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Manipulating earthworm abundance using electroshocking in deciduous forests

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ABSTRACT

Earthworms influence the biotic and abiotic characteristics of soils, but studying these effects in situ is challenging. Secondary forests in the Mid-Atlantic have abundant earthworm communities. To investigate the interaction of earthworms with the below- and aboveground part of the ecosystem, we manipulated earthworm densities in 1 m^2 enclosures located at 12 study sites within four different-aged forest stands at the Smithsonian Environmental Research Center (SERC) in Maryland, USA. The treatment plots were created by trenching around the perimeter and lining the trenches with fiberglass mesh before backfilling. Two types of untrenched plots served as control and leaf litter treatment plots. Enclosures were electroshocked between four and nine times over a two-year period to remove earthworms and to compare densities among treatment and untrenched plots. Earthworms were weighed and identified to determine whether removal by electroshocking varied depending on body size or ecological grouping. Earthworm abundances were 30–50% lower in reduced-density enclosures than in high density enclosures; however, the efficiency of the exclusion treatments varied by earthworm size and ecological group. Manipulating earthworm populations in temperate forests to assess their influence on ecological functions is feasible using electroshocking, but careful planning is essential given the amount of effort required to set up and maintain the desired experimental conditions.

Introduction

Non-native earthworms occur throughout North America as a result of human-mediated habitat disturbances, deliberate introductions during early European settlements, and accidental introductions resulting from recreational fishing and the transport of soil in plant pots and as ballast material (Lindroth 1957; Bohlen et al. 2004a; Hendrix et al. 2008). Their presence is especially apparent in previously glaciated regions, where re-colonization by native species has been slow, making it possible to detect fronts of invasion. Researchers have observed drastic changes in recently invaded forest soils, such as the elimination of the O horizon, altered mycorrhizal associations, and decreases in herbaceous plant diversity, tree seedling density, fine root biomass, and available C, N, and P as a result of increased leaching and gas fluxes (Bohlen et al. 2004a,b; Fisk et al. 2004; Hale et al. 2005, 2006; Frelich et al. 2006).

In non-glaciated regions, where both native and non-native species can occur (Hendrix et al. 2006), investigators often do not have the benefit of conducting field studies within soils lacking earthworms. In such cases, researchers often resort to laboratory mesocosm studies or, less frequently, to field manipulations intended to reduce earthworm populations as part of experimental designs analogous to exclusion studies of other organisms (e.g. birds, deer, and insect pollinators). Extracting earthworms and preventing their immigration back into an enclosure present considerable challenges. The most effective means of extraction – hand-sorting and chemical irritants (i.e. formalin or hot mustard) – have been used widely in earthworm community surveys; however, the intensity of labor required is impractical for large-scale manipulations with high levels of replication. Moreover, the unavoidable physical and chemical disturbances raise concerns regarding the inferences to undisturbed soils.

An electroshocking method of extraction (Doeksen 1950; Satchell 1955; Thielemann 1986) causes minimal physical disturbances to soil within sampling areas. Evaluations of its effectiveness have compared favorably to hand-sorting and chemical extraction (Rushton and Luff 1984; Schmidt 2001; Staddon et al. 2003; Čoja et al. 2008; Weyers et al. 2008) and there are no indications that it causes long-term negative effects on other soil fauna, plant roots, or microbial populations (Blair et al. 1995; Staddon et al. 2003; Čoja et al. 2008).

In several cases, this method has been used to create reduced earthworm density conditions in the field by means of repeated electroshocking extractions and exclusion using impenetrable

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barriers (i.e. plastic sheeting). Studies performed in agroecosystems and grasslands have reported a wide range of success (Bohlen et al. 1995, 75% density reduction in 20 m^2 enclosures; Petersen 2000, 33% reduction in 12 m^2 enclosures; Eisenhauer et al. 2008a, 40% reduction in *Lumbricus terrestris* activity in 1 m^2 enclosures). Among these, only one study (Eisenhauer et al. 2008a) had a relatively high level of replication ($n = 46$), and it was replicated within subplots at the same grassland site.

Earthworms are the most important soil invertebrate group in temperate forests affecting both below- and aboveground components of the ecosystem. Creating reduced earthworm density field manipulations is an important tool to understand these effects, especially in temperate regions where forests lacking earthworms are rare. Repeating the designs of past studies presents considerable challenges in these forests because of the tremendous effort involved in hauling equipment to remote locations and digging through numerous root and rock obstructions. In order to make inferences on an ecosystem scale, treatments have to be replicated at multiple locations within different habitat types – a level of effort that has not been attempted in the past.

In 2008, we set up a large-scale manipulation to investigate direct and indirect effects of different densities of earthworms on soil biochemical processes and interactions between mycorrhizal fungi and tree seedlings (Szlavecz et al. 2011). Earthworm manipulation plots were set up in forest stands of different successional stages and additional treatments included manipulations of the amount and type of leaf litter in the plots and plantings of tree seedlings that formed associations with different types of mycorrhizal fungi. In this paper we evaluate the effectiveness of these earthworm manipulations over a three-year period. We assessed (1) whether we were successful in creating and maintaining plots with low and high earthworm densities and (2) whether the community composition and age structure of the earthworm community changed under high and low density conditions. Finally, because the experiment required an enormous amount of time and effort, we were interested in the labor costs in terms of time and person power. The practicality of such a study, measured in terms of labor hours, is as useful to scientists as some measure of the degree to which earthworm densities can be reduced in these habitats.

Materials and methods

Study sites

The research was performed at the Smithsonian Environmental Research Center (SERC), which is located on the western shore of the Chesapeake Bay in Edgewater, MD, USA (38°53′N, 76°33′W; see Fig. 1 in Szlavecz et al. 2011 for map). Prior to its establishment as a research station in the 1960s, SERC property had been used for various agricultural practices that were abandoned at different times. As a result, the property comprises a patchwork of forests in different successional stages (Higman 1968). We chose study sites within two mature forest stands that were approximately 120–150 years old (herein referred to as "mature forests") and were ∼500 m apart. A second set of plots were placed in two forest stands that were ∼800 m apart and were approximately 50–70 years old (herein referred to as "successional forests").

