



# Litter quality, dispersal and invasion drive earthworm community dynamics and forest soil development

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## Abstract

In temperate deciduous forests of eastern USA, most earthworm communities are dominated by invasive species. Their structure and functional group composition have critical impacts on ecological properties and processes. However, the factors determining their community structure are still poorly understood, and little is known regarding their dynamics during forest succession and the mechanisms leading to these changes. Earthworm communities are usually assumed to be stable and driven by vegetation. In contrast, the importance of dispersal and ecological drift is seldom acknowledged. By analyzing a 19-year dataset collected from forest stands in eastern USA, we demonstrated that on a decadal timescale, earthworm community dynamics are shaped by the interplay of selection, dispersal, and ecological drift. We highlighted that forests at different successional stages have distinct earthworm species and functional groups as a result of environmental filtering through leaf litter quality. Specifically, young forests are characterized by soil-feeding species that rely on relatively fresh soil organic matter derived from fast-decomposing litter, whereas old forests are characterized by those feeding on highly processed soil organic matter derived from slow-decomposing litter. In addition, year-to-year species gains and losses are primarily driven by dispersal from regional to local species pools, and by local extinction resulted from competition and ecological drift. We concluded that with continued dispersal of European species and the recent “second wave” of earthworm invasion by Asian species from the surrounding landscape, earthworms at the investigated forests are well-established, and will remain as the major drivers of soil development for the foreseeable future.

**Keywords** Functional group · Long term · *Amyntas agrestis* · *Metaphire hilgendorfi* · Endogeic

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## Introduction

Understanding the assembly rules of ecological communities is crucial especially when the involved group encompasses ecologically important species, such as ecosystem engineers, keystone species or invasive species. Earthworms are widely considered to be ecosystem engineers as their behavior

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fundamentally changes the structure of the leaf litter layer and underlying soils (Hale et al. 2005; Eisenhauer et al. 2007; Holdsworth et al. 2012; Ma et al. 2014), both of which serve as habitats and provide food for many other organisms. By modifying these critical resources, the activities of earthworms, in many cases, invasive ones, affect the abundance of animals with a wide range of body sizes, including birds (Loss and Blair 2011; Loss et al. 2012), salamanders (Maerz et al. 2009; Ransom 2011), millipedes (Snyder et al. 2011), mites (Bayoumi 1978; Eisenhauer et al. 2007), enchytraeids (Dózsa-Farkas 1978) and nematodes (Yeates 1981). In North American forests, invasive earthworms have been shown to alter soil microbial community composition, redistribute soil organic matter, alter litter residue chemistry, and cause loss of soil carbon and nutrients (Filley et al. 2008; Szlavecz et al. 2011; Groffman et al. 2015; Resner et al. 2015; Chang et al. 2016b, 2017). They also substantially impact native seedling survival and plant diversity and increase cover of non-native plants (Hale et al. 2006; Dobson and Blossey 2015; Craven et al. 2017).

Ecological communities are shaped by an interplay of deterministic and stochastic processes, including selection, dispersal, ecological drift, and speciation (Vellend 2010, 2016). Selection, a deterministic fitness difference between individuals of different species (Vellend 2010), is the most widely studied process in community ecology. It is usually investigated under species–species or species–environment interactions and described in the form of niche theory, species sorting, environmental filtering, biotic interactions and ecological or environmental niche models (Chase and Myers 2011). In the case of earthworms, studies that imply selection focus on either evaluating environmental factors or understanding biotic interactions. For instance, land use and management (Ernst and Emmerling 2009; Briones and Schmidt 2017), vegetation (Eisenhauer et al. 2009; Bozarth et al. 2016; Mariotte et al. 2016) and soil properties (Eggleton et al. 2009; Crumsey et al. 2014) have been frequently shown to determine earthworm species composition. In addition, earthworm communities can be highly structured by biotic interactions, such as competition (Decaens et al. 2008; Chang et al. 2016c).

Vegetation and leaf litter quality are known to affect earthworm abundance and community structure in forest ecosystems (Reich et al. 2005; Hobbie et al. 2006; Schwarz et al. 2015; de Wandeler et al. 2016). These studies usually provide only a snapshot of the earthworm communities, assuming a steady state and perhaps slow change, given the temporal scale of vegetation change. Little is known about the temporal dynamics of earthworm assemblages in forests at different successional stages and the mechanisms driving those changes. Szlavecz and Csuzdi (2007) reported that in temperate deciduous forests at the Smithsonian Environmental Research Center (SERC) in eastern USA, young

(50–70 years) forests have higher earthworm abundance than older (120–150 years) forests, and hypothesized that these differences were caused by higher abundance of high quality leaf litter in the young forests. This relationship between forest age and earthworm abundance also was supported in a separate study conducted in a forest with similar tree species composition about 380 km north of the SERC forests (Simmons et al. 2015). In the latter study, however, there was no apparent difference in tree species composition. In theory, high abundance of high quality leaf litter should benefit species relying directly on litter as food resources. Although Szlavecz and Csuzdi (2007) and Simmons et al. (2015) did not measure leaf litter biomass, they both found higher abundance of litter-feeding earthworm species in early- and mid-successional forests. Thus, the relationship between leaf litter species composition and earthworm abundance and community structure in temperate deciduous forests of different successional stages warrants further investigation.

Previous studies investigating the effect of vegetation on earthworm community mostly adopted the space-for-time approach to investigate earthworm assemblage changes during secondary succession, or used afforestation projects/common garden experiments on previously agricultural (e.g., Pizl 1992) or mining sites (e.g., Frouz et al. 2008; Dunger and Voigtlander 2009). The space-for-time approach usually focused on the early stages of succession (i.e., fallow or grassland) and rarely had more than one forest category to represent a chronosequence of deciduous forest development (Scheu 1992; Decaens et al. 1997). Although common garden experiments and afforestation projects usually contain several tree species representing different successional stages and litter qualities, these stands are generally less than 50 years old (Muys et al. 1992; Reich et al. 2005; Hobbie et al. 2006; Dunger and Voigtlander 2009; de Schrijver et al. 2012; Schelfhout et al. 2017) and may not truly represent the old/mature forests characterized by the respective tree species. There appears to be a sizable gap between these two types of studies.

Changes in an ecosystem happen at multiple temporal scales. In forests, community responses to climatic trends, land use change, vegetation shift, and recovery after disturbances are relatively slow, and therefore, detecting those requires long-term data (Pecl et al. 2017). Only few earthworm community studies span more than a few years and those that sampled once a year over multiple years provide a snapshot with no information on seasonal dynamics of species presence/absence, abundance, or changing juvenile-to-adult ratio. Studies conducted during a single year miss potential year-to-year species composition changes through dispersal from regional to local species pools. As such, earthworm communities implicitly are assumed to be stable, and the importance of dispersal, ecological drift, and local extinction is rarely acknowledged outside the context of

early succession and earthworm invasion (Hale et al. 2005; Eijssackers 2011).

We investigated earthworm community dynamics and evaluated the importance of vegetation, soil characteristics, dispersal and local extinction on earthworm community structure in the upland forests of SERC. Our overarching questions were: (1) whether earthworm communities are locally stable over a decadal timescale, and (2) whether earthworm communities change during forest succession. Space-for-time substitution is the main approach for the latter question and we employed this method by comparing earthworm communities from ten deciduous forest stands representing different stages of secondary succession. Such chronosequence studies are not always appropriate because the other biotic and abiotic factors driving community change cannot be properly controlled (Johnson and Miyanishi 2008; Walker et al. 2010). Our long-term dataset collected during a period of 19 years (with a 6-year gap between 2000 and 2005) allows detection of changes at the same site. We hypothesized that young and old forests would differ in their earthworm abundance and species composition. Specifically, we predicted that: (1) total earthworm abundance would be higher in young forests than in old forests, (2) differences in abundance would be driven by leaf litter quality and soil properties, (3) abundance of litter-feeding species would be higher in young forests, and (4) dispersal from the regional species pool into the local species pool would lead to temporal changes in earthworm species richness at the local scale.

## Materials and methods

### Study site

The Smithsonian Environmental Research Center (Fig. S1) is located along the western shore of the Chesapeake Bay in Anne Arundel County, Maryland on the Rhode River estuary (38°53'N, 76°33'W). SERC has a mean annual rainfall of 1146 mm and a mean annual temperature of 13.0 °C (Correll et al. unpublished data), with a mean annual maximum temperature of 19.0 °C and a mean minimum temperature of 8.0 °C (NCDC database, Annapolis Police Bar Station). SERC is the site of one of the oldest colonial farms in the Mid-Atlantic region with a well-documented history of a variety of past land uses, ranging from cultivation of crops, such as tobacco, corn, wheat and alfalfa, to clear cutting or selective logging, and grazing (Higman 1968). Today, most of the upland area is covered with deciduous forests belonging to the tulip poplar association (Brush et al. 1980). However, due to different past land uses as well as timing of agricultural abandonment, the forest cover is a patchwork of stands of different ages and tree species composition. Young

forests are at least 50 years old, dominated by tulip poplar (*Liriodendron tulipifera*) and sweetgum (*Liquidambar styraciflua*), with red maple (*Acer rubrum*), black cherry (*Prunus serotina*) and box elder maple (*Acer negundo*) being the secondary species. Old forests are 120–150 years old with several species of oaks (*Quercus* spp.), American beech (*Fagus grandifolia*), and hickories (*Carya* spp.) dominating, while tulip poplar and sweetgum are still present. A few small forest patches are considered undisturbed as they have never been cut. Vegetation on these patches has been classified as the Chestnut oak-Chestnut Association (Brush et al. 1980) with chestnut oak (*Q. prinus*) as the dominant tree.

