3.77 (0.08)	*D. octaedra*	17(7)	2(1)
0–10	0.91 (0.15)	0.04 (0.01)	24.98 (0.96)	-26.24 (0.41)	2.25 (0.25)	1.02 (0.06)	3.8 (0.03)	*L. rubellus*	8(4)	5(3)
10–20	0.44 (0.07)	0.02 (<0.01)	23.15 (0.69)	-25.98 (0.18)	5.3 (0.31)	1.27 (0.04)	4.19 (0.06)	*L. terrestris*	5(5)	13(13)
Bulk Soil	3.45 (1.48)	0.14 (0.06)	24.38 (0.47)	-26.71 (0.25)	1.46 (0.92)	0.89 (0.13)	3.90 (0.05)	juveniles	21(2)	3(0)

Table 5.2: Table 2. Earthworm species density (individuals m^{-2}) and biomass (g FW m^{-2}) DIRT leaf litter manipulation treatments. Values represent means ± 1 standard error (SE).

EW Density (individuals m^{-2}) ± SE					
Treatment	*D. octaedra*	*L. rubellus*	*L. terrestris*	Juveniles	Total EW
REF	17 (7)	8 (4)	5 (5)	21 (2)	51 (4)
C	18 (4)	4 (2)	4 (3)	26 (6)	52 (14)
NL	3 (1)	0 (0)	0 (0)	3 (2)	6 (4)
DL	17 (6)	25 (9)	6 (5)	39 (10)	87 (11)
EW Biomass (g FW m^{-2}) ± SE					
Treatment	*D. octaedra*	*L. rubellus*	*L. terrestris*	Juveniles	Total EW
REF	2 (1)	5 (3)	13 (13)	3 (0)	23 (12)
C	2 (0)	3 (1)	11 (9)	4 (1)	19 (11)
NL	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)
DL	2 (1)	16 (6)	16 (14)	5 (1)	40 (9)

Table 5.3: MANOVA (statistic = Wilk's λ) and univariate analyses (statistic = F) for the effects of Block and Treatment on the density and biomass of earthworm species (** $P < 0.05$, * $P \leq 0.10$). Earthworm density and biomass data were square-root transformed prior to the analyses.

	Block			Treatment		
EW species density (individuals m^{-2})	Wilk's λ	F	*P*	Wilk's λ	F	*P*
MANOVA	0.192	1.922	0.10*	0.064	2.052	0.10*
Univariate analyses:						
D. octaedra		0.404	0.68		2.102	0.18
L. rubellus		*0.768*	*0.49*		*4.867*	*0.03***
L. terrestris		*7.459*	*0.01***		*0.458*	*0.72*
Juveniles		*0.692*	*0.53*		*5.969*	*0.02***
EW species biomass (g FW m^{-2})	Wilk's λ	F	*P*	Wilk's λ	F	*P*
MANOVA	*0.191*	*1.932*	*0.10**	*0.060*	*2.129*	*0.09**
Univariate analyses:						
D. octaedra		*0.397*	*0.69*		*2.042*	*0.19*
L. rubellus		*0.715*	*0.52*		5.159	0.03**
L. terrestris		7.467	0.01**		0.452	0.72
Juveniles		0.721	0.51		5.642	0.02**

Table 5.4: Statistical outcomes of the multiresponse permutation procedure (MRPP, statistic = δ, chance-corrected within group agreement = *A*) comparing earthworm community composition across leaf litter manipulation plots and the permutational multivariate analyses of variance (PerMANOVA; statistic = *F*) comparing treatment-level earthworm community composition nested by block, indicated by brackets [ ]; ($P < 0.05$, n = 47). Comparisons use the Bray–Curtis distances between earthworm community assemblages, visualized by the NMDS in Figure 3.

EW Community × Treatment				
MRPP	Observed δ	Expected δ	*A*	*P*
	0.364	0.569	0.360	0.011
EW Community × [Treatment]Block				
PerMANOVA	*SS*	*MS*	*F*	*P*
Similarity	1.494	0.498	6.325	0.015
Residuals	0.630	0.079		
Total	2.123			

Table 5.5: Statistical outcomes of the general linear model with repeated measures comparing soil chemical properties across soil depth increments, leaf litter manipulation treatment, and time. Error for each term is reported in the mean squares column in parentheses. The significance of the F-statistic is indicated as follows: (*** $P < 0.01$, ** $P < 0.05$, * $P < 0.15$).

		Soil C		Soil N		C:N		δ^{13}C (‰)		δ^{15}N (‰)	
Terms	df	MS	F	MS	F	MS	F	MS	F	MS	F
Depth	2	470746 (75289)	6.252 **	540.63 (99.49)	5.43	14.313 (3.422)	4.183 **	17.16 (0.75)	229.65 ***	391.49 (.823)	475.605 ***
Treatment	2	60871	1.305	22.88 (30.93)	.740	24.883 (22.98)	1.083			2.96 (1.948)	1.519
Time	2	734977 (22273)	33.00 ***	1040.94 (23.774)	43.79 ***	44.639 (9.761)	4.573 **	1.245 (.037)	33.40 ***	7.404 (.666)	11.110 ***
Treatment x Time	4	6206	.279	20.69	.870	2.433	.249	0.48	1.28	1.649	2.474 **
Depth x Time	4	576303 (47619)	12.10 ***	697.38 (51.603)	13.51 ***	16.322 (3.613)	4.518 **	.065 (.033)	.872	2.192 (.629)	2.663 ***
Depth x Treatment	4	35266	.468	40.61	.408	8.450	2.470 *	.058	1.732	5.441	8.646 **
Depth x Treatment x Time	8	40447	.849	40.28	.781	4.460	1.234	.014	.430	1.741	2.767 *

Table 5.6: Soil chemical properties (C content, N content, C:N, δ^{13}C, and δ^{15}N) averaged across depth increments (A-horizon, 0-10cm, 10-20cm) and sampling years (2004 – 2011). Different lowercase letters indicate significant differences (overall $P < 0.05$) between means for soil chemical properties, as determined by repeated measures GLM and pairwise modified Bonferroni comparison tests. Main effects and interaction terms of statistical models are given in Table B3.

Treatment	C content (g C/m^2)	N content (g N/m^2)	C:N	δ^{13}C (‰)	δ^{15}N (‰)
No Litter	684.55a (52.85)	28.77a (1.38)	23.46a (0.7)	-26.66a (0.18)	2.24a (1.03)
Control	724.53a (64.89)	30.15a (2.28)	24.04a (0.75)	-26.71a (0.22)	1.49a (0.94)
Double Litter	803.79a (59.51)	32.04a (2.17)	24.64a (0.65)	-26.62a (0.19)	2.16a (0.99)

Table 5.7: Relative contributions of different ^{13}C NMR spectral regions to total peak area and the humification index (O-alkyl C: alkyl C) of composite A-horizon soils sampled across leaf litter manipulation treatments in 2011. Delta (Δ) values represent differences in relative contributions of NMR spectral regions and the humification index from 2004 to 2011.