The mature forests, hereafter referred to as "mature 1" and "mature 2", were dominated by tulip poplar (*Liriodendron tulipifera*), oak (mostly *Quercus alba*, *Q. falcata* and *Q. velutina*), hickory (*Carya glabra* and *C. tomentosa*) and American beech (*Fagus grandifolia*). The two successional stands, hereafter referred to as "successional 1" and "successional 2", were dominated by tulip poplar, sweet gum (*Liquidambar styraciflua*), red maple (*Acer rubrum*), black cherry (*Prunus serotina*), and

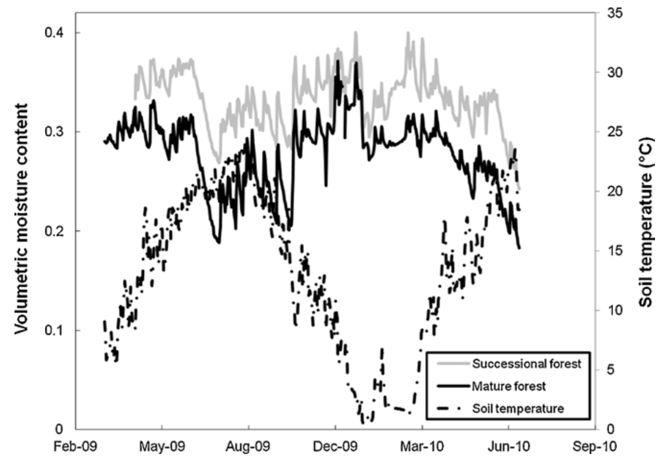

Fig. 1. Mean soil moisture (solid lines) within successional and mature forests, and mean soil temperature (dashed line) within both forest types measured using a wireless sensor network between April 2009 and July 2010. Sensors were placed at 5 cm depth.

box elder maple (*Acer negundo*). Soils at the two mature forest sites were classified as Collington sandy loam (fine-loamy mixed, active, mesic Typic Hapludult); whereas soils in the two successional stands were classified as Monmouth fine sandy loam (fine, mixed, active, mesic Typic Hapludult) (Soil Survey Staff; http://soils.usda.gov/technical/classification/osd/index.html, accessed October 2011). Average soil pH (measured in water) was 5.4 in the two mature forest stands and 5.7 in the two successional forest stands (Szlavecz and Csuzdi 2007).

SERC has a temperate climate with a mean annual temperature of 13.1 °C and mean annual precipitation of 108 cm. Monthly average temperatures range from 1.3 °C in January to 25.3 °C in July. Precipitation is relatively evenly distributed over the year with monthly average precipitation ranging from 6.9 to 11.1 cm (temperature and precipitation measured at Baltimore–Washington airport from 1987 to 2006 were obtained from the National Climatic Data Center [http://www.ncdc.noaa.gov/, accessed October 2011]). We used a wireless sensor network (Szlavecz et al. 2006; Terzis et al. 2010) to continuously monitor soil temperature and soil moisture from April 2009 until July 2010. Soils in the two successional forest stands tended to be wetter but soil temperature was very similar between the forests during the monitoring period (Fig. 1).

The composition of the earthworm communities in SERC forests has been previously described (Szlavecz and Csuzdi 2007; Crow et al. 2009; Szlavecz et al. 2011) and the information is summarized in Table 1 which was modified from Szlavecz et al. (2011). *Eisenoides loennbergi* is the only species native to North America found at our study sites. It occurred in one of the mature forest stands. *Lumbricus friendi*, a European species that was first reported in North America about a decade ago (Csuzdi and Szlavecz 2003), is the only deep-burrowing anecic earthworm at SERC; whereas *Lumbricus rubellus* is the dominant leaf litter-dwelling epigeic species found within the interior of SERC forests. Endogeic species residing in the mineral soil are more diverse than the other ecological groups at SERC and the relative abundance of each species is site-specific; however, *Aporrectodea caliginosa* is frequently the most abundant endogeic species within successional forest stands at SERC (Szlavecz and Csuzdi 2007).

Earthworm enclosures

In each of the four stands, we located three replicate sites and each site had four 1 m^2 earthworm manipulation enclosures

Table 1
Presence of earthworm species and their ecological grouping (according to Bouché 1977) and within SERC forest study sites (mature 1, 2; successional 1, 2).

Species	Ecological group	Present
Lumbricus friendi (Cognetti, 1904)	anecic	mature 1, 2; successional 1, 2
Allolobophora chlorotica (Savigny, 1826)	endogeic	successional 1
Aporrectodea caliginosa (Savigny, 1826)	endogeic	mature 1, 2; successional 1, 2
Aporrectodea rosea (Savigny, 1826)	endogeic	successional 1, 2
Eisenoides loennbergi (Michaelsen, 1894)	endogeic	mature 1
Octolasion cyaneum (Savigny, 1826)	endogeic	mature 1, 2; successional 1, 2
Octolasion lacteum (Örley, 1881)	endogeic	successional 1, 2
Lumbricus rubellus (Hoffmeister, 1843)	epigeic	mature 1, 2; successional 1, 2

Table modified from Szlavecz et al. (2011).

(described in detail below) and additional 1 m^2 plots where earthworm density was not manipulated. The maximum distance separating replicate sites was 65 m. In the spring of 2008, we installed the earthworm manipulation enclosures using a gas-powered trencher to dig 80 cm deep trenches around the perimeter of each enclosure. We lined the trenches with 1 mm mesh fiberglass screen to restrict subsurface movement of earthworms while enabling the soils in the trenched plots to be colonized by fungi that could establish mycorrhizal connections with the tree seedlings that were planted inside the plots. While backfilling, we also installed 12.7 cm high plastic commercial garden edging (Suncast Professional Landscape Edging®) stapled to aluminum window screening that protruded ~30 cm above the soil surface to provide additional barriers to below- or aboveground earthworm movements.

