

Invasive shrub cover and tree species composition influence exotic earthworms

Gwendolyn Lloyd*, Michael B. Mahon, Thomas O. Crist

Department of Biology, 700 E High Street, Miami University, Oxford, OH 45056, USA



ARTICLE INFO

Keywords:

Lonicera maackii
Tree composition
Earthworms
Leaf litter
Deciduous forest
Above-belowground interactions

ABSTRACT

Forest ecosystems are often invaded by multiple exotic species that, when combined, may amplify negative impacts on native biodiversity and ecosystem functioning. These patterns are often determined from plot-level experiments, but it is less clear how interactions among native and exotic species vary across landscapes. Amur honeysuckle (*Lonicera maackii*) is an invasive understory shrub in eastern forests, observed to reduce understory plant diversity and alter litter decomposition rates. Exotic earthworms may aid this process by increasing decomposition rates and nutrient fluxes in forest soils. We hypothesized that honeysuckle increases exotic earthworm density and biomass in forest soils by providing a preferred litter resource and beneficial abiotic conditions. We established 15 transects, each 100-m long, in a stratified random design across a 245-ha deciduous forest in southwestern Ohio, USA. We measured honeysuckle shrub cover and canopy tree cover along the entire transect, and sampled earthworms and standing litter biomass at intervals along each transect. We found little evidence for Amur honeysuckle effects on earthworm density but found a strong positive effect of honeysuckle cover on earthworm biomass. We also recorded an effect of species composition of canopy trees on earthworm density and biomass. Moreover, multivariate analyses indicated a shift of earthworm community composition along an oak-elm gradient of tree species composition. Standing litter biomass was lowest in areas of high earthworm biomass and in the forest interior. Our results demonstrate that landscape-level variation in tree species composition and honeysuckle invasion drive exotic earthworm density and biomass. Our findings suggest invasion of exotic shrubs may facilitate invasion by nonnative earthworms.

1. Introduction

Nonnative invasive species have deleterious effects where they are established, by altering ecosystem processes and reducing native biodiversity (Simberloff and Von Holle, 1999). Meta-analyses of plant invasions suggest that their impacts are variable and context dependent, but often include changes in rates of nutrient and carbon cycling and shifts in both above- and below-ground communities (Vilà et al., 2011). Plant invasion reduces abundance and diversity of native plants (Ashton et al., 2005; Vilà et al., 2011), thereby weakening mutualistic relationships between native plants and microorganisms (Seifert et al., 2009; Vogelsang and Bever, 2009). Invasive plants also alter plant community structure by reducing growth and fitness of native plant species (Vilà et al., 2011). Conversely, alien plants can increase microbial activity, litter decomposition, and available nitrogen, resulting in higher aboveground production and biomass (Ashton et al., 2005). In many cases, the presence of one invasive species facilitates future invasions, by priming the ecosystem for the invasion of other nonnative

species, resulting in invasion meltdown (Simberloff and Von Holle, 1999).

Human disturbance and horticultural practices have aided non-native plant establishment in eastern deciduous forests (Webster et al., 2006). Originally introduced for soil erosion mitigation in the early 1900s, Amur honeysuckle (*Lonicera maackii*) is one such plant (Luken and Thieret, 1996). Amur honeysuckle invasion has broad impacts on plant and animal communities, as well as ecosystem processes (McNeish and McEwan, 2016). Amur honeysuckle shades out native herbaceous plants, due to its extended leaf phenology, reducing native plant growth and reproductive success (Miller and Gorshov, 2004). Amur honeysuckle also has negative effects on native plant growth and reproduction through allelopathic interference, though these effects may be dependent on soil conditions (Bauer et al., 2012; Cipollini et al., 2012; McNeish and McEwan, 2016). Additionally, microbial communities on Amur honeysuckle leaves are distinct from those found on native litter, which may alter decomposition rates of litter and nutrient cycling in soils near honeysuckle shrubs (Arthur et al., 2012; Poulette

* Corresponding author.

E-mail address: lloydg@miamioh.edu (G. Lloyd).

<https://doi.org/10.1016/j.foreco.2019.05.049>

Received 5 February 2019; Received in revised form 17 May 2019; Accepted 18 May 2019

0378-1127/ © 2019 Elsevier B.V. All rights reserved.

and Arthur, 2012). Amur honeysuckle litter decomposes rapidly and, combined with early leaf phenology, provides an early season influx of nitrogen into forest soils (Arthur et al., 2012), altering both above and below-ground aspects of forest ecosystems (Ashton et al., 2005). Moreover, high quality Amur honeysuckle litter (low C:N) may be preferentially selected by earthworms (Hendriksen, 1990), and may facilitate earthworm invasion (Pipal, 2014).

Earthworm populations are driven by many factors, including nutrient availability, soil structure, soil moisture, large herbivores, and human activity (Curry, 2004; De Wandeler et al., 2016; Mahon and Crist 2019). Areas dominated by tree and shrub species with less palatable litter (high C:N; e.g. *Quercus* spp.) typically support lower earthworm densities, while earthworm density is greater in areas with highly palatable leaf litter (low C:N; e.g. Amur honeysuckle; Hendriksen, 1990). Invading exotic earthworms are ecosystem engineers, and accelerate leaf litter decomposition, alter nutrient availability, and stimulate microbial growth (Eisenhauer, 2010). In turn, these altered ecosystem properties benefit invasive plant species competing with native plants (Heneghan et al., 2007; Whitfield et al., 2014). Earthworm functional groups (anecic, endogeic, and epigeic) have varying impacts on ecosystem processes. Anecic earthworms have vertical burrows and move leaf litter from the surface deep into the soil column (Bohlen et al., 2004), bringing nutrients into the mineral soil and shifting microbial community composition from fungal dominated to bacteria dominated (Dempsey et al., 2011; Ferlian et al., 2018). Endogeic earthworms are found in the upper mineral soil and facilitate microbial growth by providing nutrients within their casts (Bohlen et al., 2004; Eisenhauer, 2010). Within the litter layer, epigeic earthworms facilitate litter decomposition by priming leaves for microbial growth (Ferlian et al., 2018).