Soils at SERC are Collington sandy loam, Annapolis fine sandy loam and Donlonton fine sandy loam (Yesilonis et al. 2016). Past land use and the developing vegetation after agricultural abandonment left a legacy on surface soil properties. Uncut sites also have lower pH, and much lower Ca, Mg and NO<sub>3</sub><sup>−</sup> concentrations. The Bt horizon is closest to the soil surface in the young stands, followed by the old stands and uncut sites (Yesilonis et al. 2016), which implies a greater degree of erosion of the surface soils in the younger stands. After more than 100 years since abandonment, there was only a nascent development of an O horizon in the old successional forests and none in the young forest; the O horizon is nearly exclusive to the uncut sites that are earthworm free (Ma et al. 2013).

The first earthworm sampling at SERC was conducted in 1998–1999 (Szlavecz and Csuzdi 2007). This survey found differences in abundance between old and young stands, while no earthworms were recorded in the uncut stands. Today uncut stands are still earthworm free; therefore, we will not include them in this discussion. In Szlavecz and Csuzdi (2007), three young and two old forest plots were sampled. Over time, one plot was abandoned, and six were added, resulting in five young and five old plots (Fig. S1). Each plot is 30 m in diameter with the exception of Java Trail, which was a rectangle about 30 m × 100 m.

### Earthworm sampling

Earthworms from all ten plots were sampled in April 2009, 2010, 2011, 2012 and 2016 (ten-plot dataset; Table S1). Three 50 cm × 50 cm quadrats were randomly placed inside each plot and earthworms were collected from each quadrat using the formalin extraction method (Raw 1959). Five of the plots (Canoe Shed, Front Gate, Fox Point Road, Frog Canyon and Treefall) were also sampled in June 2006, October 2008, and August and November in 2009 and 2010. Thus, a complete spring, summer and fall dataset was available for the five plots in 2009 and 2010 (five-plot dataset; Table S1). Collected earthworms were anesthetized in 10% ethanol, fixed in 10% formalin for at least 48 h, and transferred to 70% ethanol for preservation. For species names

and identification, we followed Csuzdi and Zicsi (2003), Chang et al. (2016a) and Csuzdi et al. (2017). Clitellate specimens were identified to species. Juveniles (individuals without a clitellum and/or genital markings) were identified to genera. As species-level identification is required for analyses of species composition and functional groups, juvenile individuals were not included in these analyses. Data (density and biomass) from the three quadrats were averaged, and the mean was treated as one data point.

Earthworm species were also combined into functional groups: epigeic (*Bimastos palustris*), epi-endogeic (*Lumbricus rubellus*), anecic (*L. friendi*), polyhumic endogeic (*Aporrectodea caliginosa*, *Ap. rosea*, *Allolobophora chlorotica*, and *Octolasion lacteum*), and mesohumic endogeic (*Octolasion cyaneum* and *Eisenoides lonnbergi*). The only epigeic species, *B. palustris*, appeared only during 2011 in one plot, and therefore, it was excluded from our analysis. These functional groups were based on stable isotope data from Chang et al. (2016c), a study conducted at SERC and included five of the eight species we analyzed, with support for the remaining species from stable isotope data or feeding experiments reported in Scheu and Falca (2000), Schmidt et al. (2004), Zicsi et al. (2011), Melody and Schmidt (2012), and Ferlian et al. (2014).

In summer 2010, we observed invasion of the Asian earthworm *Metaphire hilgendorfi* and *Amyntas agrestis* in the Treefall plot, the first record of these species at SERC. To better understand their point of entry and spatial distribution, and monitor their invasion, we established a 160 m × 240 m grid with grid points 10 m away from each other. We assessed the presence/absence of Asian earthworms at every other grid point in 2011 and 2014. In 2011, earthworms were sampled in May using the formalin extraction method from two 25 cm × 25 cm quadrats within a 50 cm radius of the grid point. In 2014, earthworms were sampled in August using hand sorting from a 1 m × 1 m quadrat at each grid point. The presence or absence of Asian earthworms was recorded and individuals were subsequently released.

## Litter and soil sampling

Leaf litter remaining on the forest floor at the end of August was collected from five 50 cm × 50 cm quadrats in each plot in 2010. Fall litter input from September to December was collected using seven 19-l (five gallons) buckets with an area of 0.0638 m<sup>2</sup> in each plot in 2010. Leaf litter was dried and divided into identifiable leaves and fragments. The former was further sorted into the following groups: oak (*Quercus* spp.), American beech (*F. grandifolia* Ehrh.), tulip poplar (*L. tulipifera* L.), sweetgum (*L. styraciflua* L.), maple (*Acer* spp.), and miscellaneous, which included spice bush (*Lindera benzoin*) the dominant understory woody shrub, and holly (*Ilex* spp.). The sorted litter was weighed, and grouped

into fast-decomposing and slow-decomposing litter. It is well-known that litter chemistry is a major factor determining decomposition rate. Leaf chemistry within a species can vary due to tree age, position of the leaves on the tree canopy and the time of leaf abscission. However, typically, plant detritus with low tannin and lignin (more recently called AUR: acid-unhydrolyzable residue) content and low C:N ratio are considered ‘high quality’ litter, which is more palatable to detritivores and decomposes faster. Requiring initial microbial conditioning before saprophagous soil animals consume them, litter types with high C:N ratio decompose more slowly and remain on the forest floor surface longer (Swift et al. 1979, Zhang et al. 2008, Prescott 2010). In SERC forests, fast-decomposing litter includes tulip poplar, sweetgum, and maple; slow-decomposing litter includes oak and American beech (Swift et al. 1979; Melillo et al. 1982; White et al. 1988; Cote and Fyles 1994; Ma et al. 2014; Wilson-Kokes and Skousen 2014). Additionally, the miscellaneous litter from the young forests was composed of almost exclusively fast-decomposing litter, including flowering dogwood, black cherry, red maple, and boxelder maple, whereas that from the old forests was almost exclusively slow-decomposing litter, such as hickory (*Carya* spp.), holly (*Ilex* spp.) and black gum (*Nyssa sylvatica* Marshall).

Soil temperature and moisture at 5 cm depth were measured within 50 cm of the quadrats during earthworm sampling in 2010, 2011 and 2016 (Table S2). Data of soil physical and chemical properties (0–10 cm) from the same ten plots have been previously reported and were retrieved from Yesilonis et al. (2016). Due to autocorrelation among soil variables, we selected a subset of variables for our analyses (Table S3) following criteria outlined in Yesilonis et al. (2016).

## Data analyses

All statistical analyses were conducted in R v3.4.0 (R Core Team 2017). Packages used were *lme4* (Bates et al. 2015) for mixed effect models, *glmmADMB* (Skaug et al. 2016) for generalized linear mixed models (GLMM), *multcomp* (Hothorn et al. 2008) for multiple comparisons, and *vegan* (Oksanen et al. 2016) for ordination and  $\beta$  diversity analyses. For abundance (density and biomass) data, normality was tested using the Shapiro test and by examining the Q–Q plot. Square root transformation was used to improve normality when necessary. For mixed effect models, the likelihood ratio test was used to access significant differences between nested models. For GLMM, the likelihood ratio test was not reliable for testing fixed effects under small to moderate sample sizes (Bolker et al. 2009). Therefore, the Akaike information criterion (AIC) was used instead to examine fixed effects, as suggested by Bolker



et al. (2009). These analyses were followed by the Tukey's HSD test for multiple comparisons.

Data of leaf litter remaining on the soil surface at the end of summer and leaf litter input throughout litter fall in 2010 were analyzed using ANOVA to examine differences between old and young forests. Soil moisture data were analyzed using mixed effect models with "plot" as a random effect and "forest age" and "year" as fixed effects.

The effects of forest age on total earthworm abundance (density and biomass) were examined using the ten-plot dataset and mixed effect models, with "plot" as a random effect and "year" and "forest age" as fixed effects. Functional group compositions were analyzed using GLMM with a negative binomial distribution and the same random and fixed effects.

Earthworm abundance data collected in spring, summer and fall in 2009 and 2010 (the five-plot dataset) were analyzed to examine seasonal changes using mixed effect models. Seasonal dynamics of individual species were analyzed using GLMM with a negative binomial distribution. In both analyses, "plot" was treated as a random effect, and "year" and "season" were treated as fixed effects.