Each of the trenched enclosures was randomly assigned to a "high-density" or a "reduced-density" earthworm treatment so that each site consisted of two replicates of each treatment. Our goals were to reduce earthworm density and biomass as much as possible within reduced-density enclosures; whereas, within high-density enclosures, we aimed to maintain population levels that were close to the upper range (~200 individuals m^{-2} and 80 g m^{-2}) of those observed at SERC during previous surveys (Szlavecz and Csuzdi 2007). This experimental setup had no control for the effect of trenching. However, we established two untrenched plots for the leaf litter treatments as described below.

To investigate the effect of earthworms on mycorrhizal fungi–tree seedling interactions under different litter sources, we divided each enclosure in half using garden edging. One half received tulip poplar litter while the other half received American beech leaf litter. One of the two untrenched plots received the same leaf litter treatment; these plots were set up only in mature 2 and successional 1 stands. The other untrenched plot received natural litter input, and served as a control for other biogeochemical measurements such as soil respiration, nitrogen mineralization, and enzyme activity. These plots were established in all four forest stands. In total, we established 48 trenched and 18 untrenched plots (see Supplemental material for photograph of experimental plots).

Electroshocking was used both as a means to remove earthworms from the reduced-density enclosures and to regularly monitor density and biomass within all enclosures and the untrenched plots. We permanently installed eight aluminum rods to depth of 40 cm in a 2 × 4 arrangement in each of the sixty-six 1 m^2 plots. To electroshock an enclosure, we used eight alligator clips connected to the aluminum rods to induce 110/120 V of alternating current, supplied by a portable 110/120 V gasoline generator. By changing the arrangement of the positive and negative leads (corresponding to positive and negative phases of the alternating current) connected to the rods, we were able to adjust the orientation of the electric field and maximize extraction earthworms over a period of approximately 45 min. In addition to the aluminum rods, we also used copper rods that were moved around the plot to obtain complete coverage of each plot during each electroshocking period. Prior to electroshocking, leaf litter was removed from each plot to increase visibility of emerging earthworms. Leaf litter was replaced after the electroshocking period.

After construction of all enclosures was completed, we electroshocked the 24 reduced-density enclosures five times between June and October of 2008 and three times between June and August of 2009. These earthworms were not returned to the plots. In addition, four complete electroshocking campaigns, including all 66 plots, were carried out between the fall of 2008 and summer of 2010. The objectives of these electroshocking campaigns were (1) to estimate density and biomass, (2) to evaluate the effectiveness of the manipulation treatments, and (3) to make adjustments, as necessary. During these campaigns, earthworms were identified, counted, and weighed in the field then returned to high density enclosures and unmanipulated plots. Earthworms from reduced-density enclosures were either discarded or added to high-density enclosures that fell below our density or biomass benchmarks. Occasionally, additional earthworms were collected from the surrounding area to supplement additions to high-density enclosures. No species was introduced to an enclosure it did not occur in previously. Earthworms identified to species were *Allolobophora chlorotica*, *Ap. caliginosa*, *Aporrectodea rosea*, *E. loennbergi*, *L. friendi*, and *L. rubellus*. Individuals from the genus *Octolasion* were grouped together because it was difficult for inexperienced observers to differentiate between the two co-occurring species (*O. cyaneum* and *O. lacteum*). Immature earthworms belonging to the genus *Lumbricus* were grouped into their own category; whereas, very small immature earthworms (~0.01 g) that could not be reliably identified even to genus level were grouped together as "small Lumbricidae".

As with all earthworm sampling methods, electroshocking has less than 100% extraction efficiency (Rushton and Luff 1984; Schmidt 2001; Čoja et al. 2008; Weyers et al. 2008). Therefore, densities reported throughout this study reflect relative differences between treatments.

Statistical analyses

Our study design contained a number of complicating factors that prevented us from using a simple general linear model to test for differences in density or biomass among earthworm treatments. These factors included repeated measurements on the same experimental units that occurred during different times of the year, the potential for error variances to be correlated in space such that densities and biomasses might be more similar within sites or forests, and an unbalanced design as untrenched leaf treatment plots were located only at sites within mature 2 and successional 1. Mixed effects linear models have been shown to be robust at handling ecological data collected at various scales that are complicated by unbalanced designs and concerns about the independence of observations (Bolker et al. 2008) and therefore, we used this approach to test the driving questions of this study.

To test the success of manipulating overall earthworm abundance in the two density treatments, we used mixed model analyses of variance (ANOVAs) with a repeated-measures design that treated individual enclosures as subjects (R package lme4). Time (i.e. each electroshocking campaign), forest, site, and enclosure were considered random effects, and earthworm manipulation treatment was included as a fixed effect in addition to a fixed intercept. We tested for the significance ($\alpha = 0.05$) of the earthworm manipulation treatment using likelihood ratio tests (LRTs) comparing a full model containing all random and fixed effects to a reduced model lacking the fixed earthworm manipulation effect (Bolker et al. 2008). Tukey's pairwise comparisons (R package multicomp) were used to determine which earthworm treatments had different densities and biomass. Although we were primarily interested in the differences between the reduced and high-density enclosures, we also made comparisons to the unmanipulated, untrenched plots. This analysis informed us about natural, background fluctuations of the earthworm community and helped to determine whether trenching or leaf litter manipulation affected earthworm populations independently of electroshocking.

To test whether the effectiveness of the removal method varied among earthworm species, we divided our data by species and performed similar repeated-measures mixed model ANOVAs and LRTs to test for differences in density and biomass between reduced-density enclosures, high-density enclosures, and untrenched leaf treatment plots. Untrenched background plots were excluded from this portion of the analysis because of potentially confounding effects of different litter sources. Species included in the analysis were *Ap. caliginosa*, *L. friendi*, *L. rubellus*, *Octolasion* spp., and the immature categories, *Lumbricus* juveniles and small Lumbricidae. The other species (*A. chlorotica*, *Ap. rosea*, *E. loennbergi*) were excluded from the analysis either because they occurred at low densities or were only present at a small number of sites. We used an α level of 0.01 based on the Bonferroni method to maintain a 0.05 experimentwide error rate when performing separate ANOVAs for the six earthworm categories. We inspected the results of the analysis for trends related to ecological grouping or size, which we determined by calculating the mean body mass of individuals from each species category. Maximum likelihood (ML) estimation was used for all mixed models. Diagnostic plots and Levene's tests for homogeneity indicated the residuals met the assumptions of normality and homoscedasticity.