		NO LITTER			CONTROL			DOUBLE LITTER		
	NMR spectral regions (ppm)	2004	2011	Δ	2004	2011	Δ	2004	2011	Δ
alkyl-C	0-45	31.36	30.44	-0.92	23.6	30.26	6.66	22.3	33.3	11
O-alkyl C	45-110	44.91	33.7	-11.21	52.41	39.09	-13.32	46.13	38.45	-7.68
aromatic C	110-160	12.35	19.91	7.56	16.82	16.39	-0.43	16.67	15.56	-1.11
carboxyl C	160-200	11.38	15.95	4.57	7.17	14.26	7.09	14.9	12.69	-2.21
Humification Index	Alkyl/O-alkyl	0.70	0.90	0.20	0.45	0.77	0.32	0.48	0.87	0.38

Figures:

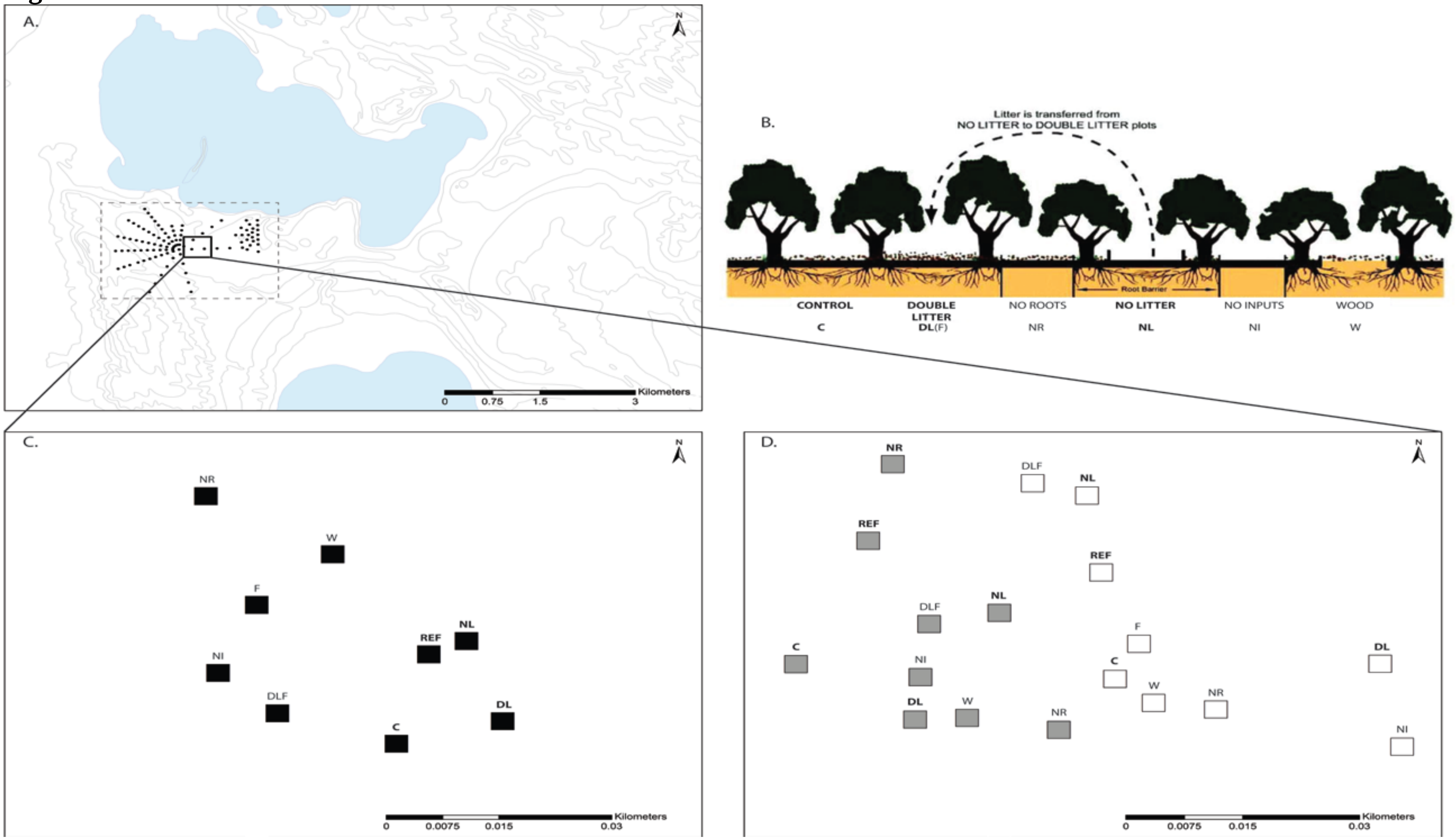

Figure 5.1: A) Dashed square represents the footprint of the UMBS Forest Ecosystem Study located along the south shore of Douglas Lake, Michigan. Dots represent permanent sampling plots that surround two atmospheric towers, established for long-term research on forest succession and ecosystem processes (See site description). Solid square represents the location of the Detritus Inputs Removal and Transfer (DIRT) experiment, established as a part of FEST in 2004. B) Conceptual diagram of litter manipulation treatments across the UMBS DIRT site (modified from Nadelhoffer et al. 2006). C and D) Treatment plots (5 m^2) are replicated in three blocks (black = Block 1, grey = Block 2, white = Block 3). Plots sampled in this study are indicated by bold text in figures B – D.