Our study aimed to understand how landscape-level variation in tree-species composition and Amur honeysuckle abundance affect invasive earthworms and standing litter biomass in a temperate, deciduous forest of southwest Ohio. We selected 15 transects in a stratified random design across areas that differed in honeysuckle cover, and along each transect we recorded the species composition of canopy trees, litter biomass, as well as earthworm abundance, composition, and biomass. We hypothesized earthworm density and biomass would be higher in areas with greater Amur honeysuckle cover. Additionally, we hypothesized a shift in earthworm community composition along a gradient of Amur honeysuckle; specifically, we expected more anecic and endogeic individuals in areas with higher honeysuckle cover. Based on the hypothesized patterns of earthworm density, we expected less standing litter biomass in areas of high Amur honeysuckle cover. Alternatively, environmental factors other than honeysuckle cover, such as dominant tree canopy species, soil moisture, and canopy cover, may be important drivers of earthworm density.

2. Methods

2.1. Study area and sampling design

The study was conducted within the mid- to late-successional deciduous forests of the Miami University Natural Areas (N 39°31'1.71", W 84°42'34.61") in southwest Ohio, USA. Between 1938 and 1995, the Natural Areas were converted from row-crop cultivation and cattle pasture to mid- and late-successional forest (Medley and Krisko, 2007). Climate of the study area is temperate continental with an average annual temperature of 11 °C and average annual rainfall of 110 cm. The area is characterized by loess soils, rolling bedrock shale, and limestone hills influenced by the Wisconsin Glaciation (Lerch et al., 1980). Dominant soils of the study area are fine, mixed active Hapludalfs (Lerch et al., 1980). Dominant hardwood species at these sites include sugar maple (*Acer saccharum*), oak (*Quercus* spp.), elm (*Ulmus* spp.), ash (*Fraxinus* spp.), and hickory (*Carya* spp.). Amur honeysuckle (*Lonicera maackii*) dominated the understory of these sites, though common

understory shrubs and trees include Ohio buckeye (*Aesculus glabra*), multiflora rose (*Rosa multiflora*), and common pawpaw (*Asimina triloba*). The study was conducted prior to the invasion of the emerald ash borer (*Agrilus planipennis*) at our study area.

In summer 2015, we placed 15 transects, each 100 m in length, in the 245-ha study area according to a stratified random design. This design includes landscape-level variation in tree species composition and honeysuckle invasion, whereas most previous studies of earthworm-honeysuckle interactions were conducted within a few forest stands or plots. The number of transects was weighted to the relative size of each forest stand, with smaller stands receiving fewer transects compared to larger stands (Fig. S1). We also avoided areas with high densities of coniferous trees, as earthworms typically avoid acidic soils under these stands (Haimi and Einbork, 1992). We chose a stratified random design for this study, rather than selecting transects along a set honeysuckle gradient, to reflect the range of variation in the distribution of honeysuckle in this region. These sites span the existing topographic variation and differences in land-use history; sampling designs using gradients of tree species composition or honeysuckle invasion would not represent the variation of these factors across the larger landscape. The stratified random design of transect placement resulted in a range of understory honeysuckle cover from 6.1 to 66.6%.

2.2. Transect measurements

Along each 100-m transect, we used the line intercept method to provide an estimate of honeysuckle shrub cover and canopy tree composition. Honeysuckle cover and canopy tree cover were recorded as segments of each transect where shrub and tree canopies vertically intersected the transect. Percent cover was calculated from the summed length of these segments divided by the total transect length (Caratti, 2006). Using ArcGIS (version 10.3), we found distance to forest edge for each transect by finding the shortest distance from the midpoint of each transect to the delineated forest edge. Elevation and percent slope for each sampling point were found using Digital Elevation Model (DEM) data with a spatial resolution of 2.5 m. We calculated a mean normalized difference vegetation index (NDVI) value from buffer area of 10 m surrounding each transect using Landsat 8 OLI imagery from 4 August 2015 using ENVI 5.4.

2.3. Earthworm and litter sampling

We collected leaf litter, earthworms, and soil samples at three points at 16.7, 50, and 83.3 m along each transect. Samples were collected between 12 June 2015 and 9 July 2015. All samples from a transect were collected on the same day. Soil cores were collected at a depth of 10 cm using a 2-cm diameter corer. Leaf litter and earthworms were collected from 0.25-m² quadrats at each sampling point along each transect. Prior to earthworm sampling, we collected leaf litter and woody detritus so that only the bare mineral soil remained. We sampled earthworms using the mustard extraction technique (Gunn, 1992) with 3.1 L of mustard solution applied to each 0.25-m² quadrat at a ratio of 10 g mustard powder per liter of water. Earthworms that came to the soil surface within 20 min of mustard solution application were collected and placed in 70% ethanol. Earthworms were later identified to genus and species when possible (only mature earthworms can reliably be identified to species). Overall earthworm biomass (ethanol wet mass) was found for each sample by placing earthworms on paper towels and allowing them to air dry. We dried soil samples at 60 °C to constant mass for a measure of gravimetric soil moisture content. We removed woody detritus from the leaf litter and sifted leaf litter through a no. 4 sieve (4.75 mm openings) to remove dirt and small litter particles. Leaf litter was then dried at 60 °C to constant mass as a measure of standing litter biomass.