To determine whether repeated removal by electroshocking had altered earthworm community composition, we used relative densities and relative biomasses of the five most common earthworms (*A. chlorotica*, *Ap. caliginosa*, *L. friendi*, *L. rubellus*, and *Octolasion* spp.) and two immature categories (*Lumbricus* juveniles and small Lumbricidae) within reduced-density enclosures, high-density enclosures, and untrenched leaf treatment plots (mature 2 and successional 1 forests only). Average relative densities and biomasses were calculated for each enclosure treatment within each forest for spring and autumn electroshocking campaigns and we used these in separate non-metric multidimensional scaling (NMDS) ordinations (R package vegan) to examine differences in community composition. Bray–Curtis distance matrices were used to rank differences in community composition among our season-forest enclosure means. We tested the hypotheses that relative density and biomass differed by season, forest, and enclosure type using an analysis of similarity (ANOSIM, Clarke 1993) based on 1000 randomizations. Weighted species scores were used to identify patterns related to changes in relative abundance or biomass of a species. All analyses were performed using program R (R Development Core Team 2011; http://www.R-project.org).

Results

Reductions in density and biomass within reduced-density enclosures

Total density (LRT: $\chi^2 = 45.8$, df = 3, $P < 0.01$) and biomass (LRT: $\chi^2 = 84.1$, df = 3, $P < 0.01$) of earthworms differed among the enclosure manipulations (Fig. 2). Pairwise comparisons indicated that reduced-density enclosures had lower densities and biomass than all other treatments ($P < 0.05$); whereas, untrenched background plots had higher biomass than all other treatments ($P < 0.05$).

Overall mean density and biomass (averaged over the four full shocking campaigns) within reduced-density enclosures was 61.4 ± 5.5% and 56.2 ± 2.3% of high-density enclosures in the two mature forest stands and 66.1 ± 7.1% and 58.2 ± 10.7% of high-density enclosures in the two successional forest stands. The greatest differences were achieved in the fall of 2008 (mature stands: 58.7% and 46.8%; successional stands: 48.1% and 52.9% for

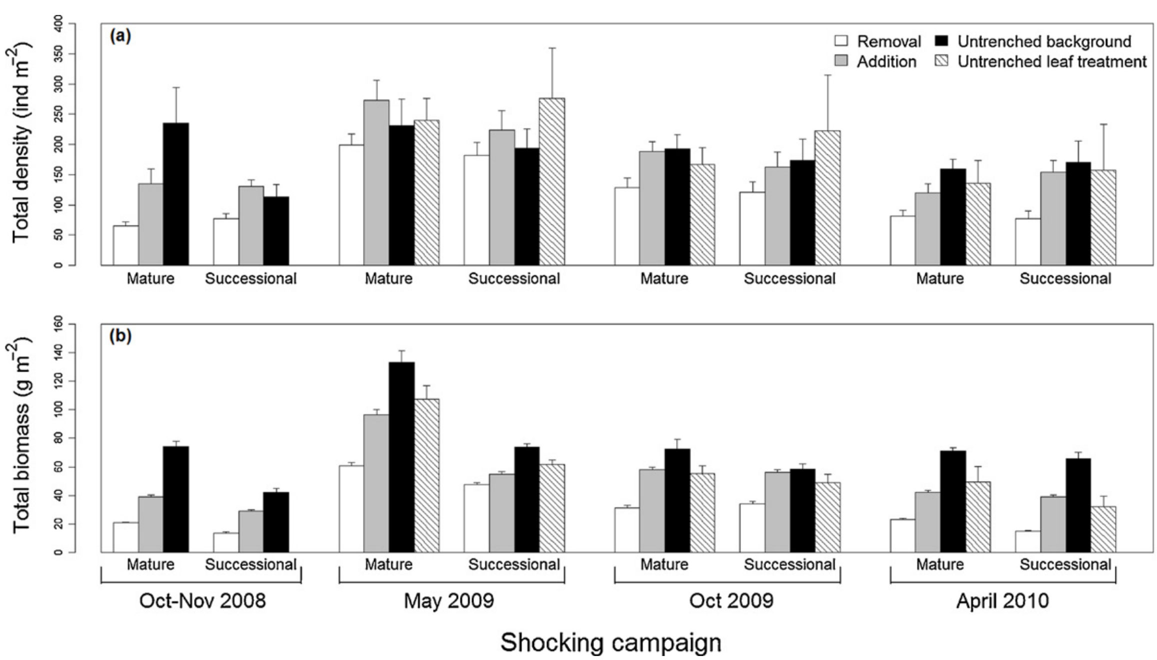

Fig. 2. (a) Mean (±SE) total earthworm density (individuals m^{-2}) in earthworm manipulation enclosures averaged within forest types and electroshocking campaigns. (b) Mean (±SE) total earthworm biomass (g m^{-2}) in earthworm manipulation enclosures averaged within forest types and electroshocking campaign. Untrenched leaf treatment plots received the same leaf litter as earthworm manipulation enclosures, but earthworm abundance was not manipulated. Untrenched background received natural, mixed leaf litter input.

density and biomass, respectively), prior to which removal electroshocking was most frequent.

Averaging all electroshocking events, the high-density enclosures had 104.8% of the density and 93.5% of the biomass of untrenched leaf treatments within the mature forests and 84.1% and 108.2% within the successional forests. Biomass within the high-density enclosures was 66.0% and 74.6% of the untrenched background plots with natural litter input, despite similarities in density between the two treatments.