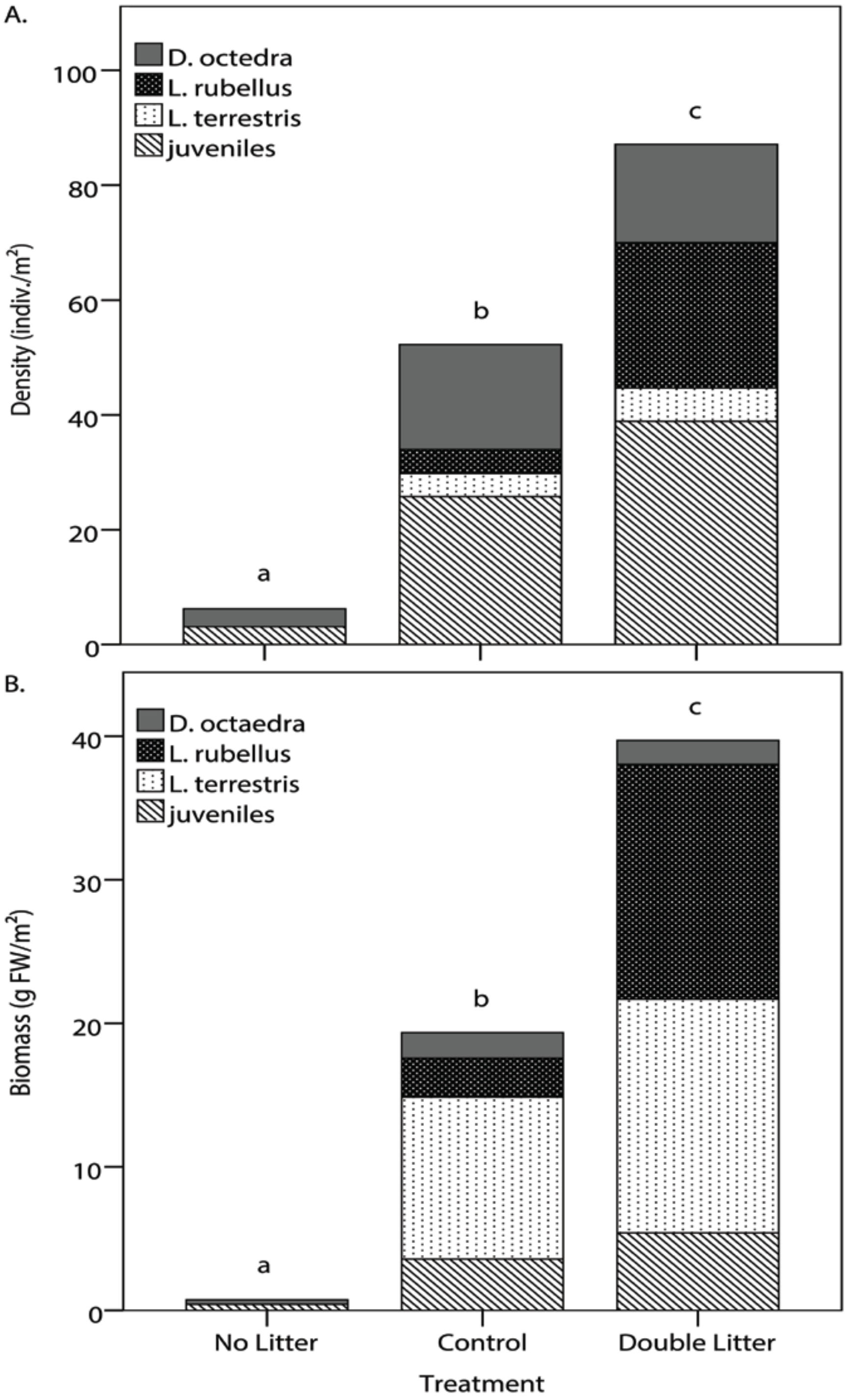

Figure 5.2: Average earthworm species (A) density (individuals m^{-2}) and (B) biomass (g FW m^{-2}) across treatment plots sampled in 2011 (n = 3). Different lower case letters above bars represent significant treatment-level differences in the total earthworm density and biomass, determined by Kruskal-Wallis H tests with nonparametric multiple comparisons ($P < 0.05$). Differences in earthworm species densities and biomass are analyzed by MANOVA (Table A3).

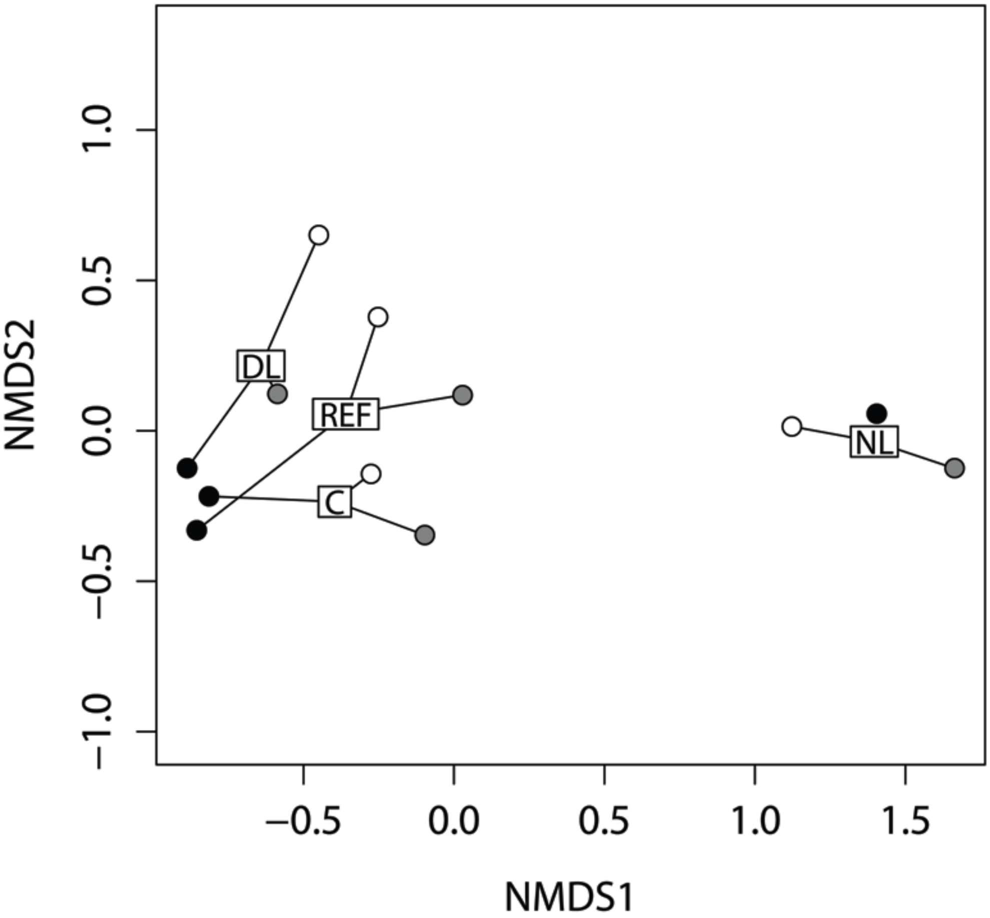

Figure 5.3: Non-metric multidimensional scaling (NMDS) analysis based on Bray-Curtis dissimilarity measures of earthworm community composition across leaf litter manipulation plots. Treatment abbreviations (No Litter = NL, C = Control, DL = Double Litter, REF = Reference) represent the weighted plot centroid of earthworm community composition across plots in 2011. Each point represents a plot-level earthworm community for each treatment across sampling blocks (black = Block 1, grey = Block 2, white = Block 3; Figure 1), with communities of similar composition being located close together in the NMDS ordination space. Statistical outcomes of the MRPP and PerMANOVA testing treatment and block effects on earthworm community composition are presented in Table B2.