2.4. Statistical analyses

We conducted statistical analyses on earthworm density, earthworm biomass, and standing litter biomass using the R programming language version 3.5 (R Core Team, 2018). Data on the species composition of dominant canopy trees were reduced to multivariate axes using principal component analysis (PCA; `prcomp` function in base R). We defined dominant tree species as those with > 5% mean canopy cover across all transects. The first two PCA axes (those accounting for most of the variation) were used as predictor variables in our regression analyses. Prior to regression analyses, we tested random effects of transect and forest stand, but found that these did not account for any additional variance, so we dropped random terms from our models. Therefore, we used generalized linear models (GLMs) with a negative binomial distribution (`glm.nb` function, MASS package, R; Venables and Ripley, 2002) for analysis of earthworm density, and a Gaussian distribution for analysis of earthworm biomass and standing litter biomass (`glm` function, stats package, R; R Core Team, 2018). Earthworm biomass and standing litter biomass were natural-log transformed prior to all analyses. Predictor variables included the first two PCA axes, honeysuckle cover, elevation, percent slope, soil moisture, and distance to forest edge. We also included earthworm biomass as a predictor variable in standing litter biomass analyses. We used the lowest bias-corrected Akaike's Information Criterion (AICc) to select best and competing models ($\leq 2 \Delta AICc$). To compare best models against null models (intercept only), we used likelihood ratio tests and inferred statistical significance with p -values < 0.05.

To determine the role of dominant tree composition, honeysuckle cover, and forest productivity on earthworm community composition, we conducted a constrained ordination using the first two axes from the canopy tree PCA, honeysuckle cover, and NDVI in a distance-based redundancy analysis (dbRDA) with Bray–Curtis dissimilarity using square-root transformed abundance data (McArdle and Anderson, 2001). We used Akaike's Information Criterion (AIC) to select the best-fitting ordination model and obtained P -values using random permutations (9999 permutations). The dbRDA was conducted using the “`dbRDA`” function in `vegan`, R (Okansen et al., 2018). Since the species by sample matrix would have been too sparse without juvenile earthworms, we included juveniles identified to genus in multivariate analyses. We removed singleton earthworms (*Aporrectodea longa*) to reduce the effect of rare species on the analyses. We pooled data from the three samples taken along each transect, and all 15 transects were included in the community analyses.

3. Results

3.1. Tree canopy composition

The first two axes of the PCA analysis of canopy tree species explained 60.0% of total variance and revealed two gradients of tree species composition (Fig. 1). PCA axis 1 (36.3% of variance) was positively correlated with elm, but negatively correlated with oak. PCA axis 2 (23.7% of variance) was positively associated with sugar maple and ash and negatively associated with hickory.

3.2. Earthworms

We collected 979 individuals from nine species within three earthworm genera (*Aporrectodea*, *Lumbricus*, and *Octolasion*; Table S1). The endogeic juveniles of *Aporrectodea* comprised 23% of individuals collected. We identified five species of *Aporrectodea* (4% of individuals): *A. longa*, *A. rosea*, *A. trapezoides*, *A. tuberculata*, and *A. turgida*. We collected only a single individual of the anecic *Aporrectodea longa*, while another anecic earthworm, *Lumbricus terrestris* represented 4% of individuals collected. *Lumbricus rubellus* was the most common mature earthworm collected (7% of individuals). Juvenile *Lumbricus* were the most common earthworms, representing 55% of individuals collected. *Octolasion* earthworms were less common (7%), with *O. cyaneum* and *O.*

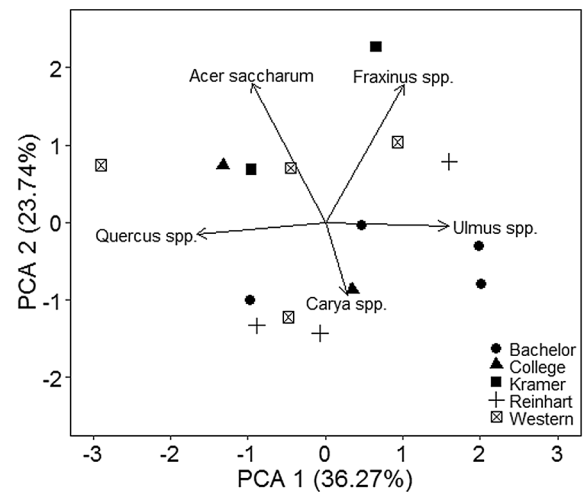


Fig. 1. Principal Component Analysis (PCA) using the five dominant canopy tree species recorded along 15 transects 100 m in length. Shapes represent the five forest stands in which transects were located (circles – Bachelor Preserve, triangles – College Woods, filled squares – Kramer Woods, plus – Reinhart Preserve, square with x – Western Woods).

tyrtaeum collected.

Earthworm density ranged from 1 to 79 individuals 0.25 m^{-2} , while earthworm biomass ranged from 0.03 to $13.81 \text{ g } 0.25 \text{ m}^{-2}$. Density was best explained by a model with PCA axis 1, PCA axis 2, and NDVI (Fig. 2; $w = 0.24$; Adj. $R^2 = 0.39$; $LR_{3,41} = 13.66$, $p = 0.003$), and was