Effectiveness of electroshocking in relation to ecological grouping and body size of earthworms

There were differences in density and biomass (Fig. 3) among the reduced-density, high-density, and untrenched leaf treatments for the largest-bodied species, *L. friendi* (density: LRT: $\chi^2=8.9$, df = 2, $P=0.01$; biomass: LRT: $\chi^2=8.4$, df = 2, $P<0.01$), and endogeic species *Ap. caliginosa* (density: LRT: $\chi^2=26.1$, df = 2, $P<0.01$; biomass: LRT: $\chi^2=26.0$, df = 2, $P<0.01$) and *Octolasion* spp. (density: LRT: $\chi^2=7.7$, df = 2, $P=0.02$; biomass: LRT: $\chi^2=16.6$, df = 2, $P<0.01$). Pairwise comparisons indicated that abundances of these species were lowest in reduced-density enclosures ($P<0.05$) and that differences between high-density enclosures and untrenched leaf treatment plots were not significant ($P>0.05$). Density of *Lumbricus* juveniles differed among the treatments (LRT: $\chi^2=12.4$, df = 2, $P<0.01$); however, pairwise comparisons indicated that the only significant difference existed between high-density and untrenched leaf treatment plots ($P<0.01$). There were no differences in biomass of *Lumbricus* juveniles (LRT: $\chi^2=3.7$, df = 2, $P=0.15$) or abundance of the epigeic *L. rubellus* (density: LRT: $\chi^2=0.8$, df = 2, $P=0.69$; biomass: LRT: $\chi^2=1.0$, df = 2, $P=0.61$), or small Lumbricidae (density: LRT: $\chi^2=1.5$, df = 2, $P=0.47$).

Changes in community composition

Analyses of similarity based on NMDS ordinations (Fig. 4) of relative density and biomass indicated that community composition differed between seasons (density: $P<0.01$; biomass: $P<0.01$) and sites (density: $P<0.01$; biomass: $P<0.01$), but not among earthworm enclosure treatments (density: $P=0.15$; biomass: $P=0.21$). Small Lumbricidae comprised a greater proportion of total density and *Lumbricus* juveniles comprised a greater proportion of total biomass during autumn electroshocking campaigns within all enclosure types. *Ap. caliginosa* was more abundant and accounted for more biomass in the successional forest (successional 1) than in the mature forest (mature 2). The density ordination (Fig. 4a)

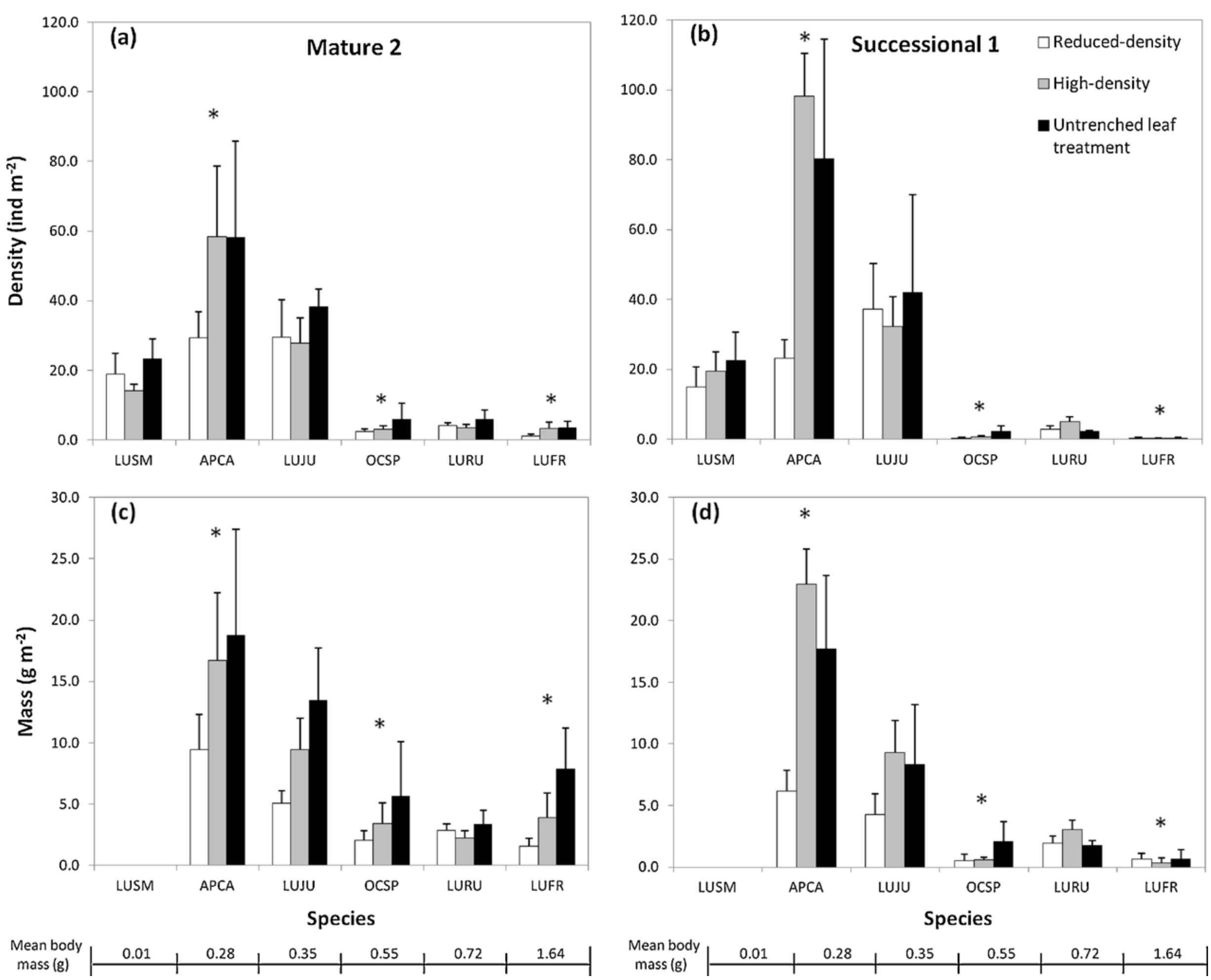

Fig. 3. Effectiveness of removal for different species and groups. Mean (±SE) density within mature 2 (a) and successional 1 sites (b) and mean biomass within mature 2 (c) and successional 1 sites (d) of most common species are compared within reduced-density, high-density, and leaf treatments during the final electroshocking campaign. Significant differences between the treatments were determined using mixed linear models and are indicated by asterisks. Species are organized by mean body mass (g) per individual as indicated by scale bar at the bottom. Species codes: LUSM, small Lumbricidae; APCA, *Aporrectodea caliginosa*; LUJU, *Lumbricus* juveniles; OCSP, *Octolasion* spp.; LURU, *Lumbricus rubellus*; LUFR, *Lumbricus friendi*. Untrenched leaf treatment plots received the same leaf litter as earthworm manipulation enclosures, but earthworm abundance was not manipulated. Untrenched background received natural, mixed leaf litter input.