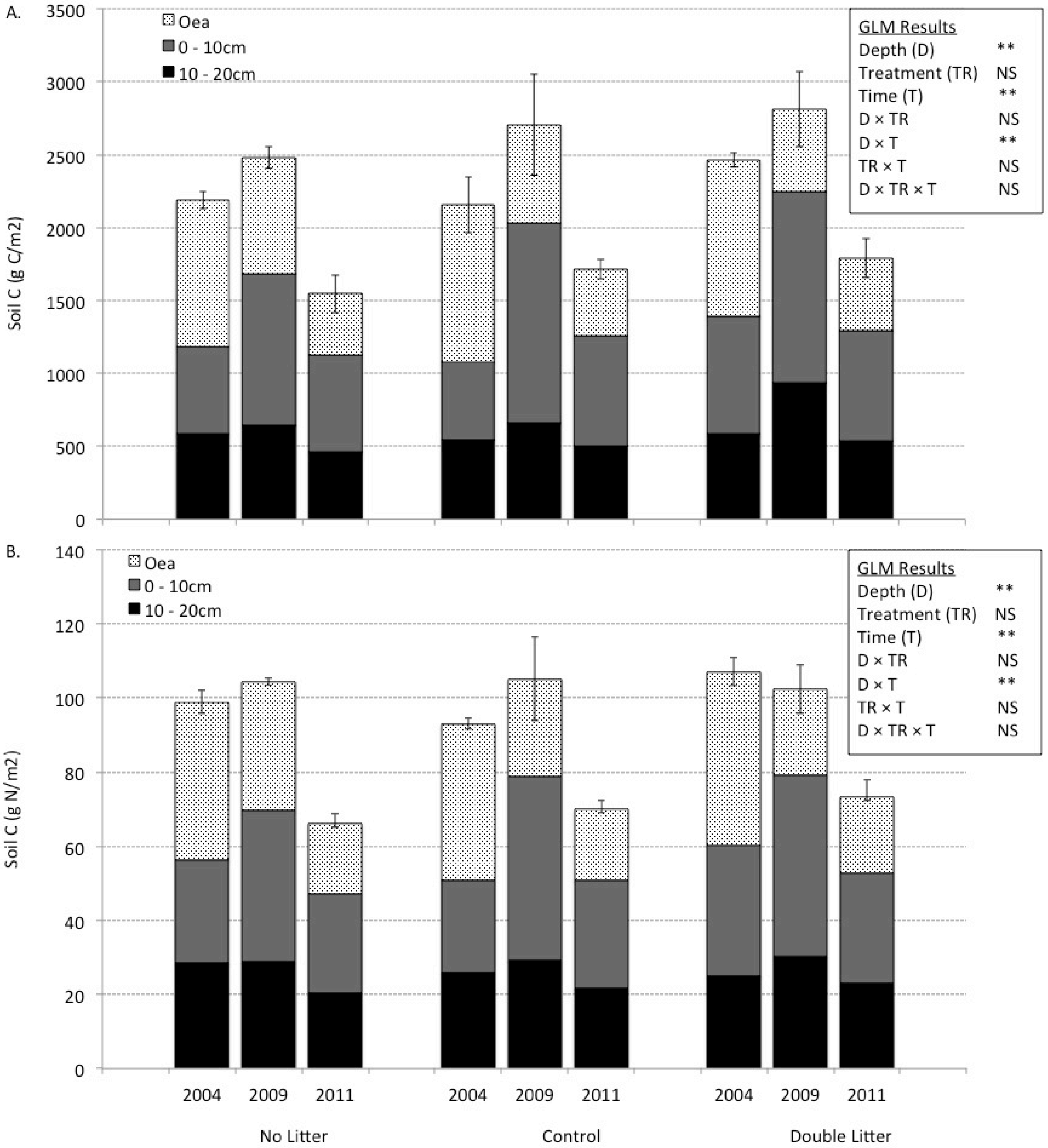

Figure 5.4: A) Soil C pool (g C/m^2) and B) N pool (g N/m^2) across DIRT plots, clustered by year (2004 to 2011), depth increment (A-horizon, 0 – 10 cm, and 10 – 20cm), and leaf litter manipulation treatment (No Litter, Control, Double Litter). Statistical outcomes evaluating main and interactive effects of soil depth increment (D), treatment (TR), and time (T) of the general linear model with repeated measures are given as an inset in each figure (** $P < 0.05$, NS = $P > 0.05$, also see Table A3). Error bars represent the mean ± 1 standard error of total soil C and N pools, respectively.