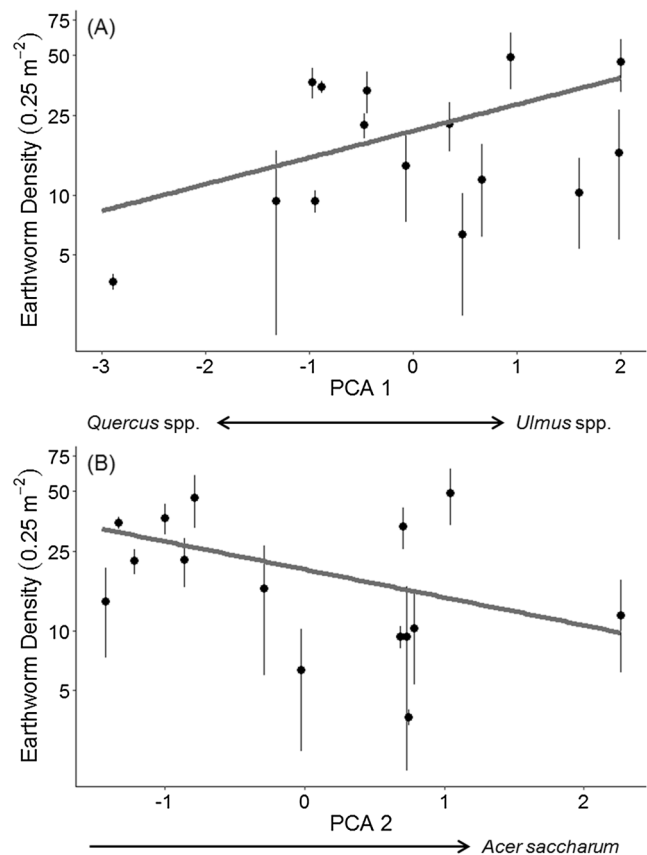


Fig. 2. Partial regression plots of earthworm density and principal components analysis of tree species composition, PCA 1 (A) and PCA 2 (B). Values for earthworm density represent the mean of each transect, with error bars representing standard error. Trendlines represent the coefficient from best model via AICc model selection. Note y-axes are on natural log scale.

Table 1

Model selection using lowest bias-corrected Akaike's Information Criterion (AICc) to select best and competing models ($\leq 2 \Delta AICc$). (–) in front of a predictor indicates a negative model coefficient.

	df	AICc	$\Delta AICc$	w	Adj. R ²
Earthworm Density					
PCA1 + (–) PCA2 + (–) NDVI	5	364.96	0	0.24	0.387
Earthworm Biomass					
HSCover + PCA1 + (–) PCA2	5	149.07	0	0.12	0.369
PCA1 + (–) PCA2	4	149.74	0.66	0.09	0.338
PCA1 + (–) PCA2 + Slope	5	149.98	0.91	0.08	0.356
HSCover + PCA1 + (–) PCA2 + Slope	6	150.28	1.21	0.07	0.374
Standing Litter Biomass					
(–) DistEdge + (–) Worm Mass	4	115.38	0	0.07	0.370
(–) DistEdge + (–) HSCover	4	115.45	0.07	0.06	0.369
(–) DistEdge + (–) Worm Mass + (–) HSCover	5	116.22	0.84	0.04	0.379
(–) DistEdge + (–) Worm Mass + (–) PCA1	5	116.42	1.04	0.04	0.376
(–) DistEdge + (–) PCA1	4	116.83	1.45	0.03	0.349
(–) DistEdge + (–) HSCover + (–) PCA1	5	116.92	1.54	0.03	0.369
(–) DistEdge + NDVI + (–) Worm Mass	5	117.17	1.78	0.03	0.365

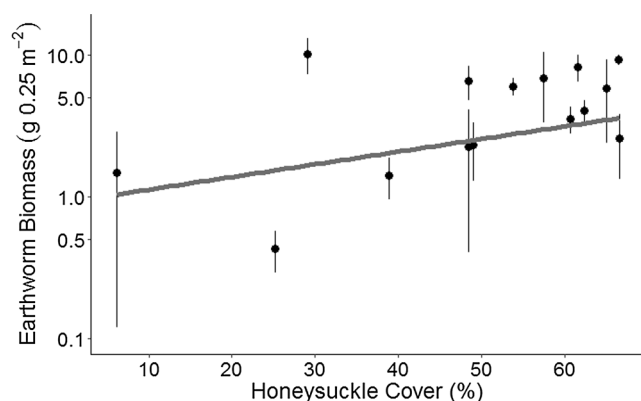


Fig. 3. Conditional plot of earthworm biomass and honeysuckle cover in a general linear model that also included tree species composition (PCA 1 and 2). Points are transect mean earthworm biomass, error bars are \pm SE. Note y-axis is on natural log scale.

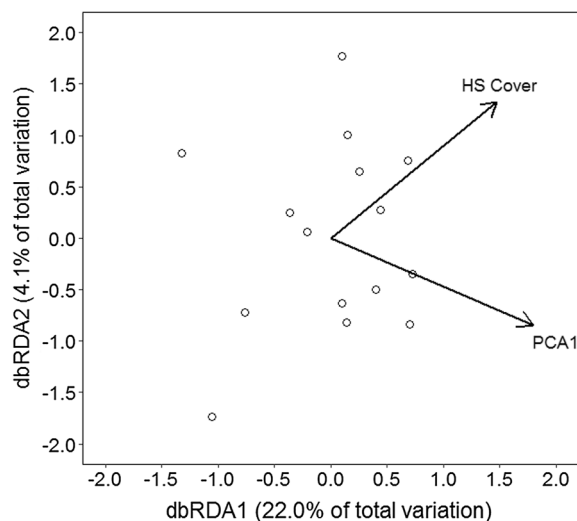


Fig. 4. Constrained multivariate ordination (dbRDA) of earthworm community composition across 15 transects. Symbols indicate site scores of transects. Arrows are biplot correlations of the significant predictor variable (PCA 1) and established honeysuckle cover gradient (HS Cover). PCA 1 and honeysuckle cover explain 26.1% of variation in earthworm community composition.

higher in forest stands with more elm than oak (PCA 1; Fig. 2A) and with less maple (PCA 2; Fig. 2B). Earthworm density was lower in areas of higher NDVI as a measure of total canopy cover. There were no competing models (Table 1).

Overall earthworm biomass was best explained by honeysuckle cover, PCA axis 1, and PCA axis 2 (Fig. 3; $w = 0.12$; Adj. $R^2 = 0.37$; $F_{3,41} = 9.56$, $p < 0.001$). There were several competing models that included these variables and slope (Table 1). Like earthworm density, overall earthworm biomass was positively related to PCA axis 1 and negatively related to PCA axis 2. Earthworm biomass was higher in forest stands with more elm and hickory, while earthworm biomass was lower in forest stands with more oak, maple, and ash. Earthworm biomass was positively related to honeysuckle cover across the study transects (Fig. 3). Finally, there was a weak positive association between earthworm biomass and slope, over the range of slopes within our study area.