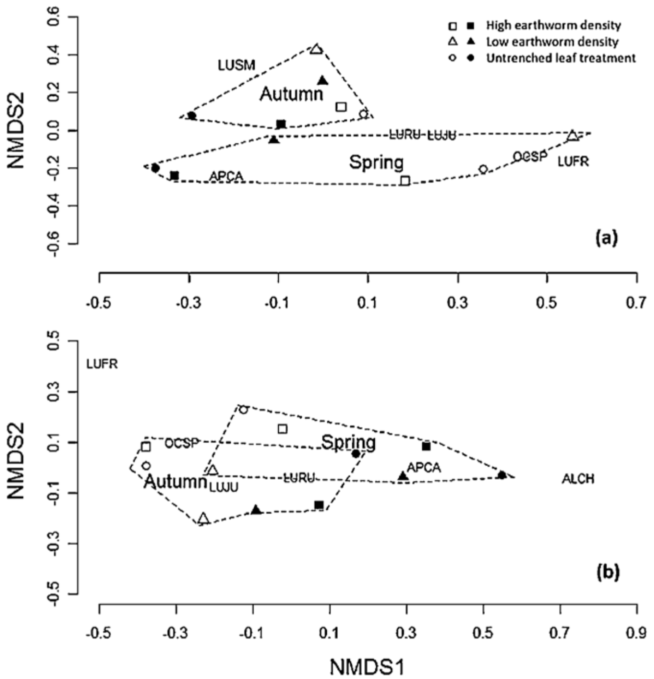

Fig. 4. (a) NMDS of earthworm community composition at mature 2 (open symbols) and successional 1 (filled symbols) based on average relative densities (proportion of total within an enclosure) of earthworm species observed during spring ($n=2$) and autumn ($n=2$) electroshocking campaigns. (b) NMDS of earthworm community composition based on average relative contributions to total biomass by each earthworm species. Weighted species scores are plotted to illustrate differences in community composition. (ALCH, *Allolobophora chlorotica*; APCA, *Aporrectodea caliginosa*; LUSM, small Lumbricidae; LUFR, *Lumbricus friendi*; LUJU, *Lumbricus* juveniles; LURU, *L. rubellus*; OCSP, *Octolasion* spp.)

indicated a weak trend for reduced relative density of *Ap. caliginosa* and an increased relative density of small Lumbricidae within reduced-density enclosures. Similarly, there was a trend for reduced *Ap. caliginosa* relative biomass, reduced *L. friendi* biomass, and increased *Lumbricus* juvenile biomass within reduced-density enclosures according to the biomass ordination (Fig. 4b).

Discussion

Manipulating earthworm abundance using electroshocking is far from being a standard method. While electroshocking has been used widely in assessing earthworm abundance, few studies have attempted to use this method to exclude earthworms in long-term experimental plots. Most of the studies were conducted in non-forested ecosystems, such as grasslands and agricultural fields (Bohlen et al. 1995; Petersen 2000; Eisenhauer et al. 2008b). Moreover, studies differed in the length of the experiment (5 months to 3 years), size and number of experimental units ($n = 4$–46), the frequency of repeated electroshockings (every three months to twice a year), and the protocol used to drive earthworms out of the ground (e.g. using constant vs. gradually increasing voltage, 120–600 V). Our study was distinctive in several ways: (1) it was conducted in spatio-temporally heterogeneous temperate forests; (2) to our knowledge, it was the first study to use semi-permeable barriers for long-term exclusion barriers; and (3) with a total of 66 plots used to manipulate and monitor earthworms in several forest stands of different ages, it was the largest-scaled project both in terms of its level of replication and its scope of inference.

Several factors make earthworm exclusion particularly difficult in forest soils in comparison to agricultural fields or grasslands. A high diversity of woody species with a wide size distribution and large root biomass that extends deep into the soil makes forests structurally more complex than any other ecosystem in all three dimensions. Consequently, spatial heterogeneity of soil characteristics, such as aggregate structure, porosity, and moisture tends to be greater, affecting conductivity and thus introducing additional sources of variation. Maintenance of the experimental plots is more labor intensive in forests than in grasslands or agricultural fields. For example, we had to place netting over the plots to exclude the introduction of natural leaf litter and other input (twigs, seeds, fruits) into the plots. Furthermore, forest sites, especially with a diverse topography, can be less accessible than those located in open spaces.

In the present study, we achieved density and biomass reductions in total earthworms by 50% after two years of electroshocking, which falls in the middle of the efficiency values reported for long-term manipulations (Bohlen et al. 1995; Petersen 2000; Eisenhauer et al. 2008a). To our knowledge, the only exclusion experiment in forest ecosystems was conducted in a tropical forest (Liu and Zou 2002) where there was a 90% reduction in earthworm density; however, this was achieved by killing most of the earthworms during the initial electroshocking. The dead earthworms then remained in the soil.

Our findings demonstrate that the effectiveness of the manipulation was species and size dependent. Small Lumbricidae were not affected by the manipulation, which is not surprising given the semi-permeable barriers. Endogeic and anecic species were efficiently reduced but the epigeic *L. rubellus* was not.

Several factors may have affected our ability to exclude specific earthworm groups using our methods. Foremost was our selection of fiberglass mesh to line the perimeter of the enclosures. Choosing a different material, such as PVC sheets used in past studies (Bohlen et al. 1995; Petersen 2000; Liu and Zou 2002; Eisenhauer et al. 2008a), may have provided a more impenetrable barrier; however, our study required growth of fungal hyphae into our enclosures, and thus, some degree of permeability was necessary. The mesh also allowed fine root growth which is a significant component of carbon turnover in forest ecosystems. Keeping soil moisture in the enclosures similar to the surrounding soil is difficult in forests, and trenching may damage tree roots and alter local hydrology. We were not able to compare this aspect of our manipulation to published studies, but feel that using solid walls as barriers would likely cause significant microhabitat changes in forest soils.