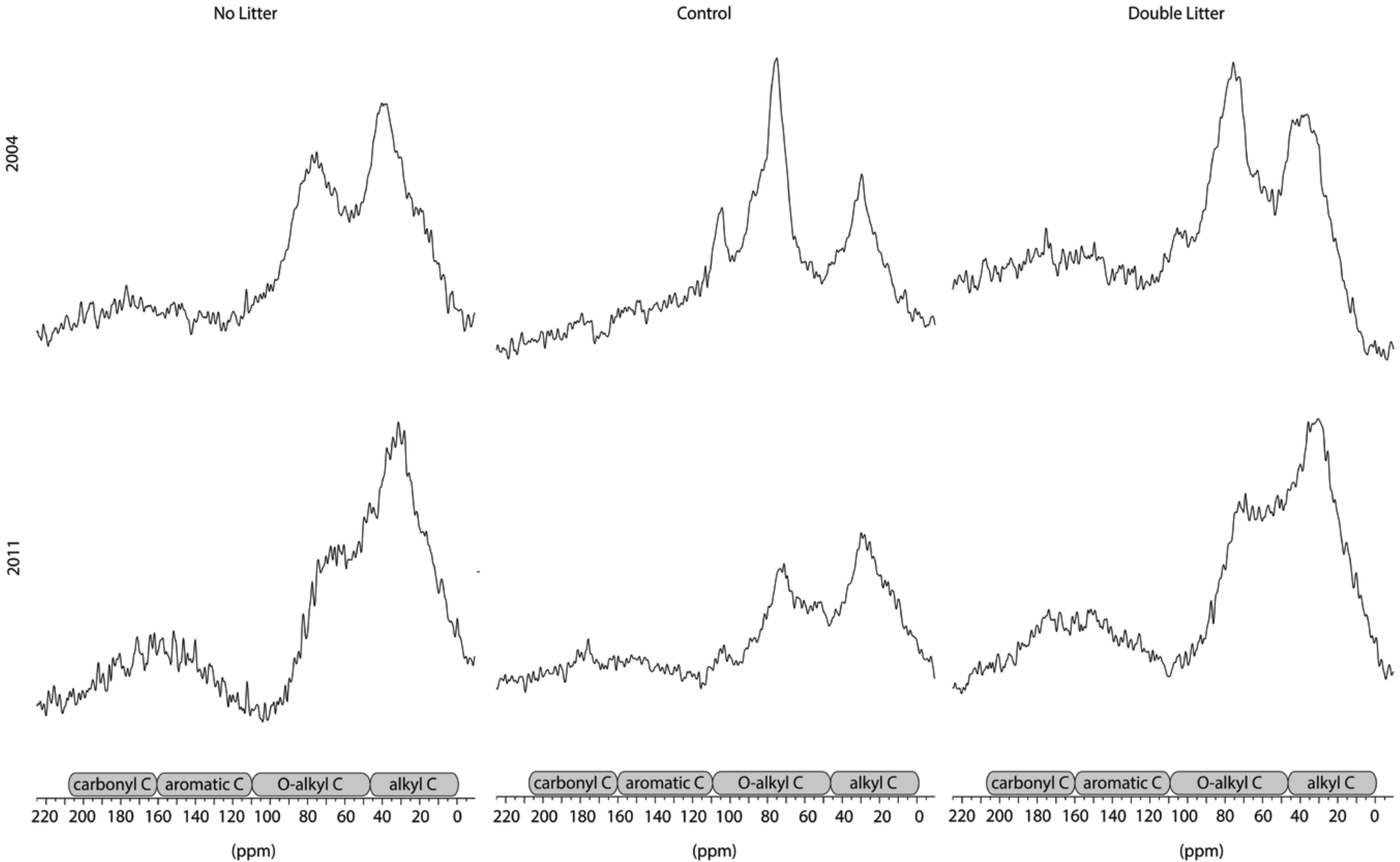

Figure 5.5: Solid-state CP-MAS ^{13}C NMR spectra of A-horizon soils from leaf litter manipulation treatments (No Litter, Control, Double Litter) in 2004 and 2011. Carbon contained in chemical structures in order of decreasing recalcitrance is differentiated on the basis of chemical shift values (i.e. carboxyl C = 200–160 ppm, aromatic C = 160–110 ppm, O-alkyl C = 110–60 ppm, and alkyl C = 45–0 ppm).

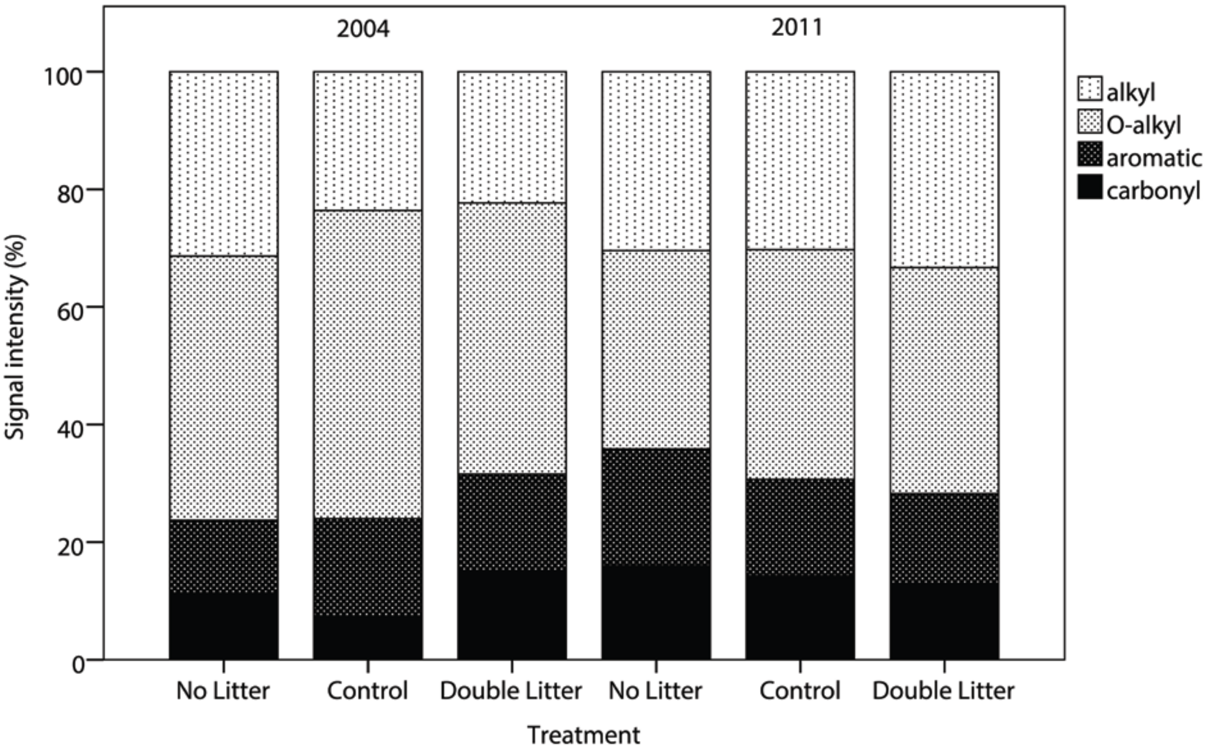

Figure 5.6: Relative mean proportion in percent of total signal intensity of alkyl C, O-alkyl C, aromatic C and carbonyl C derived from ^{13}C CPMAS NMR spectra of A-horizon soils.