Earthworm community structures were analyzed using the density data and visualized using non-metric multidimensional scaling (NMDS) based on the Bray–Curtis distance. To investigate differences in species composition and  $\beta$  diversity between old and young forests, we analyzed individual year separately using the ten-plot dataset. Bray–Curtis distances were calculated. Differences in species compositions between old and young forests were tested for each year independently using PERMANOVA (Anderson 2001) with 999 permutations.  $\beta$  diversity was evaluated by testing the homogeneity of multivariate dispersions (Anderson et al. 2006, 2011) using the Bray–Curtis distance. PERMDISP (Anderson et al. 2006) with 999 permutations was used to test for differences between old and young forests. To test whether species compositions and  $\beta$  diversities varied among different years, we combined data from all years and used "year" as the grouping factor.

Earthworm species compositions based on mean spring (April) densities were analyzed using constrained correspondence analysis (CCA) with soil properties and fall litter inputs as constrained variables. CCA was done without rare species, which are defined as species meeting one of the following two criteria: (1) a species that was present in only one data point in our ten-plot dataset, or (2) a species whose mean spring density was lower than one individual per square meter. Permutation tests with 999 permutations were used to test the significances of the overall models, the constrained axes, soil properties and fall litter inputs.

## Comparison with historical data

Mixed effect models were used to compare the spring abundance data we collected in 2009–2016 with those from 1999 and 2006 reported by Szlavecz and Csuzdi (2007). As we later found significant interactions between "year" and "forest age", we analyzed old and young forests separately with "plot" as a random effect and "year" as a fixed effect. As only two old and two young forest plots were sampled in Szlavecz and Csuzdi (2007) and species composition data were only available for the spring 1999 sample, we refrained from analyzing long-term changes in abundance of individual species.

## Results

### Earthworm species richness and composition

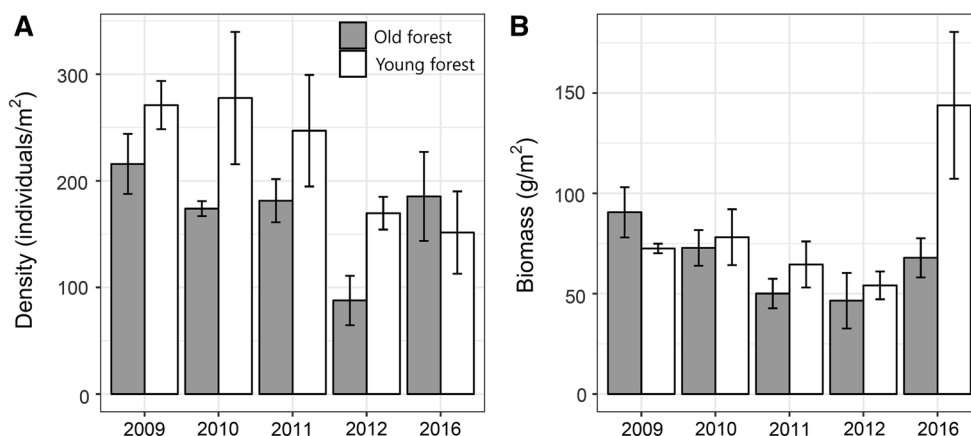
Our long-term and grid-sampling assessment recorded a total of 14 species of earthworms (Table S4). Seven newly recorded species (two Lumbricidae and five Megascolecidae) were added to the list in Szlavecz and Csuzdi (2007), increasing the species richness by 37% (Table S5). Numbers of species recorded each year ranged between 8 and 11 (Fig. S2), with 8 species in most years. In the quadrat samples, four species, *Amyntas corticis*, *B. palustris*, *Lumbricus terrestris*, and *L. castaneus*, met our criteria of rare species (Table S4). They appeared in only a few samples with low numbers and were not repeatedly recovered from the same plots.

### Earthworm density and biomass

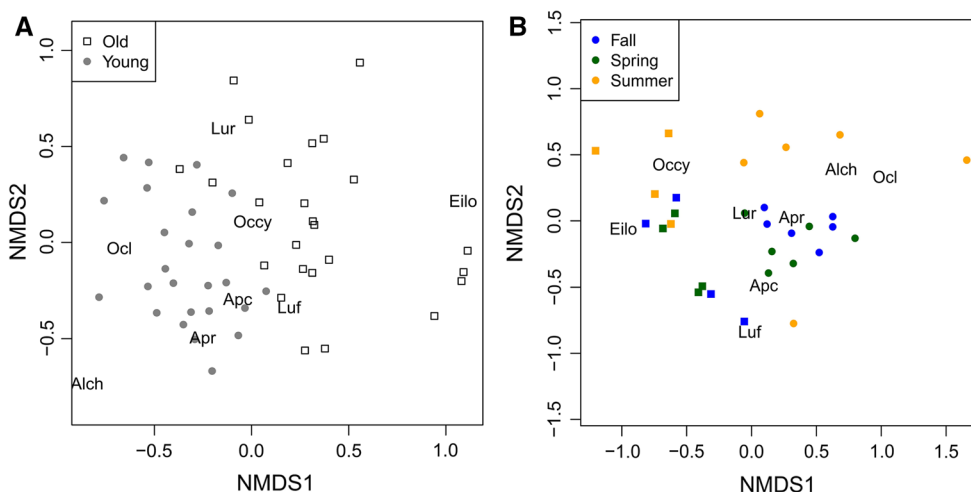
Earthworm density varied from 25 individuals/m<sup>2</sup> at the Tower plot (old forest) in spring 2012 to 467 individuals/m<sup>2</sup> at the Canoe Shed plot (young forest) in fall 2009. Earthworm biomass varied from 13.1 g/m<sup>2</sup> at the Front Gate plot (young forest) in summer 2010 to 240.0 g/m<sup>2</sup> at the Fox Point Road plot (young forest) in spring 2016. Juveniles (individuals without clitellum) made up 68% (SD = 13%) of the total number.

Earthworm density was higher in the young forests than in the old forests. However, the effect of forest age was only nearly significant ( $P=0.059$ ) (Fig. 1). The effect of forest age was not significant for biomass ( $P=0.188$ ) (Fig. 1). Both earthworm density and biomass were higher in spring and fall than in summer ( $P<0.001$  in both cases; Fig. S3). The mean volumetric water content at the time of sampling in summer was high ( $30.2 \pm 2.4\%$  in 2009 and  $35.2 \pm 2.4\%$  in 2010); therefore, the seasonal differences in abundance

**Fig. 1** Earthworm density (a) and biomass (b) in old (gray) and young (white) forests between 2009 and 2016 (mean  $\pm$  SE). In most years, density was higher in young than in old forests, but the model was only nearly significant ( $P=0.059$ )



**Fig. 2** NMDS based on Bray–Curtis distances of density data comparing the two forest age groups (a) and different seasons (b). Old and young forests are marked with square and circles, respectively. Spring, summer and fall are marked in green, orange and blue, respectively (see online version for colors). Lur: *Lumbricus rubellus*; Luf: *Lumbricus friendi*; Apc: *Aporrectodea caliginosa*; Apr: *Aporrectodea rosea*; Alch: *Allolobophora chlorotica*; Ocl: *Octolasion lacteum*; Occy: *Octolasion cyaneum*; Eilo: *Eisenoides lonnbergi*



were likely due to a combination of high soil temperature (22–23 °C at 10 cm) driving earthworms to deeper layers and obligatory aestivation of *Ap. caliginosa*.

### Earthworm community structure

NMDS based on spring density showed separation between the two forest age groups (Fig. 2a). PERMANOVA generally supported this observation ( $P=0.001$ , 0.135, 0.125, 0.012 and 0.016 for 2009, 2010, 2011, 2012 and 2016, respectively). These differences were primarily driven by the absence or low abundance of *O. lacteum*, *Ap. rosea*, and *Al. chlorotica* in the old forests, and *E. lonnbergi* in the young forests (Table S4). PERMDISP showed marginally higher  $\beta$  diversity in old than in young forests in 2009 ( $P=0.077$ ) and 2012 ( $P=0.089$ ). Overall, earthworm species compositions and  $\beta$  diversities were not significantly different among different years ( $P=0.840$  and 0.913, respectively). Regarding functional group compositions, the densities of epi-endogeic and anecic earthworms were not different between old and young forests (Table 1).