Another significant factor impacting the efficiency is the different life histories of the earthworm species. In general, epigeic earthworms have higher fecundity and shorter incubation time, and mature faster than endogeic and anecic species (Satchell 1980). Cocoon production of *L. rubellus* and *Ap. caliginosa* was 80–106 and 27–30 cocoons per individual, respectively, during two years of observation (Evans and Guild 1948). Many environmental factors can influence life cycles (Evans and Guild 1948; Uvarov et al. 2011), yet it is possible that high cocoon production of *L. rubellus* helped the populations recover faster than other species between electroshocking campaigns. Previous studies using electroshocking to manipulate earthworm abundances did not have *L. rubellus* present in their system and, therefore, did not report on this issue.

The effectiveness of electroshocking itself depends on the activity of earthworms, which is affected by soil temperature and moisture. Schmidt (2001) recommended the minimal temperatures for electroshocking to be between 6 and 10 °C in temperate regions. Soil moisture also varies throughout the year and needs to be monitored to decide the appropriate timing for sampling. Soil moisture can become too low during summer for efficient earthworm sampling in temperate forests, and obligatory aestivation of certain species can present additional difficulties, as presented by Eisenhauer et al. (2008b) and Bohlen et al. (1995) for *Ap. caliginosa* and *Ap. tuberculata*, respectively. Even though in the mid-Atlantic regions of USA precipitation is evenly distributed, soil moisture drops in the summer (Fig. 1), and this significantly reduces earthworm extraction efficiency. Accordingly, spring and autumn are more suitable seasons for electroshocking.

The frequency of earthworm electroshocking should strike a balance between achieving high efficiency and minimizing disturbance of the experimental area. On the one hand, multiple electroshocking campaigns might be necessary to remove earthworms from the exclusion plots, and to monitor the remainder of the experimental plots. As indicated by the NMDS ordination, different groups (species or age groups) drive the community structure in different seasons, and adjustments in the high-density plots had to be made accordingly. On the other hand, even though electroshocking has the least physical impact on the soil ecosystem, some disturbance still may occur. For instance, prior to electroshocking the leaf litter has to be removed carefully to minimize damage to fungal colonies and earthworm middens.

Time and labor are potential limiting factors that investigators must consider when designing an exclusion experiment. During the 2.5 years of our study, a considerable amount of effort went into the initial set up of enclosures (~750 person-hours), electroshocking and earthworm identification (~3000 person-hours), and maintenance (~700 person-hours) of the enclosures and conditions specific to the aims of our study (i.e. repairs, removal of debris, leaf litter additions, etc.). We found that using a gas-powered trencher was the most efficient way to install the enclosures, but its size restricted us from creating enclosures smaller than 1 m^2 without disturbing soil within the treatments. This size further complicated earthworm electroshocking as at least two people were needed to collect earthworms from a single enclosure without being overwhelmed. Thus, large field crews (ideally eight people or more) were necessary during complete electroshocking campaigns.

Safety is an important consideration when operating any electrical device and it should be a top concern. A person familiar with electrical wiring (e.g. electrical engineer or electrician) should be sought for help when building electroshocking kits. Our system used low amperages (less than 1) and did not increase voltage beyond 120 V. Great care should be taken not to contact the probes with loose clothing, jewelry, or body parts while the device is in operation. Rubber gloves provide some protection from incidental contact of the apparatus. Weyers et al. (2008) provide detailed description of precautions that should be taken to maximize safety for those involved.

Increased rates of land use conversion and ongoing species introductions present dynamic changes in the structure and function of many temperate forests. Earthworms are a keystone group in these ecosystems and in situ density manipulation is a direct way to study their effects. Our study revealed that electroshocking can be an effective means to establishing and maintaining earthworm manipulation treatments, but vast investments of time and manual labor were required. Modifications to the study design likely would increase the effectiveness of electroshocking and reduce labor costs. When possible, digging a smaller enclosure may reduce assembly time and the large number of personnel required for earthworm collection during electroshocking. Locating study sites in easily accessible areas reduces travel times and the level of exertion involved with transporting heavy equipment. Careful consideration of sample sizes and logistics is paramount to any study intending to use this approach in forest ecosystems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pedobi.2012.08.008.

References

Blair, J.M., Bohlen, P.J., Edwards, C.A., Stinner, B.R., McCartney, D.A., Allen, M.F., 1995. Manipulation of earthworm populations in field experiments in agroecosystems. Acta Zoologici Fennici 196, 48–51.

Bohlen, P.J., Parmelee, R.W., Blair, J.M., Edwards, C.A., Stinner, B.R., 1995. Efficacy of methods for manipulating earthworm populations in agroecosystems with different N sources. Soil Biology and Biochemistry 27, 993–999.

Bohlen, P.J., Groffman, P.M., Fahey, T.J., Fish, M.C., Suárez, E., Pelletier, D.M., Fahey, R.T., 2004a. Ecosystem consequences of exotic earthworm invasion of north temperate forests. Ecosystems 7, 1–12.

Bohlen, P.J., Scheu, S., Hale, C.M., McLean, M.A., Migge, S., Groffman, P.M., Parkinson, D., 2004b. Non-native invasive earthworms as agents of change in northern temperate forests. Frontiers in Ecology and the Environment 2, 427–435.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.S.S., 2008. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24, 127–134.

Bouché, M.B., 1977. Strategies lombriciennes. In: Lohm, U. (Ed.), Soil Organisms as Components of Ecosystems. Ecological Bulletins (Stockholm) 25, 122–132.

Clarke, K.R., 1993. Nonparametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18, 117–143.

Čoja, T., Zehetner, K., Bruckner, A., Watzinger, A., Meyer, E., 2008. Efficacy and side effects of five sampling methods for soil earthworms (Annelida, Lumbricidae). Ecotoxicology and Environmental Safety 71, 552–565.