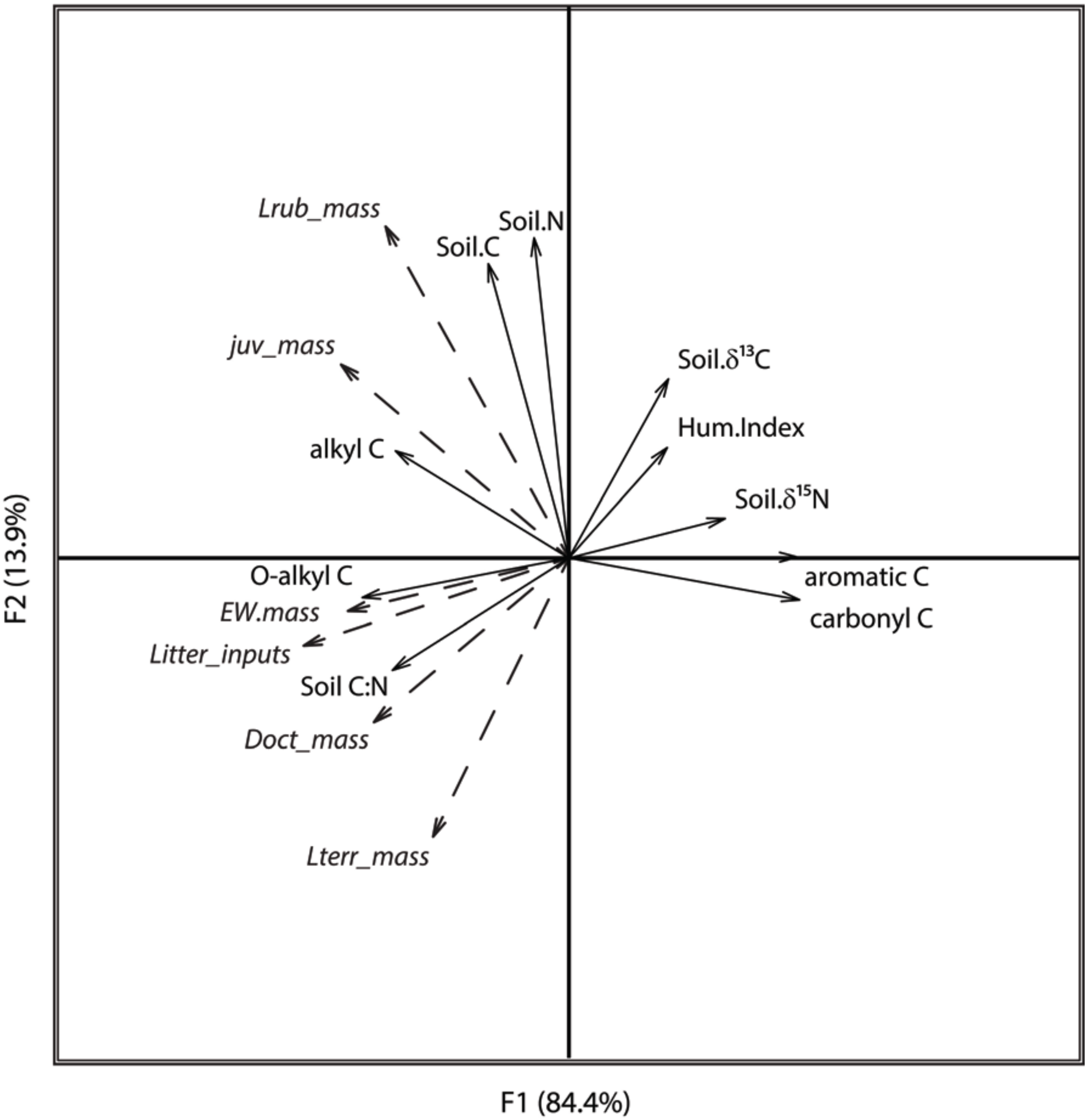

Figure 5.7: Relationships between leaf litter inputs, earthworm species biomass (dashed vectors, italicized text), and soil chemical properties (solid vectors, plain text) determined by co-inertia analysis. Eigenvalues corresponding to the first two co-inertia axes are equal to 9.72 and 1.60. The % of total inertia explained by the first (F1) and second (F2) co-inertia axes are given in parentheses, and axis scales are given in bottom right corner of the plot. Earthworm biomass measures include *L. terrestris* (*Lterr_mass*), *L. rubellus* (*Lrub_mass*), *D. octaedra* (*Doct_mass*), and total earthworm biomass (*EW.mass*). Manipulated leaf litter inputs across treatment plots are shown as *Litter_inputs*. A-horizon chemical properties include carbon content (Soil.C), nitrogen content (Soil.N), C:N, δ^{13}C, δ^{15}N, and the mean proportion in percent of total signal intensity of alkyl C, O-alkyl C, aromatic C and carbonyl C derived from ^{13}C CPMAS NMR spectra of surface soils. The humification index (Hum.Index) is calculated as the ratio of O-alkyl to alkyl C intensities.

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Chapter 6

Conclusions and Implications

This dissertation research focuses on exotic earthworm introductions as drivers of soil C dynamics in North American temperate forests, with two overall goals:

a) Establish fundamental baseline data (in the form of soil C budgets) to compare earthworm community impacts on soil C content;
b) Link variations in the magnitude and direction of change in soil C processes to the functional diversity of earthworm communities, soil properties, and interactions between these two factors.

To accomplish these goals, I integrated field surveys, laboratory experiments, and historical data to describe relationships between earthworm species distributions and environmental factors (e.g., leaf litter inputs, soil physical and chemical properties), and to determine community specific impacts on all major components of soil C budgets. Tools from community ecology, ecosystem ecology, and soil ecology used in this dissertation research included multivariate ordination techniques, elemental and isotopic mass balance, soil macrostructure imaging by X-ray computed tomography, and characterization of soil organic matter molecular structure by ^{13}C CPMAS NMR. Using two laboratory experiments, I applied elemental and isotopic mass balance to quantify the impacts of functionally diverse earthworm communities on total soil C storage and C redistribution through measurements of: (1) cumulative C losses from earthworm mesocosms as CO_2 and DOC, (2) leaf litter mass losses (3) changes in soil C pool sizes (4) and the redistribution of isotopically-enriched leaf litter into soil pools. In the second experiment, I also characterized soil texture controls on soil C processes and differences in earthworm community impacts on soil C budgets. Surveys of earthworm species densities and community composition were conducted within study areas of previously established long-term field experiments, and were used in conjunction with historical data (Murchie 1954, 1956, Nielsen and Hole 1963, 1964) to improve our understanding of earthworm species responses and effects on soil carbon dynamics across northern temperate forest landscapes. Below I summarize the main conclusions from my four primary chapters and discuss implications for future research.

Main Findings

1. Soil-dwelling earthworm communities remain dominated by exotic species; shifts in earthworm community composition in upland forest soils have occurred over 60 years. Present-day earthworm communities in my study region are dominated by five exotic species also present in the early to mid-1900s, though shifts in earthworm community composition of upland forest soils, particularly introductions of *Lumbricus terrestris* have occurred more recently.