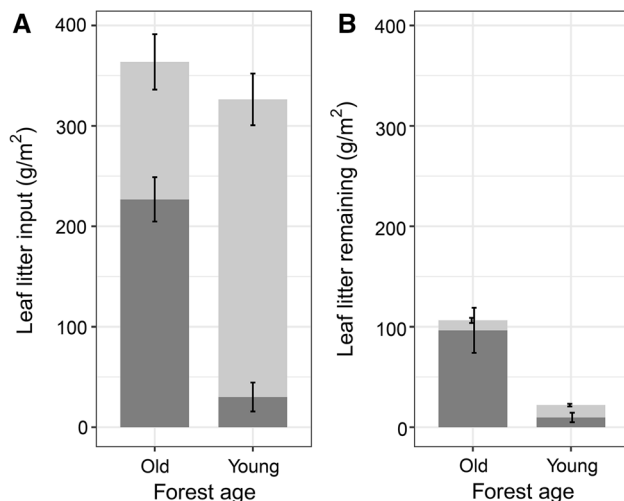
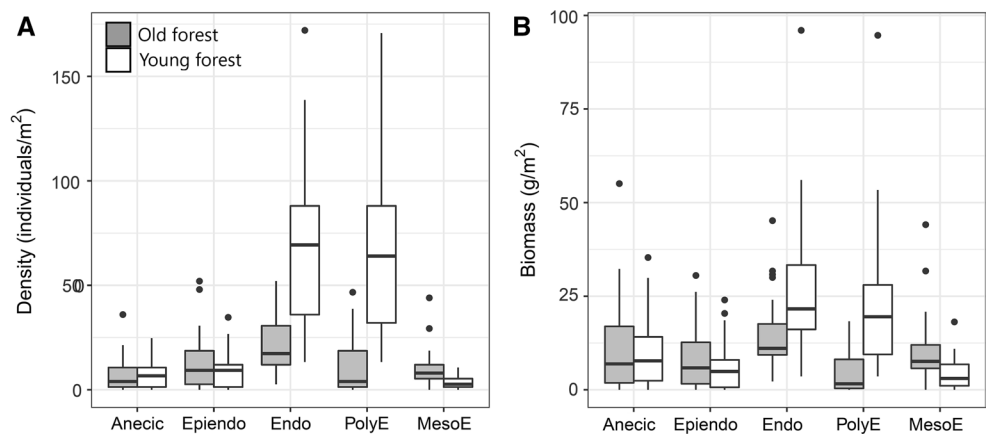
**Table 1** Generalized linear mixed models testing for the effects of forest age on the earthworm functional groups based on density

Functional group	Full model AIC	Reduced model AIC	$\Delta$ AIC <sup>a</sup>
Epi-endogeic	346.6	345.3	1.4
Anecic	310.9	308.9	1.9
Endogeic	453.7	464.6	<b>–10.9</b>
Polyhumic endogeic	442.2	454.6	<b>–11.5</b>
Mesohumic endogeic	279.7	282.1	<b>–2.5</b>

<sup>a</sup>Full models include both ‘year’ and ‘forest age’ as fixed effect; reduced models include only ‘year’. To test for the effect of forest age,  $\Delta$ AIC values were calculated as ‘full model AIC’ – ‘reduced model AIC’.  $\Delta$ AIC values smaller than –2 are marked in bold

However, the densities of polyhumic endogeic earthworms were higher in young than in old forests, whereas those of mesohumic endogeic earthworms were higher in old than in young forests (Fig. 3). Overall, endogeic earthworm densities were higher in young forests (Table 1).

**Fig. 3** Box plots of density (a) and biomass (b) of earthworm functional groups in old (gray) and young (white) forests. *Anecic anecic*, *Epiendo epi-* endogeic, *Endo endogeic*, *PolyE* polyhumic endogeic, *MesoE* mesohumic endogeic



**Fig. 4** Leaf litter input during fall senescence (a) and leaf litter remaining on the soil surface at the end of summer (b) in old and young forests at SERC. Light gray (top): fast-decomposing litter; dark gray (bottom): slow-decomposing litter. Values are mean  $\pm$  SE,  $N=5$  for both forest types. See Figure S1 for more details

Earthworm community structures in summer clearly separated from those in spring and fall (Fig. 2b). Densities of *L. rubellus*, *L. friendi* and *O. lacteum* were lowest in summer (Table S6). In addition, *L. friendi* had higher density in spring than in fall ( $P=0.007$ ), and *O. lacteum* had higher density in fall than in spring ( $P=0.021$ ). We did not fit a GLMM for *Ap. caliginosa* due to zero abundance in summer in all plots.

### Leaf litter

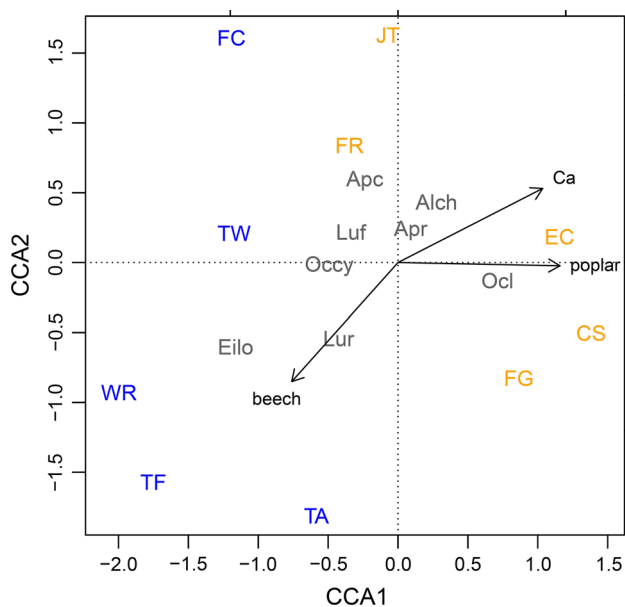
The biomass of leaf litter input throughout litter fall in 2010 did not differ between old and young forests ( $P=0.213$ ). However, fast-decomposing litter input was higher in young than in old forests ( $P=0.002$ ), and slow-decomposing litter input was higher in old than in young

forests ( $P<0.001$ ) (Fig. 4a). These differences were driven by higher abundance of tulip poplar litter in the young forests ( $P=0.006$ ) and American beech litter in the old forests ( $P=0.012$ ). There were no significant differences regarding oak, maple, and sweetgum litter (Fig. S4).

At the end of summer 2010, 61–88% of the leaf litter remaining on the ground was unidentifiable fragments. The biomass of leaf litter was significantly higher in old than in young forests ( $P=0.016$ ). Among leaf litter that was identifiable, slow-decomposing litter biomass was significantly higher in old than in young forests ( $P=0.005$ ), while fast-decomposing litter was not significantly different ( $P=0.415$ ) (Figs. 4b, S4).

### Constrained ordination

Constrained correspondence analysis using soil properties as constraints showed that the constrained axes explained 28.7% of the variance (Fig. 5). The overall model was significant ( $P=0.013$ ). Among the constrained variables,  $\text{Ca}^{2+}$  had a significant effect ( $P=0.014$ ), while the effects of the other soil properties were not significant. Constrained correspondence analysis using leaf litter input as a constraint showed that the constrained axes explained 48.8% of the variance. The overall model was significant ( $P=0.003$ ). Among the constrained variables, tulip poplar ( $P=0.008$ ) and American beech ( $P=0.049$ ) had significant effects, but the effects of the other leaf litter species were not significant (Fig. 5). When soil properties and leaf litter were considered together, the constrained axes with  $\text{Ca}^{2+}$  and tulip poplar and American beech litter explained 52.5% of the variance. This was a mere 3.7% increase from the litter-only model, and  $\text{Ca}^{2+}$  became non-significant ( $P=0.810$ ). Tulip poplar litter input and soil  $\text{Ca}^{2+}$  concentration were highly correlated (adjusted  $R^2=0.65$ ,  $P=0.003$ ).



**Fig. 5** CCA (constrained correspondence analysis) plot of earthworm community structure constrained by leaf litter input and soil properties. While significant when soil properties alone were used as constraints, soil  $\text{Ca}^{2+}$  was dropped from the final model due to its high correlation with biomass of tulip poplar leaf litter. *Poplar* tulip poplar, *beech* American beech, *Ca* soil  $\text{Ca}^{2+}$ . Lur: *Lumbricus rubellus*; Luf: *Lumbricus friendi*; Apc: *Aporrectodea caliginosa*; Apr: *Aporrectodea rosea*; Alch: *Allolobophora chlorotica*; Ocl: *Octolasion lacteum*; Occy: *Octolasion cyaneum*; Eilo: *Eisenoides lonnbergi*. TW, WR, TF, TA and FC are old forests; JT, FR, EC, CS and FG are young forests; see Table S3 for abbreviations

### Comparison with historical data

Mixed effect models comparing the earthworm abundance in 2009–2016 with historical data from 1999 and 2006 showed that with the exception of 2012, earthworm density was significantly higher during 2009–2016 than during 1999 and 2006 in the old forests ( $P < 0.001$  for the overall model; Table S7). In contrast, earthworm density in the young forests was not significantly different among the years ( $P = 0.130$ ). Similarly, in the old forests, earthworm biomass was significantly higher in 2009, 2010, 2011 and 2016 than in 1999 and in 2009 than in 2006 ( $P < 0.001$  for the overall model; Table S7). In the young forests, earthworm biomass during 2009–2016 was not significantly different from that in 1999 and 2006 (Table S7).