Constrained ordinations of earthworm community composition using dbRDA showed that the best model included a single predictor variable with PCA 1 of tree composition (Pseudo- $F = 3.01$, $p = 0.017$, $R^2 = 0.19$; Fig. 4). Honeysuckle cover explained 14% of the variation in earthworm species composition, but permutation tests indicated that it was not statistically significant at the $p < 0.05$ (HS Cover, Pseudo- $F = 2.12$, $p = 0.069$, $R^2 = 0.14$). PCA 2 of canopy tree composition and NDVI were less important and not significant predictors of earthworm species composition (PCA 2, Pseudo $F = 1.24$, $p = 0.300$, $R^2 = 0.09$; NDVI, Pseudo $F = 0.84$, $p = 0.546$, $R^2 = 0.06$).

3.3. Standing litter biomass

Standing litter biomass ranged from 1.1 to 141.1 g per 0.25 m^{−2} (Table S2). Variation in standing litter biomass was best explained by earthworm biomass and distance to forest edge (Fig. 5; $w = 0.07$; $R^2 = 0.37$; $F_{2,42} = 13.92$, $p < 0.001$). Competing models included honeysuckle cover and PCA axis 1 (Table 1). Standing litter biomass was strongly negatively related to distance to edge, with more leaf litter found near the forest edge than in the forest interior. Standing litter biomass was also strongly negatively related to earthworm biomass. There was a weak negative relationship between standing litter biomass and both honeysuckle cover and PCA axis 1. Conversely, standing litter biomass tended to be higher in areas with greater canopy cover (NDVI).

4. Discussion

Our results support our hypothesis that areas of high honeysuckle cover contain higher earthworm biomass. However, there was little support for our hypothesis that honeysuckle cover would be positively

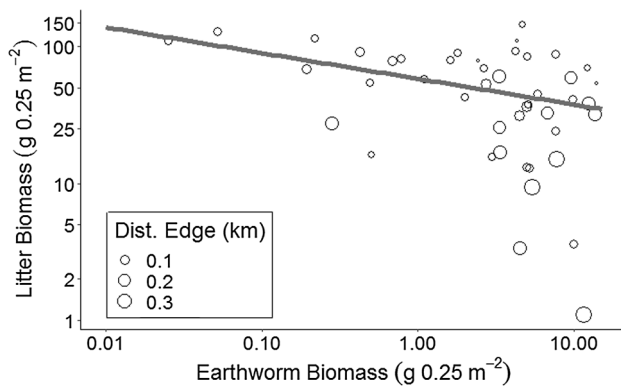


Fig. 5. Conditional plot of standing litter biomass with earthworm biomass in a general linear model that also included distance to forest edge. Point size corresponds to distance to forest edge. Note x- and y-axes are on natural log scales.

related to earthworm density, as density was associated with the canopy tree species composition and total canopy cover as measured by NDVI. These findings support our alternative hypothesis, which states that environmental factors other than honeysuckle cover may have a stronger influence on earthworm density. We also found little support for our hypothesis that honeysuckle cover directly reduces standing litter biomass. Multivariate analyses indicated earthworm community composition is related to dominant tree composition. The positive relationship observed between exotic earthworms and invasive honeysuckle is consistent with previous studies (Madrith and Lindroth, 2009; Pipal, 2014). Our study is the first to demonstrate that landscape-level variation in invasive plant abundance corresponds with areas of greater biomass of invasive earthworms, adding to a growing body of work investigating the relationship between invasive plants and earthworms (Bohlen et al., 2004; Dávalos et al., 2015; Heneghan et al., 2007; Madritch and Lindroth, 2009; Pipal, 2014).

Earthworm biomass and density were best explained by PCA axis 1 and PCA axis 2. The PCA axes represent stand-level variation in the dominant canopy trees, which, in turn, should reflect differences in leaf litter quality on the forest floor. There was greater earthworm density and biomass at sites that were elm dominated compared to those that were oak dominated. Additionally, there was lower earthworm density at sites dominated by maples. Moreover, these changes in abundance and biomass along the oak-elm gradient (PCA axis 1) correspond with a shift in earthworm community composition, indicating that earthworm species composition is partially structured by dominant canopy trees. Much of the unexplained variation in earthworm community composition is likely attributable to time since earthworm invasion, as earthworm community composition follows a predictable, generalized pattern after initial invasion (Hale et al. 2005). Nonetheless, the partial structuring of earthworm community composition by dominant canopy trees is supported by previous studies that have shown tree identity is a strong driver of earthworm communities (De Wandeler et al., 2018; Neiryck et al., 2000).

Due to previous land-use history of these forests and the patchiness of current tree species composition, this PCA gradient likely represents both topographic variation and a heterogeneous legacy of disturbance, as some oaks may be remnant shade trees from pastures, while elms were more dominant along stream terraces. Alternatively, changes in tree species composition along the first PCA axis 1 may correspond to high lignin oak litter to low lignin elm leaves (Kucera, 1959; Melillo et al., 1982), though we did not specifically measure lignin content in litter samples. Prior studies have found that areas dominated by trees with higher lignin leaves (e.g. oak) have lower earthworm abundance relative to areas mainly with trees containing low lignin content leaves (e.g. elm; De Wandeler et al., 2018; Hendriksen, 1990). Moreover, growth rates of *L. terrestris* are negatively related to lignin content of

litter (Kasurinen et al., 2007). The PCA axis 2 may reflect changes in soil calcium, as hickory leaves have higher calcium content than both maple and ash leaves (Côté and Fyles, 1994). Reich et al. (2005) reported an increase across all earthworm functional groups in areas where calcium rich litter was added. In addition to tree species composition, total canopy cover, as measured by average NDVI along each transect, was a strong predictor of earthworm density. This suggests that canopy cover (or aboveground productivity) may limit earthworm density, possibly due to the litter resources available (Campana et al., 2002).