2. Soil nutrients, leaf litter inputs, and roads are key drivers of earthworm species distributions. Spatial variations in earthworm species densities and community composition are attributed to species-specific responses to environmental factors: soil moisture and texture were key drivers of earthworm species abundance in historical surveys, though associations with soil C were only evident for *Aporrectodea* spp. Contemporary associations between earthworm species and soil C and N content suggest greater nutrient limitation in upland forest soils, while the importance of plot-to-road distance suggests the persistence of dispersal limitation and repeated introductions as a mechanism maintaining population densities.

3. Earthworm species differences in resource use and burrowing behaviors lead to community-specific effects on leaf litter degradation and organic matter redistribution. Anecic species accelerate litter C mass loss by 30 – 40% with differential mass loss of litter types (*A. rubrum* > *P. grandidentata* > *F. grandifolia* > *Q. rubra* ≥ *P. strobus*) indicative of leaf litter preference. Isotopic tracers used to determine leaf-litter derived organic matter redistribution into forest soils, showed A-horizons were dominant sinks for leaf litter C and N, with ^{13}C and ^{15}N recoveries significantly higher in soils containing both endogeic and anecic species (30 – 40%). Earthworm communities containing endogeic species also increased burrow soil ^{13}C and ^{15}N recovery by 10 – 15% relative to epigeic populations of equal biomass.
   .

4. Organic matter redistribution is controlled by the production and use of dense burrow networks in the A-horizon and vertical burrows extending into the B-horizon by earthworm

species. Sub–surface burrow system structure (volume, continuity, size distribution) controls vertical redistribution of litter–derived organic material into the A-horizon as indicated by strong correlations between (1) sub-surface 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.. Burrow systems are associated with CO_2 and DOC losses during initial burrow system production and in the months immediately following the onset of earthworm activity; relationships between burrow systems structure and C losses are not observed after soils have over-wintered and earthworm activity has declined.

5. Earthworm effects on soil C losses are largest when earthworms are first introduced to soils; the magnitude of effects on C losses depends on earthworm community composition. In the first mesocosm experiment, 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 mono-specific 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.

6. Soil-texture controls temporal trajectories of C losses following earthworm community additions and transfer of leaf litter-derived organic matter into earthworm burrow systems. In the second mesocosm experiment, we observed a difference in the onset of earthworm community-enhanced CO_2 release, with fine-textured soils showing a longer temporal lag prior to maximum respiration than coarse-textured soils. Isotopic tracers showed that A-horizons were dominant sinks for leaf litter C and N, with ^{13}C and ^{15}N recoveries significantly higher in fine-textured soil (50 – 85%) and in coarse-textured soil containing both endogeic and anecic species (30 – 40%).

7. Earthworm community activity results in only modest shifts in net soil C storage in the short-term; shifts in long-term trajectories of soil C storage may take longer to observe in coarse-textured soils. Field-based studies assessing earthworm impacts on soil C stocks of fine-textured soils generally show significant declines in soil C stocks within 1 – 5 years (e.g., Nielsen and Hole 1964, Alban and Berry 1994, Bohlen et al. 2004b, Fahey et al.

2013b). Comparable measurements in coarse-textured soils, show no change in C content after seven years. The importance of short-term changes in soil C chemistry associated with earthworm community activity on turnover time of soil C pools remains unknown.

Future Directions

The capacity of individual forest ecosystems to sequester atmospheric carbon dioxide (CO_2) varies considerably. Exotic earthworm introductions represent only one of a number of factors determining forest soil C storage. Also important are factors including plant composition, soil type, climate (i.e. precipitation, temperature, solar radiation), and other external drivers including atmospheric CO_2 enrichment (Finzi et al. 2001, Beedlow et al. 2004, Hoosbeek and Scarascia-Mugnozza 2009, Talhelm et al. 2012), land use change (Houghton et al. 1999, Goodale and Aber 2001, Groffman et al. 2006, Nave et al. 2010), and nitrogen (N) deposition (Nadelhoffer et al. 1999, Bowden et al. 2004, Waldrop et al. 2004, Zak 2010). Tree species and leaf litter chemistry effects (e.g., C:N) on soil properties and interactions with earthworm communities (Scheu 1997, McInerney and Bolger 2000, Reich et al. 2005, Melvin and Goodale 2013); and the role of land use history (Lee 1985, Bohlen et al. 2004b, Ma et al. 2013) have been studied extensively. Interactions between earthworm communities and the remaining controls have received less attention, and though understanding these interactions are critical to improving our ability to predict changes in forest carbon storage capacity across space and time.

This dissertation research answers previously unresolved questions concerning net changes in soil carbon budgets following exotic earthworm introductions, and community-specific impacts on soil carbon processes. Importantly, the establishment of fundamental baseline data (in the form of soil C budgets) to compare earthworm community impacts on soil C content, and evaluation of earthworm species distribution following regional spread across the landscape, will contribute to the growing literature on biological invasions in north temperate forests of the Midwestern and Northeastern U.S., and will advance our general knowledge of exotic earthworm invasions and their impacts.

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APPENDICES

Appendix A: Earthworm species and associated ecological groups present at the University of Michigan Biological Station (Pellston, MI).

Table A1: Ecological groups of earthworm (EW) species and associated ecological behaviors (Bouché 1978, Lavelle 1983, 1988).

Functional Group	Physiology	Foraging Activity	Burrowing Patterns	EW Species
Epigeic	1-7cm in length, dark-colored; parthenogenic reproduction	bacteria and fungi in forest litter; particulate organic matter; surface cast production	soil-litter interface; ephemeral burrows;	*Dendrobaena octaedra, Lumbricus rubellus*
Endogeic	2-12cm in length; lightly pigmented or unpigmented	mineral soil and associated organic matter; subsurface cast production	semi-permanent networks of burrows in upper mineral soils	*Aporrectodea caliginosa, Aporrectodea trapezoides*
Anecic	8-15 cm in length; dark anterior pigmentation;	surface organic matter; surface and subsurface cast production	vertical, unbranching burrows 1-2m deep	*Lumbricus terrestris*