### Grid sampling of Asian earthworm invasion

Grid sampling of Asian earthworms showed that the invasive species were present in a higher proportion of the grid points in 2014 than in 2011 (Fig. S5), suggesting ongoing invasion. We acknowledged that sampling between the 2 years were conducted using different methods and in different seasons.

Therefore, only presence/absence data can be meaningfully compared. Our timing of sampling did not allow us to identify individual specimens to species, as most individuals were still juveniles. However, recent earthworm samples collected from the same site have documented the presence of two Asian species, *A. agrestis* and *M. hilgendorfi* (Chang et al. 2018).

### Discussion

Unlike the northern regions in North America and Canada, earthworm assemblages in the mid-Atlantic are a mixture of native and introduced species. Most forests are secondary, recovering from intensive logging and/or agriculture. European lumbricids dominate these forests, but we can only speculate when colonization took place. SERC is one of the oldest farms in the region (Yesilonis et al. 2016), thus we can assume that most species have been established for at least a century. The issue is how stable these local communities are given climatic fluctuation as well as different resource quality and soil conditions between young and old forests (Yesilonis et al. 2016).

Encompassing a period of 19 years, our long-term data documented spatial and temporal dynamics of earthworm communities in a temperate deciduous forest in eastern USA, including changes in species presence, increased earthworm abundance in old forests, invasion of Asian *Amyntas* and *Metaphire* earthworms, and functional differences in earthworm communities in forests of different ages. Our data did not support previous observations that earthworm abundance is higher in young than in old forests (hypothesis 1). Instead, we documented differences in earthworm species compositions and community structures between the two forest age groups.

### Leaf litter quality and earthworm community structure and function

The two forest age groups differ in the abundance of fast-decomposing and slow-decomposing litter, especially tulip poplar and American beech. This contrasting pattern in litter quality explains the differences in earthworm abundance and community structures between old and young forests, but not as stated in hypothesis 2, which following hypothesis 1, expects litter quality to be the main driver of the (hypothesized) higher total earthworm abundance in young forests. In addition, contrary to our hypothesis 3, the abundance of litter-feeding (epi-endogeic and anecic) species did not differ between the two forest age groups. Instead, the abundance of soil-feeding (endogeic) species was higher in the young forests. Differences in earthworm functional groups affect how surface detritus is incorporated into soil fractions. Our



finding is consistent with Ma et al. (2014), who documented that in young forests, wood detritus is incorporated primarily as particulate organic matter, and into deeper soil, presumably due to the higher mixing and activity of endogeic earthworms. Moreover, our data further demonstrated that within the soil-feeding species, the young forests are dominated by species relying on relatively fresh soil organic matter (polyhumic endogeic species), whereas the old forests are dominated by species feeding on highly processed soil organic matter (mesohumic endogeic species). These results are clear evidence of two systems with functional differences driven by detritus inputs.

American beech and tulip poplar, two dominant litter species in old and young forests, represent two distinct types of leaf litter. Tulip poplar has low lignin, but high Ca contents (White et al. 1988; Ma et al. 2014; Wilson-Kokes and Skousen 2014); American beech is the opposite (Melillo et al. 1982; Cote and Fyles 1994). Litter quality, particularly lignin content, is known to influence the soil microbial communities (Cleveland et al. 2014; Moorhead et al. 2014; Fanin et al. 2016) and microbially mediated decomposition (Reich et al. 2005; Hobbie et al. 2006), leading to differences in soil organic matter properties and biogeochemistry (Filley et al. 2008; Crow et al. 2009; Ma et al. 2013). Leaf litter is also an important driver of soil Ca content (Reich et al. 2005). Ca contents in litter and soil are known to be positively correlated with earthworm abundance (Ponge et al. 1999; Reich et al. 2005; Hobbie et al. 2006). In our case, soil Ca was highly correlated with the input of tulip poplar leaf litter. Differences in litter Ca among tree species can change soil Ca content, pH and fertility, and are a key driver of changes in soil properties (Reich et al. 2005; De Schrijver et al. 2012; Ma et al. 2014; Schelfhout et al. 2017), as high soil Ca content is often associated with higher microbial biomass, and has the potential to accelerate litter decomposition (Ma et al. 2014; Schelfhout et al. 2017) and enhance tree growth (Ponge et al. 1999). Altogether, we believe that leaf litter quality, particularly the interplay of lignin and Ca contents, leads to differences in microbially mediated decomposition and soil organic matter properties in old and young forests. These differences could explain our observed differences in functional group compositions of soil-feeding earthworms between the two forest age groups, and documented the importance of selection, in the form of environmental filtering (Vellend 2010, 2016; Chase and Myers 2011), in shaping earthworm communities. In the young forests, the fresh soil organic matter derived directly from fast-decomposing leaf litter is readily accessible to polyhumic endogeic earthworms, such as *Ap. caliginosa* and *O. lacteum*, whereas in the old forests, soil organic matter derived from the slow-decomposing American beech leaf litter is not consumed by mesohumic endogeic earthworms, such as *O. cyaneum* and *E. lonnbergi*, until it becomes highly processed by soil

microbes. These findings are the first demonstration that soil-feeding (endogeic) earthworms in forests of different ages or successional stages comprise species that differ not only in their identities, but also in their functional ecology.

Leaf litter quality has been shown to be the main driver of earthworm abundance in temperate deciduous forests (Reich et al. 2005; Hobbie et al. 2006; de Schrijver et al. 2012; Schelfhout et al. 2017), but little is known regarding how it affects functional diversity of earthworm assemblages. Hobbie et al. (2006) documented that litter quality, particularly Ca content, is positively related to the abundance of litter-feeding anecic species. Our results further extended the observation of this phenomenon to include the soil-feeding endogeic species, and highlighted contrasting patterns between two distinct subgroups (polyhumic and mesohumic) within the endogeic earthworms. Two reasons explain why this structural and functional contrast has never been documented before. First, many published studies are monoculture common garden experiments (Reich et al. 2005; Hobbie et al. 2006; de Schrijver et al. 2012; Schelfhout et al. 2017) that lasted for 30–40 years. The relatively short period of time might not be enough for soil to develop to allow multiple mesohumic endogeic earthworms to colonize the plots. They lack the complexity of our diverse natural forests that with a mixture of fast-decomposing and slow-decomposing leaf litter have been established for more than 120 years. In fact, although our old forests are dominated by slow-decomposing leaf litter, fast-decomposing litter still constitutes about one-third of leaf litter input in fall. Second, earthworm functional groups have rarely been rigorously categorized in most ecological studies. Most studies use a simple, three-group system that lump epi-endogeic species, such as *L. rubellus*, and the true epigeic species, such as *Dendrobaena octaedra*, together as “epigeic species”, and do not differentiate among groups within endogeic species. While we recognize the limitations of using the concept of functional groups in earthworms (Neilson et al. 2000; Zicsi et al. 2011; Chang et al. 2016c), we also believe that our use of functional group categorization can also be more refined to reflect up-to-date knowledge of earthworm feeding ecology (e.g., Zicsi et al. 2011; Ferlian et al. 2014; Chang et al. 2016c; Moretti et al. 2017).

The high abundance of polyhumic endogeic species in the young forests may explain our observation that litter-feeding earthworms (epi-endogeic and anecic species) were not more abundant in the young forests than in the old forests, despite higher biomass of fast-decomposing leaf litter in the former. Polyhumic endogeic species are highly plastic. They feed not only on soil organic matter, but also on plant materials, which can even be an important part of their diet (Xia et al. 2011; Ferlian et al. 2014). One of the two dominant polyhumic endogeic species at SERC, *O. lacteum*, is also known to consume significant amount of leaf litter (Chang

et al. 2016c). The plasticity of polyhumic endogeic species may help them avoid intense competition with epi-endogeic species, and at the same time reduce the amount of leaf litter resource available for the co-existing epi-endogeic species, limiting their abundance.

Although our discussion focused on leaf litter and soil organic matter, other potential resources may be important. Belowground input of carbon from roots and root exudates has been shown to be a major energy source for the soil food web in temperate forests (Pollierer et al. 2007). Further studies are needed to understand how the quality of root-derived resources varies among tree species, and to quantify the contribution of root-derived carbon to the diet of earthworms, especially root-associated polyhumic endogeic species throughout the growing season.

### Increasing earthworm abundance in the old forests

The earthworm community at SERC is a dynamic system with year-to-year fluctuations. These fluctuations could be explained by the interplay of stochastic and deterministic factors such as leaf litter input, weather patterns and soil moisture (Eggleton et al. 2009). During the 19-year period, the increase in abundance in the old forests is particularly intriguing. Resource allocation of trees at SERC has been known to vary from one year to another due to precipitation pattern and drought during the growing season. Additionally, a greater than expected increase in forest biomass was detected and attributed to increasing temperature and CO<sub>2</sub> levels (McMahon et al. 2010). These changes are species-specific, and may disproportionally affect leaf litter inputs of different tree species to the soil ecosystem, which in turn, as we noted earlier, could have disproportional impacts on different earthworm species and/or functional groups, leading to distinct earthworm abundance dynamics in old and young forests. Further research with multi-year earthworm and leaf litter sampling will be required to test this hypothesis.