We observed a positive relationship between exotic earthworms and invasive honeysuckle in the study area, suggesting that honeysuckle invasion may alter the ecosystem in such a way that may facilitate an increase in invasive earthworm biomass. Alternatively, the inverse effect is possible, as earthworms may alter the soil composition in a way that facilitates honeysuckle establishment. Increased nutrient availability, caused by longer leaf senescence period, benefits exotic earthworms that prefer the nutrient rich leaf litter (low C:N), as it provides a more readily available food source with a lower energy investment for more nutrients (Pipal, 2014). Compared to native shrub species, Amur honeysuckle is a higher quality litter source, as it decomposes more rapidly and has a higher amount of nitrogen than native tree species (Arthur et al., 2012). In other southwest Ohio forests similar to the study area, Amur honeysuckle leaves decomposed 21 times more rapidly than red oak leaves, causing changes in soil nutrient fluxes at times of the year when nutrients found within native leaf litter are inaccessible to detritivores (Blair and Stowasser, 2009).

The influence of canopy tree composition and honeysuckle cover on earthworm biomass corresponded to reductions in standing litter biomass. Standing litter biomass was best explained by earthworm biomass and distance to forest edge, while there was a closely competing model of honeysuckle cover and distance to forest edge. These findings are unique to our study, as the relationship between earthworms and honeysuckle has been studied smaller scales rather than at a forest level. Prior studies have found increased litter decomposition in the presence of higher earthworm biomass (Heneghan et al., 2007; Pipal, 2014) and with greater density of honeysuckle shrubs (Blair and Stowasser, 2009; Trammell et al., 2012). Additionally, previous studies have found a relationship between the distance to forest edge and decomposition mediated through changes in soil moisture (Riutta et al., 2012). Mesic areas typically have greater earthworm density and more microbial activity than xeric environments, resulting in increased litter decomposition (Szlavecz et al., 2011). Seasonal effects may alter the microbial community through environmental conditions, resulting in similar conditions at the forest edge and forest interior in drier seasons (Riutta et al., 2012). Moreover, the weak positive relationship between NDVI and litter biomass, while not improving model fit, does provide some support for differences in litter fall influencing standing litter biomass (Yang et al., 2017).

5. Conclusion

Invasive plants and exotic earthworms are major drivers of environmental change, specifically native plant loss and alterations to ecosystem processes (Frelich et al., 2006; Vilà et al., 2011). Our landscape-level study shows that invasive honeysuckle cover and invasive earthworm biomass are linked, supporting the findings of Madritch and Lindroth (2009) and Pipal (2014). Our findings also support a relationship between dominant canopy trees and exotic earthworm community composition (De Wandeler et al., 2018). While previous studies have investigated the effects of invasive plant removal on earthworms, our study is the first to investigate exotic earthworms along a landscape gradient of invasive plant cover. The honeysuckle gradient that was randomly sampled for this study reflects the topographic variation and land-use history in this region. These results, together with studies that show earthworm invasion facilitates plant

invasion (Madritch and Lindroth, 2009; Pipal, 2014), support the possibility of invasion meltdown occurring between invasive earthworms and Amur honeysuckle. Future studies should investigate the possible beneficial reciprocal relationship between honeysuckle and exotic earthworms, specifically along the honeysuckle invasion front. Additionally, while we focused on a single landscape, having a regional perspective may provide greater insight into the invasion patterns of both organisms. Understanding the large-scale drivers between honeysuckle and earthworms are necessary to improve management strategies across invasion gradients in eastern deciduous forests.

Declaration of Competing Interest

None. No funding sources had any involvement in study design or analysis and interpretation of data.

Acknowledgements

We would like to thank T. Meyer for assistance in the collection of field samples. We are grateful for the Miami University Natural Areas for permitting us to sample earthworms within the boundary of their preserves.

Funding

This work was supported by the National Science Foundation [NSF DBI-1460518]; Miami University.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2019.05.049>.