Crow, S.E., Filley, T.R., McCormick, M., Szlavecz, K., Stott, D.E., Gamblin, D., Conyers, G., 2009. Earthworms, stand age, and species composition interact to influence particulate organic matter chemistry during forest succession. Biogeochemistry 92, 61–82.

Csuzdi, C., Szlavecz, K., 2003. *Lumbricus friendi* Cognetti, 1904 a new exotic earthworm in North America. Northeastern Naturalist 10, 77–82.

Doeksen, J., 1950. An electrical method of sampling soil for earthworms. Transactions of the 4th International Congress of Soil Science 2, 129–131.

Eisenhauer, N., Milcu, A., Sabais, A.C.W., Scheu, S., 2008a. Animal ecosystem engineers modulate the diversity–invasibility relationship. PLoS ONE 3, e3489.

Eisenhauer, N., Straube, D., Scheu, S., 2008b. Efficiency of two widespread nondestructive extraction methods under dry soil conditions for different ecological earthworm groups. European Journal of Soil Biology 44, 141–145.

Evans, A.C., Guild, W.J.Mc., 1948. Studies on the relationship between earthworms and soil fertility. IV. Some notes on reproduction in British earthworms. Annals of Applied Biology 35, 471–484.

Fisk, M.C., Fahey, T.J., Groffman, P.M., Bohlen, P.J., 2004. Earthworm invasion, fineroot distributions, and soil respiration in north temperate forests. Ecosystems 7, 55–62.

Frelich, L.E., Hale, C.M., Scheu, S., Holdsworth, A.R., Heneghan, L., Bohlen, P.J., Reich, P.B., 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. Biological Invasions 8, 1235–1245.

Hale, C.M., Frelich, L.E., Pastor, J., 2005. Effects of European earthworm invasion on soil characteristics in northern hardwood forests of Minnesota, USA. Ecosystems 8, 911–927.

Hale, C.M., Frelich, L.E., Reich, P.B., 2006. Changes in hardwood forest understory plant communities in response to European earthworm invasions. Ecology 87, 1637–1649.

Hendrix, P.F., Baker, G.H., Callaham, M.A., Damoff, G.A., Fragoso, C., González, G., James, S.M., Lachnicht, S.L., Winsome, T., Zou, X., 2006. Invasion of exotic earthworms into ecosystems inhabited by native earthworms. Biological Invasions 8, 1287–1300.

Hendrix, P.F., Mac, A., Callaham Jr., M.C., Drake, J.M., Huang, C.-Y., James, S.W., Snyder, B.A., Zhang, W., 2008. Pandora's Box contained bait: the global problem of introduced earthworms. Annual Review of Ecology, Evolution, and Systematics 39, 593–613.

Higman, D., 1968. An Ecologically Annotated Checklist of the Vascular Flora at the Chesapeake Bay Center for Field Biology, with Keys. Office of Ecology, Smithsonian Institution, Washington, DC.

Lindroth, C.H., 1957. The Faunal Connections Between Europe and North America. John Wiley & Sons, Inc., New York, p. 344.

Liu, Z.G., Zou, X.M., 2002. Exotic earthworms accelerate plant litter decomposition in a Puerto Rican pasture and a wet forest. Ecological Applications 12, 1406–1417.

Petersen, H., 2000. Collembola populations in an organic crop rotation: population dynamics and metabolism after conversion from clover-grass ley to spring barley. Pedobiologia 44, 502–515.

Rushton, S.P., Luff, M.L., 1984. A new electrical method for sampling earthworm populations. Pedobiologia 26, 15–19.
Satchell, J.E., 1955. An electrical method of sampling earthworm populations. In: Kevan, D.K. (Ed.), Soil Zoology. Butterworth, London, pp. 356–364.
Satchell, J.E., 1980. R worms and K worms: a basis for classifying earthworm strategies. In: Dindal, D.L. (Ed.), Soil Biology as Related to Land Use Practices. EPA Publ., Washington, DC, pp. 848–863.
Schmidt, O., 2001. Appraisal of the electrical octet method for estimating earthworm populations in arable land. Annals of Applied Biology 2001, 231–241.
Staddon, P.L., Ostle, N., Fitter, A.H., 2003. Earthworm extraction by electroshocking does not affect canopy CO_2 exchange, root respiration, mycorrhizal fungal abundance or mycorrhizal fungal vitality. Soil Biology and Biochemistry 35, 421–426.
Szlavecz, K., Csuzdi, C., 2007. Land use change affects earthworm communities in Eastern Maryland, USA. European Journal of Soil Biology 43, 79–85.
Szlavecz, K., McCormick, M., Xia, L., Saunders, J., Morcol, T., Whigham, D., Filley, T., Csuzdi, C., 2011. Ecosystem effects of non-native earthworms in Mid-Atlantic deciduous forests. Biological Invasions 13, 1165–1182.
Szlavecz, K., Terzis, A., Ozer, S., Cogan, Musăloiu-E., Small, J., Burns, S., Gray, R., Szalay, J.A., 2006. Life under your feet: an end-to-end soil ecology sensor network, database, web server, and analysis service. Microsoft Technical Report, 2006.
Terzis, A., Musăloiu, E., Cogan, R., Szlavecz, J., Szalay, K., Gray, A., Ozer, J., Liang, S., Gupchup, C.J.M., Burns, J.R., 2010. Wireless sensor networks for soil science. International Journal of Sensor Networks 7, 53–70.
Thielemann, U., 1986. Elektrischer Regenwurmfang mit der Oktett-Methode. Pedobiologia 29, 296–302.
Uvarov, A.V., Tiunov, A.V., Scheu, S., 2011. Effects of seasonal and diurnal temperature fluctuations on population dynamics of two epigeic earthworm species in forest soil. Soil Biology and Biochemistry 43, 559–2570.
Weyers, S.L., Schomberg, H.H., Hendrix, P.F., Spokas, K.A., Endale, D.M., 2008. Construction of an electrical device for sampling earthworm populations in the field. Applied Engineering in Agriculture 24, 391–397.