### The ‘second wave’ of earthworm invasion

Our grid sampling clearly showed that the Asian earthworms, presumably *Am. agrestis* and *M. hilgendorfi*, are well-established and invading into some of the SERC forest stands. Currently, these species are rapidly spreading in northeastern USA. Their high abundance (Görres et al. 2014) and impacts on the leaf litter layer, soil properties and biogeochemistry (Snyder et al. 2011; Greiner et al. 2012; Chang et al. 2016b, c, 2017; Qiu and Turner 2017) have become a source of changes in ecosystem processes and functions. With the possibility of outcompeting European earthworms, such as *L. rubellus* (Chang et al. 2016b; Laushman et al. 2018), and interrupting the interspecific interaction between *L. rubellus* and *O. lacteum*, they can

increase microbial biomass, potentially leading to soil carbon loss (Chang et al. 2017). Their activity also accelerates the release of nutrients from the leaf litter layer into surface soil, resulting in loss of nutrients through leaching (Qiu and Turner 2017). It is too early to predict what impacts the Asian earthworms may have on SERC forests. Given the different life histories and activity peaks between the European and Asian species, the two may coexist as long as sufficient leaf litter is on the ground throughout summer. Only long-term monitoring of the ongoing invasion and its impacts on soil biogeochemical processes will provide insight to the dynamics of the two non-native earthworm groups.

### Long-term dynamics of earthworm community

Four of the 14 species reported in this study occurred in our plots only once or twice during a time-span of nearly 20 years. Another two species that were only recently detected in the grid sampling were showing range expansion. Presumably, the occurrence of these species was the result of dispersal between regional and local species pools, supporting our hypothesis 4. Except for the invasion of the two Asian species, dispersal did not affect the long-term trajectory of earthworm communities, as ecological drift or competition pushed the involved species to local extinction. Although it is not possible to distinguish between competition and ecological drift as the cause of local extinction, we noted the potential importance of the latter in year-to-year differences. The potential importance of ecological drift applies not only to the species that appeared in only 1 year, such as *B. palustris* and *Am. corticis*, but also to abundance changes in species that were present regularly in our samples, such as *O. cyaneum* and *E. lonnbergi*. Although these factors cannot be directly tested or disentangled in our dataset, we conclude that earthworm communities at SERC are structured by not only selection (environmental filtering and competition), but also ecological drift and dispersal, with the latter two processes playing presumably important roles. These two processes not only might be important in maintaining a relatively high abundance, but also provide an alternative mechanism to explain the observed increase in the old forests.

Previous chronosequence studies on earthworm communities assumed that species composition and abundance of the undisturbed or climax vegetation is the endpoint of the successional trajectory. Earthworm abundance decreases as the forest reaches subclimax or climax stage (Pizl 1992). On the forest floor, succession is associated with accumulation of organic layer and a shift from mull to moder humus (Muys et al. 1992; Trap et al. 2011), presumably driven by changes in tree composition associated with a decrease in litter decomposition rate. Such transformations have been observed as early as 20 years after grassland afforestation

(Muys et al. 1992). At SERC we see no evidence of such successional trajectories. On one hand, lumbricid earthworms at SERC are well-established and we predict their long-term existence even in 150-year-old forests; the community is in an alternative stable state. The continuous presence of non-native earthworms also affects forest floor development. Previous work on soil properties indicates interplay between land use history and forest age at SERC (Ma et al. 2014; Yesilonis et al. 2016). At the same time, soils are on a different developmental trajectory from what would be anticipated solely based upon vegetation succession, and this different trajectory is primarily controlled by earthworm activity. On the other hand, some stands are experiencing drastic changes due to Asian earthworm invasion. SERC is surrounded by continuously increasing suburban residential and commercial areas that provide a continuous pool of synanthropic, peregrine species already existing at SERC or new to its forests. In these stands, the earthworm community is dynamic. To predict the outcome of the second wave of earthworm invasion requires further monitoring. Either way, we conclude that earthworms at SERC will remain a key group driving leaf litter decomposition, and the dynamics of soil organic matter and nutrients.

## Conclusion

We demonstrated that spatial and temporal earthworm community dynamics are determined by the interplay of selection, dispersal and ecological drift in a temperate deciduous forest in eastern USA during a period of 19 years, and highlighted that these dynamics have important implications for carbon cycling in these systems. Specifically, forests of different ages or at different successional stages are characterized by distinct species and functional groups of soil-feeding (endogeic) earthworms. We showed that these differences are a result of environmental filtering and are driven by leaf litter quality, particularly the abundance of slow-decomposing and fast-decomposing litter with distinct Ca content. In addition, we postulated that dispersal from the regional to the local species pools and local extinction caused by competition and ecological drift are the main cause of year-to-year species gains and losses. Contrary to most previous studies that earthworm abundance is greatly reduced in a climax forest, we predict that with continued dispersal and the ongoing second wave of earthworm invasion by Asian species, earthworms at SERC are well-established and their abundance is likely to stay stable or increase for the foreseeable future. Earthworms as a keystone group of soil fauna play an important role in the plant–soil feedback loop (Ponge et al. 1999) driving the system to different successional pathways. At SERC, with the development of moder humus and accumulation of O layer impeded by earthworm activity,

the trajectory of soil and forest floor development has been irreversibly altered.

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## References

- Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 26:32–46
- Anderson MJ, Ellingsen KE, McArdle BH (2006) Multivariate dispersion as a measure of beta diversity. *Ecol Lett* 9:683–693. <https://doi.org/10.1111/j.1461-0248.2006.00926.x>
- Anderson MJ et al (2011) Navigating the multiple meanings of beta diversity: a roadmap for the practicing ecologist. *Ecol Lett* 14:19–28. <https://doi.org/10.1111/j.1461-0248.2010.01552.x>
- Bates D, Maechler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48
- Bayoumi BM (1978) Significance of the microhabitat on the distribution of oribatid mites in a hornbeam-oak mixed forest. *Opuscula Zoologica (Budapest)* 15:51–57
- Bolker BM et al (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–135. <https://doi.org/10.1016/j.tree.2008.10.008>
- Bozarth MA, Farrish KW, Damoff GA, VanKley J, Young JL (2016) Spatial distribution of earthworms in an east Texas forest ecosystem. *Appl Soil Ecol* 104:91–103. <https://doi.org/10.1016/j.apsoil.2016.03.005>
- Briones MJI, Schmidt O (2017) Conventional tillage decreases the abundance and biomass of earthworms and alters their community structure in a global meta-analysis. *Glob Change Biol* 23:4396–4419. <https://doi.org/10.1111/gcb.13744>
- Brush GS, Lenk C, Smith J (1980) The natural forests of Maryland: an explanation of the vegetation map of Maryland. *Ecol Monogr* 50:77–92. <https://doi.org/10.2307/2937247>
- Chang CH, Snyder BA, Szlavecz K (2016a) Asian pheretimoid earthworms in North America north of Mexico: an illustrated key to the genera *Amyntas*, *Metaphire*, *Pithemera*, and *Polypheretima* (Clitellata: Megascotocidae). *Zootaxa* 4179:495–529. <https://doi.org/10.11646/zootaxa.4179.3.7>
- Chang CH, Szlavecz K, Buyer JS (2016b) Species-specific effects of earthworms on microbial communities and the fate of litter-derived carbon. *Soil Biol Biochem* 100:129–139. <https://doi.org/10.1016/j.soilbio.2016.06.004>
- Chang CH, Szlavecz K, Filley T, Buyer JS, Bernard MJ, Pitz SL (2016c) Belowground competition among invading detritivores. *Ecology* 97:160–170. <https://doi.org/10.1890/15-0551.1>