References

- Arthur, M.A., Bray, S.R., Kuchle, C.R., McEwan, R.W., 2012. The influence of the invasive shrub, *Lonicera maackii*, on leaf decomposition and microbial community dynamics. *Plant Ecol.* 213, 1571–1582. <https://doi.org/10.1007/s11258-012-0112-7>.
- Ashton, I.W., Hyatt, L.A., Howe, K.M., Gurevitch, J., Lerdau, M.T., 2005. Invasive species accelerate decomposition and litter nitrogen loss in a mixed deciduous forest. *Ecol. Appl.* 15, 1263–1272. <https://doi.org/10.1890/04-0741>.
- Bauer, J.T., Shannon, S.M., Stoops, R.E., Reynolds, H.L., 2012. Context dependency of the allelopathic effects of *Lonicera maackii* on seed germination. *Plant Ecol.* 213, 1907–1916. <https://doi.org/10.1007/s11258-012-0036-2>.
- Blair, B.C., Stowasser, A., 2009. Impact of *Lonicera maackii* on decomposition rates of native leaf litter in a southwestern Ohio woodland. *Ohio J. Sci.* 109, 43–47. <http://hdl.handle.net/1811/52617>.
- Bohlen, P.J., Scheu, S., Hale, C.M., McLean, M.A., Migge, S., Groffman, P.M., Parkinson, D., 2004. Non-native invasive earthworms as agents of change in northern temperate forests. *Front. Ecol. Environ.* 2, 427–435. [https://doi.org/10.1890/1540-9295\(2004\)002\[0427:NIEAAO\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2004)002[0427:NIEAAO]2.0.CO;2).
- Campana, C., Gauvin, S., Ponge, J.F., 2002. Influence of ground cover on earthworm communities in an unmanaged beech forest: linear gradient studies. *Eur. J. Soil Biol.* 38 (2), 213–224.
- Caratti, J.F., 2006. Point intercept (PO). In: Lutes, Duncan C., Keane, Robert E., Caratti, John F., Key, Carl H., Benson, Nathan C., Sutherland, Steve, Gangi, Larry J. (Eds.), FIREMON: Fire Effects Monitoring and Inventory System. US Department Of, Fort Collins, CO Gen. Tech. Rep. RMRS-GTR-164-CD.
- Cipollini, K., Titus, K., Wagner, C., 2012. Allelopathic effects of invasive species (*Alliaria petiolata*, *Lonicera maackii*, *Ranunculus ficaria*) in the Midwestern United States. *Allelopath. J.* 29, 63–76.
- Côté, B., Fyles, J.W., 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.* 24, 192–196. <https://doi.org/10.1139/x94-027>.
- Curry, J.P., 2004. Factors affecting the abundance of earthworms in soils. *Earthworm Ecol.* 9, 91–113. <https://doi.org/10.1201/9781420039719.pt3>.
- Dávalos, A., Nuzzo, V., Blosssey, B., 2015. Single and interactive effects of deer and earthworms on non-native plants. *For. Ecol. Manage.* 351, 28–35. <https://doi.org/10.1016/J.FORECO.2015.04.026>.
- De Wandler, H., Bruelheide, H., Dawud, S.M., Dänilä, G., Domisch, T., Finer, L., Hermy, M., Jaroszewicz, B., Joly, F.-X., Müller, S., Ratcliffe, S., Raulund-Rasmussen, K., Rota, E., Van Meerbeek, K., Vesterdal, L., Muys, B., 2018. Tree identity rather than tree diversity drives earthworm communities in European forests. *Pedobiologia (Jena)* 67, 16–25. <https://doi.org/10.1016/J.PEDOBIO.2018.01.003>.
- De Wandler, H., Sousa-Silva, R., Ampoorter, E., Bruelheide, H., Carnol, M., Dawud, S.M., Dänilä, G., Finer, L., Hättenschwiler, S., Hermy, M., Jaroszewicz, B., Joly, F.-X., Müller, S., Pollastrini, M., Ratcliffe, S., Raulund-Rasmussen, K., Selvi, F., Valladares, F., Van Meerbeek, K., Verheyen, K., Vesterdal, L., Muys, B., 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>.
- Dempsey, M.A., Fisk, M.C., Fahey, T.J., 2011. Earthworms increase the ratio of bacteria to fungi in northern hardwood forest soils, primarily by eliminating the organic horizon. *Soil Biol. Biochem.* 43, 2135–2141. <https://doi.org/10.1016/J.SOILBIO.2011.06.017>.
- Eisenhauer, N., 2010. The action of an animal ecosystem engineer: identification of the main mechanisms of earthworm impacts on soil microarthropods. *Pedobiologia (Jena)* 53, 343–352. <https://doi.org/10.1016/J.PEDOBIO.2010.04.003>.
- Ferlian, O., Eisenhauer, N., Aguirrebengoa, M., Camara, M., Ramirez-Rojas, I., Santos, F., Tanalgo, K., Thakur, M.P., 2018. Invasive earthworms erode soil biodiversity: a meta-analysis. *J. Anim. Ecol.* 87, 162–172. <https://doi.org/10.1111/1365-2656.12746>.
- Frellich, Lee E., Hale, Cindy M., Reich, Peter B., Holdsworth, Andrew R., Scheu, Stefan, Heneghan, Liam, Bohlen, Patrick J., 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. In: Hendrit, Paul F. (Ed.), *Biological Invasions Belowground: Earthworms as Invasive Species*. Springer, Netherlands, Dordrecht, pp. 35–45. <https://doi.org/10.1007/978-1-4020-5429-7.5>.
- Gunn, A., 1992. The use of mustard to estimate earthworm populations. *Pedobiologia (Jena)* 36, 65–67.
- Haimi, J., Einbork, M., 1992. Effects of endogeic earthworms on soil processes and plant growth in coniferous forest soil. *Biol. Fertil. Soils* 13, 6–10. <https://doi.org/10.1007/BF00337230>.
- Hale, C.M., Frellich, L.E., Reich, P.B., Pastor, J., 2005. Effects of European earthworm invasion on soil characteristics in northern hardwood forests of Minnesota, USA. *Ecosystems* 8, 911–927. <https://doi.org/10.1007/s10021-005-0066-x>.
- Hendriksen, N.B., 1990. Leaf litter selection by detritivore and geophagous earthworms. *Biol. Fertil. Soils* 10, 17–21. <https://doi.org/10.1007/bf00336119>.
- Heneghan, L., Steffen, J., Fagen, K., 2007. Interactions of an introduced shrub and introduced earthworms in an Illinois urban woodland: impact on leaf litter decomposition. *Pedobiologia (Jena)* 50, 543–551. <https://doi.org/10.1016/J.PEDOBIO.2006.10.002>.
- Kasurinen, A., Peltonen, P.A., Julkunen-Tiitto, R., Vapaavuori, E., Nuutinen, V., Holopainen, T., Holopainen, J.K., 2007. Effects of elevated CO₂ and O₃ on leaf litter phenolics and subsequent performance of litter-feeding soil macrofauna. *Plant Soil* 292, 25–43. <https://doi.org/10.1007/s11104-007-9199-3>.
- Kucera, C.L., 1959. Weathering characteristics of deciduous leaf litter. *Ecology* 40, 485–487. <https://doi.org/10.2307/1929769>.
- Lerch, N.K., Hale, W.F., Lemaster, D.D., 1980. Soil survey of Butler County, Ohio. Department of Agriculture, Soil Conservation Service.
- Luken, J.O., Thieret, J.W., 1996. Amur honeysuckle, its fall from grace. *Bioscience* 46, 18–24. <https://doi.org/10.2307/1312651>.
- Madritch, M.D., Lindroth, R.L., 2009. Removal of invasive shrubs reduces exotic earthworm populations. *Biol. Invas.* 11, 663–671. <https://doi.org/10.1007/s10530-008-9281-7>.
- Mahon, M.B., Crist, T.O., 2019. Invasive earthworm and soil litter response to the experimental removal of white-tailed deer and an invasive shrub. *Ecology* 100, e02688. <https://doi.org/10.1002/ecy.2688>.
- McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* 82, 290–297. [https://doi.org/10.1890/0012-9658\(2001\)082\[0290:FMTCDD\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[0290:FMTCDD]2.0.CO;2).
- McNeish, R.E., McEwan, R.W., 2016. A review on the invasion ecology of Amur honeysuckle (*Lonicera maackii*, Caprifoliaceae) a case study of ecological impacts at multiple scales. *J. Torrey Bot. Soc.* 143, 367–385. <https://doi.org/10.3159/TORREY-D-15-00049.1>.
- Medley, K.E., Krisko, B., 2007. Physical site conditions and land use history as factors influencing the conservation of regrowth forests in a southwest Ohio nature reserve. *Nat. Areas J.* 27, 31–41. [https://doi.org/10.3375/0885-8608\(2007\)27\[31:PSCALU\]2.0.CO;2](https://doi.org/10.3375/0885-8608(2007)27[31:PSCALU]2.0.CO;2).
- Melillo, J.M., Aber, J.D., Muratore, J.F., 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* 63, 621–626. <https://doi.org/10.2307/1936780>.
- Miller, K.E., Gorchov, D.L., 2004. The invasive shrub, *Lonicera maackii*, reduces growth and fecundity of perennial forest herbs. *Oecologia* 139, 359–375. <https://doi.org/10.1007/s00442-004-1518-2>.
- Neirynck, J., Mirtcheva, S., Sioen, G., Lust, N., 2000. Impact of *Tilia platyphyllos* Scop., *Fraxinus excelsior* L., *Acer pseudoplatanus* L., *Quercus robur* L. and *Fagus sylvatica* L. on earthworm biomass and physico-chemical properties of a loamy topsoil. *For. Ecol. Manage.* 133, 275–286. [https://doi.org/10.1016/S0378-1127\(99\)00240-6](https://doi.org/10.1016/S0378-1127(99)00240-6).
- Okansen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P. R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2018. *vegan: Community Ecology Package*.
- Pipal, R., 2014. Earthworm, Microbial Biomass, and Leaf Litter Decay Responses After Invasive Honeysuckle Shrub Removal from Urban Woodlands. Theses Diss. University of Louisville. <https://doi.org/10.18297/etd/1759>.
- Poulette, M.M., Arthur, M.A., 2012. The impact of the invasive shrub *Lonicera maackii* on the decomposition dynamics of a native plant community. *Ecol. Appl.* 22, 412–424. <https://doi.org/10.1890/11-1105.1>.
- Core Team, R., 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Reich, P.B., Oleksyn, J., Modrzyński, J., Mrozinski, P., Hobbie, S.E., Eissenstat, D.M., Chorover, J., Chadwick, O.A., Hale, C.M., Tjoelker, M.G., 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>.