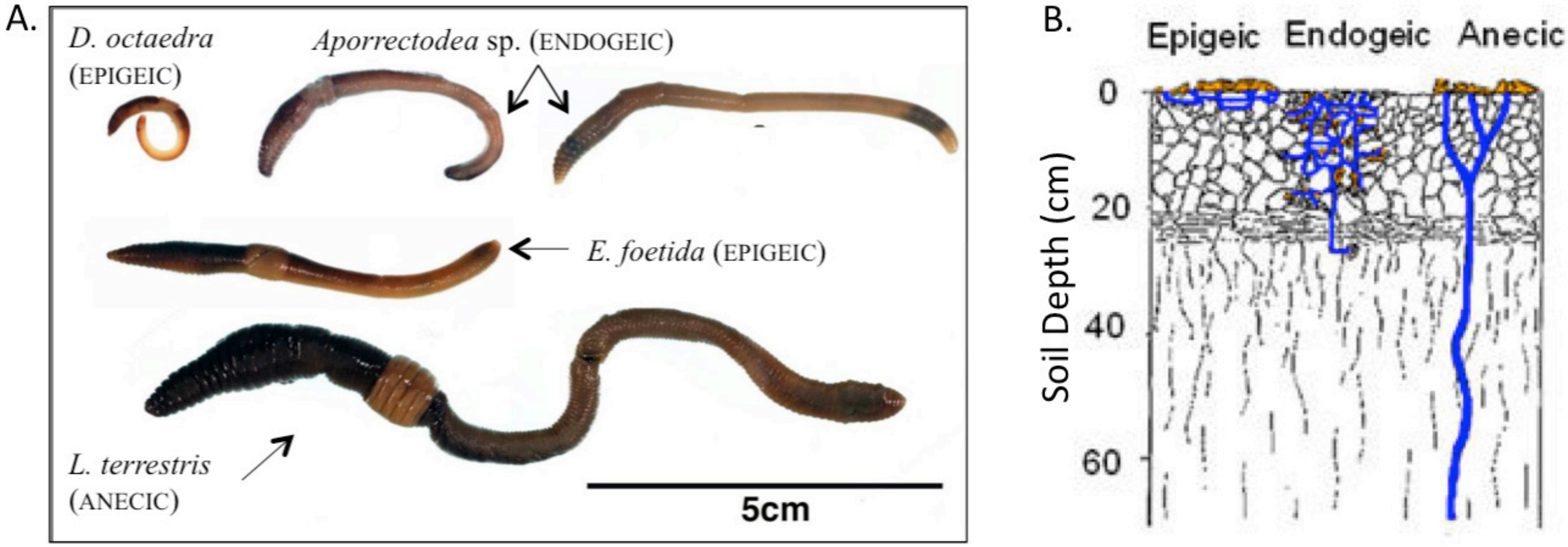

Figure A1: [A] Earthworm species present at the University of Michigan Biological Station with ecological group assignments given in parentheses. Earthworm species include *Dendrobaena octaedra* (Savigny), *Lumbricus rubellus* (Hoffmeister), *Lumbricus terrestris* (Linneus), and *Aporrectodea caliginosa* (Savigny), and *Aporrectodea trapezoides* (Dugès). Photo credit: David Bay, University of Michigan [B] Burrowing patterns of earthworm species assigned to different ecological groups (figure modified from Fraser and Boag 1998); associated physiology and foraging activities are provided in Table A1.

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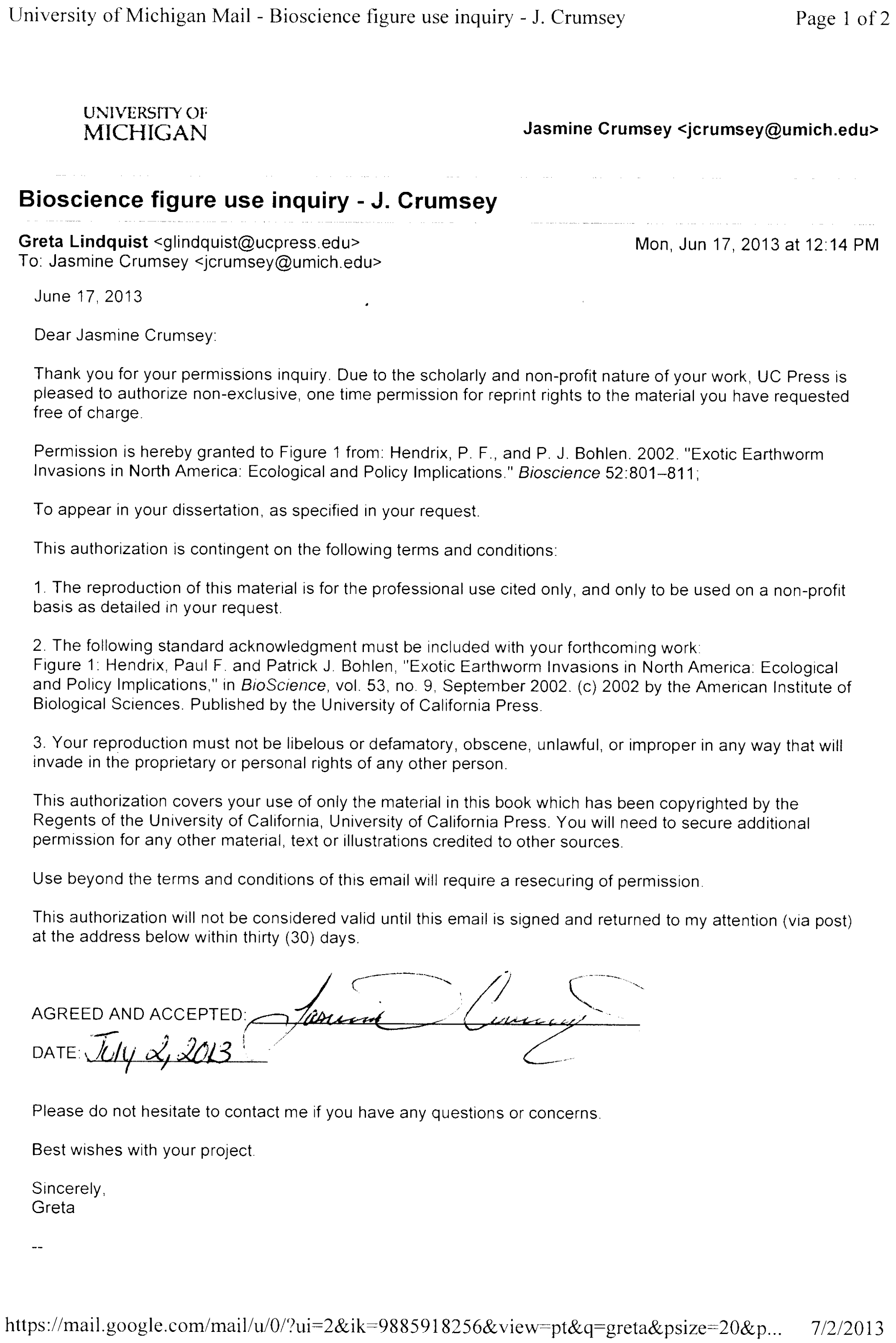

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