- Chang CH, Szlavecz K, Buyer JS (2017) *Amyntas agrestis* invasion increases microbial biomass in Mid-Atlantic deciduous forests. *Soil Biol Biochem* 114:189–199. <https://doi.org/10.1016/j.soilbio.2017.07.018>
- Chang C-H, Johnston M, Görres J, Davalos A, McHugh D, Szlavecz K (2018) Co-invasion of three Asian earthworms, *Metaphire hilgendorfi*, *Amyntas agrestis* and *Amyntas tokioensis* in the USA. *Biol Invasions*. <https://doi.org/10.1007/s10530-017-1607-x>
- Chase JM, Myers JA (2011) Disentangling the importance of ecological niches from stochastic processes across scales. *Philos Trans R Soc B Biol Sci* 366:2351–2363. <https://doi.org/10.1098/rstb.2011.0063>
- Cleveland CC et al (2014) Litter quality versus soil microbial community controls over decomposition: a quantitative analysis. *Oecologia* 174:283–294. <https://doi.org/10.1007/s00442-013-2758-9>
- Cote B, Fyles JW (1994) Nutrient concentration and acid-base status of leaf litter of tree species characteristic of the hardwood forest of southern Quebec. *Can J For Res Revue Canadienne De Recherche Forestiere* 24:192–196. <https://doi.org/10.1139/x94-027>
- Craven D et al (2017) The unseen invaders: introduced earthworms as drivers of change in plant communities in North American forests (a meta-analysis). *Glob Change Biol* 23:1065–1074. <https://doi.org/10.1111/gcb.13446>
- Crow SE et al (2009) Earthworms, stand age, and species composition interact to influence particulate organic matter chemistry during forest succession. *Biogeochemistry* 92:61–82. <https://doi.org/10.1007/s10533-008-9260-1>
- Crumsey JM, Le Moine JM, Vogel CS, Nadelhoffer KJ (2014) Historical patterns of exotic earthworm distributions inform contemporary associations with soil physical and chemical factors across a northern temperate forest. *Soil Biol Biochem* 68:503–514. <https://doi.org/10.1016/j.soilbio.2013.10.029>
- Csuzdi C, Zicsi A (2003) Earthworm of Hungary. Hungarian Natural History Museum, Budapest
- Csuzdi C, Chang C-H, Pavlíček T, Szederjesi T, Esopi D, Szlavecz K (2017) Molecular phylogeny and systematics of native North American lumbricid earthworms (Clitellata: Megadrili). *PLoS ONE* 12(8):e0181504. <https://doi.org/10.1371/journal.pone.0181504>
- de Schrijver A et al (2012) Tree species traits cause divergence in soil acidification during four decades of postagricultural forest development. *Glob Change Biol* 18:1127–1140. <https://doi.org/10.1111/j.1365-2486.2011.02572.x>
- De Wandeler H et al (2016) Drivers of earthworm incidence and abundance across European forests. *Soil Biol Biochem* 99:167–178. <https://doi.org/10.1016/j.soilbio.2016.05.003>
- Decaens T, Dutoit T, Alard D (1997) Earthworm community characteristics during afforestation of abandoned chalk grasslands (Upper Normandy, France). *Eur J Soil Biol* 33:1–11
- Decaens T, Margerie P, Aubert M, Hedde M, Bureau F (2008) Assembly rules within earthworm communities in North-Western France—a regional analysis. *Appl Soil Ecol* 39:321–335. <https://doi.org/10.1016/j.apsoil.2008.01.007>
- Dobson A, Blossey B (2015) Earthworm invasion, white-tailed deer and seedling establishment in deciduous forests of north-eastern North America. *J Ecol* 103:153–164. <https://doi.org/10.1111/1365-2745.12350>
- Dózsa-Farkas K (1978) Ecological importance of microhabitats in the distribution of some enchytraeid species. *Pedobiologia* 18:366–372
- Dunger W, Voigtlander K (2009) Soil fauna (Lumbricidae, Collembola, Diplopoda and Chilopoda) as indicators of soil eco-subsystem development in post-mining sites of eastern Germany—a review. *Soil Org* 81:1–51
- Eggleton P, Inward K, Smith J, Jones DT, Sherlock E (2009) A six year study of earthworm (Lumbricidae) populations in pasture woodland in southern England shows their responses to soil temperature and soil moisture. *Soil Biol Biochem* 41:1857–1865. <https://doi.org/10.1016/j.soilbio.2009.06.007>
- Eijssackers H (2011) Earthworms as colonizers of natural and cultivated soil environments. *Appl Soil Ecol* 50:1–13. <https://doi.org/10.1016/j.apsoil.2011.07.008>
- Eisenhauer N, Partsch S, Parkinson D, Scheu S (2007) Invasion of a deciduous forest by earthworms: changes in soil chemistry, microflora, microarthropods and vegetation. *Soil Biol Biochem* 39:1099–1110. <https://doi.org/10.1016/j.soilbio.2006.12.019>
- Eisenhauer N et al (2009) Plant community impacts on the structure of earthworm communities depend on season and change with time. *Soil Biol Biochem* 41:2430–2443. <https://doi.org/10.1016/j.soilbio.2009.09.001>
- Ernst G, Emmerling C (2009) Impact of five different tillage systems on soil organic carbon content and the density, biomass, and community composition of earthworms after a ten year period. *Eur J Soil Biol* 45:247–251. <https://doi.org/10.1016/j.ejsobi.2009.02.002>
- Fanin N, Fromin N, Bertrand I (2016) Functional breadth and home-field advantage generate functional differences among soil microbial decomposers. *Ecology* 97:1023–1037. <https://doi.org/10.1890/15-1263.1>
- Ferlian O, Cesarz S, Marhan S, Scheu S (2014) Carbon food resources of earthworms of different ecological groups as indicated by  $^{13}\text{C}$  compound-specific stable isotope analysis. *Soil Biol Biochem* 77:22–30. <https://doi.org/10.1016/j.soilbio.2014.06.002>
- Filley TR et al (2008) Comparison of the chemical alteration trajectory of *Liriodendron tulipifera* L. leaf litter among forests with different earthworm abundance. *J Geophys Res Biogeosci*. <https://doi.org/10.1029/2007jg000542>
- Frouz J et al (2008) Interactions between soil development, vegetation and soil fauna during spontaneous succession in post mining sites. *Eur J Soil Biol* 44:109–121. <https://doi.org/10.1016/j.ejsobi.2007.09.002>
- Görres JH, Melnichuk RDS, Bellitürk K (2014) Mortality pattern relative to size variation within *Amyntas agrestis* (Goto & Hatai 1899) (Oligochaeta: Megascolecidae) populations in the Champlain Valley of Vermont, USA. *Megadrilologica* 16:9–14
- Greiner HG, Kashian DR, Tiegs SD (2012) Impacts of invasive Asian (*Amyntas hilgendorfi*) and European (*Lumbricus rubellus*) earthworms in a North American temperate deciduous forest. *Biol Invasions* 14:2017–2027. <https://doi.org/10.1007/s10530-012-0208-y>
- Groffman PM et al (2015) Earthworms increase soil microbial biomass carrying capacity and nitrogen retention in northern hardwood forests. *Soil Biol Biochem* 87:51–58. <https://doi.org/10.1016/j.soilbio.2015.03.025>
- Hale CM, Frelich LE, Reich PB (2005) Exotic European earthworm invasion dynamics in northern hardwood forests of Minnesota, USA. *Ecol Appl* 15:848–860. <https://doi.org/10.1890/03-5345>
- Hale CM, Frelich LE, Reich PB (2006) Changes in hardwood forest understory plant communities in response to European earthworm invasions. *Ecology* 87:1637–1649. [https://doi.org/10.1890/0012-9658\(2006\)87\[1637:cihfup\]2.0.co;2](https://doi.org/10.1890/0012-9658(2006)87[1637:cihfup]2.0.co;2)
- Higman D (1968) An ecologically annotated checklist of the vascular flora at the Chesapeake Bay Center for field biology, with keys. Smithsonian Institution, Washington, DC
- Hobbie SE et al (2006) Tree species effects on decomposition and forest floor dynamics in a common garden. *Ecology* 87:2288–2297. [https://doi.org/10.1890/0012-9658\(2006\)87\[2288:tseoda\]2.0.co;2](https://doi.org/10.1890/0012-9658(2006)87[2288:tseoda]2.0.co;2)
- Holdsworth AR, Frelich LE, Reich PB (2012) Leaf litter disappearance in earthworm-invaded northern hardwood forests: role of tree species and the chemistry and diversity of litter. *Ecosystems* 15:913–926. <https://doi.org/10.1007/s10021-012-9554-y>
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biom J* 50(3):346–363