- Riutta, T., Slade, E.M., Beber, D.P., Taylor, M.E., Malhi, Y., Riordan, P., Macdonald, D.W., Morecroft, M.D., 2012. Experimental evidence for the interacting effects of forest edge, moisture and soil macrofauna on leaf litter decomposition. *Soil Biol. Biochem.* 49, 124–131. <https://doi.org/10.1016/j.soilbio.2012.02.028>.
- Seifert, E.K., Bever, J.D., Maron, J.L., 2009. Evidence for the evolution of reduced mycorrhizal dependence during plant invasion. *Ecology* 90, 1055–1062. <https://doi.org/10.1890/08-0419.1>.
- Simberloff, D., Von Holle, B., 1999. Positive interactions of nonindigenous species: invasional meltdown? *Biol. Invas.* 1, 21–32. <https://doi.org/10.1023/A:1010086329619>.
- 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. *Biol. Invas.* 13, 1165–1182. <https://doi.org/10.1007/s10530-011-9959-0>.
- Trammell, T.L.E., Ralston, H.A., Scroggins, S.A., Carreiro, M.M., 2012. Foliar production and decomposition rates in urban forests invaded by the exotic invasive shrub, *Lonicera maackii*. *Biol. Invasions* 14, 529–545. <https://doi.org/10.1007/s10530-011-0093-9>.
- Venables, W.N., Ripley, B.D., 2002. *Modern Applied Statistics with S*, fourth ed. Springer, New York.
- Vilà, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarošík, V., Maron, J.L., Pergl, J., Schaffner, U., Sun, Y., Pyšek, P., 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecol. Lett.* 14, 702–708. <https://doi.org/10.1111/j.1461-0248.2011.01628.x>.
- Vogelsang, K.M., Bever, J.D., 2009. Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. *Ecology* 90, 399–407. <https://doi.org/10.1890/07-2144.1>.
- Webster, C.R., Jenkins, M.A., Jose, S., 2006. Woody invaders and the challenges they pose to forest ecosystems in the Eastern United States. *J. For.* 104, 366–374. <https://doi.org/10.1093/jof/104.7.366>.
- Whitfield, T.J.S., Roth, A.M., Lodge, A.G., Eisenhauer, N., Frelich, L.E., Reich, P.B., 2014. Resident plant diversity and introduced earthworms have contrasting effects on the success of invasive plants. *Biol. Invas.* 16, 2181–2193. <https://doi.org/10.1007/s10530-014-0657-6>.
- Yang, H., Yang, X., Heskell, M., Sun, S., Tang, J., 2017. Seasonal variations of leaf and canopy properties tracked by ground-based NDVI imagery in a temperate forest. *Sci. Rep.* 7 (1), 1267. <https://doi.org/10.1038/s41598-017-01260-y>.