- Johnson EA, Miyanishi K (2008) Testing the assumptions of chronosequences in succession. *Ecol Lett* 11:419–431. <https://doi.org/10.1111/j.1461-0248.2008.01173.x>
- Laushman KM, Hotchkiss SC, Herrick BM (2018) Tracking an invasion: community changes in hardwood forests following the arrival of *Amyntas agrestis* and *Amyntas tokioensis* in Wisconsin. *Biol Invasions*. <https://doi.org/10.1007/s10530-017-1653-4>
- Loss SR, Blair RB (2011) Reduced density and nest survival of ground-nesting songbirds relative to earthworm invasions in northern hardwood forests. *Conserv Biol* 25:983–992. <https://doi.org/10.1111/j.1523-1739.2011.01719.x>
- Loss SR, Niemi GJ, Blair RB (2012) Invasions of non-native earthworms related to population declines of ground-nesting songbirds across a regional extent in northern hardwood forests of North America. *Landsc Ecol* 27:683–696. <https://doi.org/10.1007/s10980-012-9717-4>
- Ma Y, Filley TR, Johnston CT, Crow SE, Szlavecz K, McCormick MK (2013) The combined controls of land use legacy and earthworm activity on soil organic matter chemistry and particle association during afforestation. *Org Geochem* 58:56–68. <https://doi.org/10.1016/j.orggeochem.2013.02.010>
- Ma Y, Filley TR, Szlavecz K, McCormick MK (2014) Controls on wood and leaf litter incorporation into soil fractions in forests at different successional stages. *Soil Biol Biochem* 69:212–222. <https://doi.org/10.1016/j.soilbio.2013.10.043>
- Maerz JC, Nuzzo VA, Blossey B (2009) Declines in woodland salamander abundance associated with non-native earthworm and plant invasions. *Conserv Biol* 23:975–981. <https://doi.org/10.1111/j.1523-1739.2009.01167.x>
- Mariotte P, Le Bayon RC, Eisenhauer N, Guenat C, Buttler A (2016) Subordinate plant species moderate drought effects on earthworm communities in grasslands. *Soil Biol Biochem* 96:119–127. <https://doi.org/10.1016/j.soilbio.2016.01.020>
- McMahon SM, Parker GG, Miller DR (2010) Evidence for a recent increase in forest growth. *Proc Natl Acad Sci USA* 107:3611–3615. <https://doi.org/10.1073/pnas.0912376107>
- Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* 63:621–626. <https://doi.org/10.2307/1936780>
- Melody C, Schmidt O (2012) Northward range extension of an endemic soil decomposer with a distinct trophic position. *Biol Lett* 8:956–959. <https://doi.org/10.1098/rsbl.2012.0537>
- Moorhead D, Lashermes G, Recous S, Bertrand I (2014) Interacting microbe and litter quality controls on litter decomposition: a modeling analysis. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0108769>
- Moretti M et al (2017) Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits. *Funct Ecol* 31:558–567. <https://doi.org/10.1111/1365-2435.12776>
- Muys B, Lust N, Cranval PH (1992) Effects of grassland afforestation with different tree species on earthworm communities, litter decomposition and nutrient status. *Soil Biol Biochem* 24:1459–1466
- Neilson R, Boag B, Smith M (2000) Earthworm delta C-13 and delta N-15 analyses suggest that putative functional classifications of earthworms are site-specific and may also indicate habitat diversity. *Soil Biol Biochem* 32:1053–1061. [https://doi.org/10.1016/S0038-0717\(00\)00013-4](https://doi.org/10.1016/S0038-0717(00)00013-4)
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2016) *vegan: community ecology package*. R package version 2.3-5. <http://CRAN.R-project.org/package=vegan>
- Pecl GT et al (2017) Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science* 355:1389. <https://doi.org/10.1126/science.aai9214>
- Pizl V (1992) Succession of earthworm populations in abandoned fields. *Soil Biol Biochem* 24:1623–1628. [https://doi.org/10.1016/0038-0717\(92\)90160-y](https://doi.org/10.1016/0038-0717(92)90160-y)
- Pollierer MM et al (2007) The underestimated importance of below-ground carbon input for forest soil animal food webs. *Ecol Lett* 10:729–736. <https://doi.org/10.1111/j.1461-0248.2007.01064.x>
- Ponge JF et al (1999) Interactions between earthworms, litter and trees in an old-growth beech forest. *Biol Fertil Soils* 29:360–370. <https://doi.org/10.1007/s003740050566>
- Prescott CE (2010) Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry* 101:133–149. <https://doi.org/10.1007/s10533-010-9439-0>
- Qiu JX, Turner MG (2017) Effects of non-native Asian earthworm invasion on temperate forest and prairie soils in the Midwestern US. *Biol Invasions* 19:73–88. <https://doi.org/10.1007/s10530-016-1264-5>
- Ransom TS (2011) Earthworms, as ecosystem engineers, influence multiple aspects of a salamander's ecology. *Oecologia* 165:745–754. <https://doi.org/10.1007/s00442-010-1775-1>
- Raw F (1959) Estimating earthworm populations by using formalin. *Nature* 184:1661–1662
- Reich PB et al (2005) Linking litter calcium, earthworms and soil properties: a common garden test with 14 tree species. *Ecol Lett* 8:811–818. <https://doi.org/10.1111/j.1461-0248.2005.00779.x>
- Resner K et al (2015) Invasive earthworms deplete key soil inorganic nutrients (Ca, Mg, K, and P) in a northern hardwood forest. *Ecosystems* 18:89–102. <https://doi.org/10.1007/s10021-014-9814-0>
- R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.R-project.org/>
- Schelfhout S et al (2017) Tree species identity shapes earthworm communities. *Forests*. <https://doi.org/10.3390/f8030085>
- Scheu S (1992) Changes in the lumbricid coenosis during secondary succession from a wheat field to a beechwood on limestone. *Soil Biol Biochem* 24:1641–1646
- Scheu S, Falca M (2000) The soil food web of two beech forests (*Fagus sylvatica*) of contrasting humus type: stable isotope analysis of a macro- and a mesofauna-dominated community. *Oecologia* 123:285–296
- Schmidt O et al (2004) Dual stable isotope analysis ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of soil invertebrates and their food sources. *Pedobiologia* 48:171–180. <https://doi.org/10.1016/j.pedobi.2003.12.003>
- Schwarz B et al (2015) Non-significant tree diversity but significant identity effects on earthworm communities in three tree diversity experiments. *Eur J Soil Biol* 67:17–26. <https://doi.org/10.1016/j.ejsobi.2015.01.001>
- Simmons W, Davalos A, Blossey B (2015) Forest successional history and earthworm legacy affect earthworm survival and performance. *Pedobiologia* 58:153–164. <https://doi.org/10.1016/j.pedobi.2015.05.001>
- Skaug H, Fournier D, Bolker B, Magnusson A, Nielsen A (2016) Generalized linear mixed models using 'AD Model Builder'. R package version 0.8.3.3
- Snyder BA, Callahan MA Jr, Hendrix PF (2011) Spatial variability of an invasive earthworm (*Amyntas agrestis*) population and potential impacts on soil characteristics and millipedes in the Great Smoky Mountains National Park, USA. *Biol Invasions* 13:349–358. <https://doi.org/10.1007/s10530-010-9826-4>
- Swift MJ, Heal OW, Anderson JM (1979) *Decomposition in terrestrial ecosystems*. Blackwell, Oxford
- Szlavecz K, Csuzdi C (2007) Land use change affects earthworm communities in Eastern Maryland, USA. *Eur J Soil Biol* 43:S79–S85. <https://doi.org/10.1016/j.ejsobi.2007.08.008>
- Szlavecz K et al (2011) Ecosystem effects of non-native earthworms in Mid-Atlantic deciduous forests. *Biol Invasions* 13:1165–1182. <https://doi.org/10.1007/s10530-011-9959-0>



- Trap J et al (2011) Does moder development along a pure beech (*Fagus sylvatica* L.) chronosequence result from changes in litter production or in decomposition rates? *Soil Biol Biochem* 43:1490–1497. <https://doi.org/10.1016/j.soilbio.2011.03.025>
- Vellend M (2010) Conceptual synthesis in community ecology. *Q Rev Biol* 85:183–206
- Vellend M (2016) *The theory of ecological communities*. Princeton University Press, Princeton
- Walker LR, Wardle DA, Bardgett RD, Clarkson BD (2010) The use of chronosequences in studies of ecological succession and soil development. *J Ecol* 98:725–736. <https://doi.org/10.1111/j.1365-2745.2010.01664.x>
- White DL, Haines BL, Boring LR (1988) Litter decomposition in southern Appalachian black locust and pine hardwood stands—litter quality and nitrogen dynamics. *Can J For Res* 18:54–63. <https://doi.org/10.1139/x88-009>
- Wilson-Kokes L, Skousen J (2014) Nutrient concentrations in tree leaves on brown and gray reclaimed mine soils in West Virginia. *Sci Total Environ* 481:418–424. <https://doi.org/10.1016/j.scitotenv.2014.02.015>
- Xia L, Szlavecz K, Swan CM, Burgess JL (2011) Inter- and intra-specific interactions of *Lumbricus rubellus* (Hoffmeister, 1843) and *Octolasion lacteum* (Orley, 1881) (Lumbricidae) and the implication for C cycling. *Soil Biol Biochem* 43:1584–1590. <https://doi.org/10.1016/j.soilbio.2011.04.009>
- Yeates GW (1981) Soil nematode populations depressed in the presence of earthworms. *Pedobiologia* 22:191–195
- Yesilonis I, Szlavecz K, Pouyat R, Whigham D, Xia L (2016) Historical land use and stand age effects on forest soil properties in the Mid-Atlantic US. *For Ecol Manag* 370:83–92. <https://doi.org/10.1016/j.foreco.2016.03.046>
- Zhang D, Hui D, Luo Y, Zhou G (2008) Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *J Plant Ecol* 1:85–93. <https://doi.org/10.1093/jpe/rtn002>
- Zicsi A, Szlavecz K, Csuzdi C (2011) Leaf litter acceptance and cast deposition by peregrine and endemic European lumbricids (Oligochaeta: Lumbricidae). *Pedobiologia* 54:S145–S152. <https://doi.org/10.1016/j.pedobi.2011.09.004>