

Invasive earthworm and soil litter response to the experimental removal of white-tailed deer and an invasive shrub

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Abstract. Recent studies have shown that complex species interactions can regulate above- and belowground processes in terrestrial systems. Ungulate herbivory and invasive species are known to have strong effects on plant communities in some systems, but their impacts on soil biota and belowground processes are lesser known. Growing evidence suggests white-tailed deer (*Odocoileus virginianus*) and invasive plants facilitate increased abundance of exotic earthworms in temperate forests of the eastern United States. We conducted an experimental study that manipulated deer access and the presence of an invasive understory shrub in an eastern deciduous forest of southwestern Ohio, USA, from 2013 to 2017. Earthworm density and biomass, and standing litter biomass were measured in five paired deer access and enclosure plots, each with a split-plot removal of Amur honeysuckle (*Lonicera maackii*). Earthworm density declined in response to the experimental exclusion of deer, with earthworm density decreasing over time in the deer enclosure plots relative to deer access plots. Deer exclusion produced greater variation in earthworm species composition relative to access plots. Multivariate analyses indicated that larger earthworms in the genus *Lumbricus* were associated with deer enclosure plots, while smaller endogeic species were ubiquitous in both treatments. Standing litter biomass decreased over time in the deer-access plots. In contrast, honeysuckle removal had little effect on earthworm density and standing litter biomass. There was an interaction between deer and honeysuckle treatments on earthworm biomass, with honeysuckle removal reducing earthworm biomass when deer were excluded. Our results demonstrate strong effects of herbivores on invasive earthworms and ecosystem processes, but indicate a weaker influence of invasive shrubs. Further, our findings suggest that the effects of deer overabundance in forest ecosystems are potentially reversible with long-term intervention.

Key words: aboveground–belowground interactions; earthworms; invasion; leaf litter; *Lonicera maackii*; temperate deciduous forest; white-tailed deer.

INTRODUCTION

Above- and belowground components of terrestrial ecosystems are typically studied independently, but growing evidence suggests that these biota and processes are interlinked (Bardgett and Wardle 2003, Wardle et al. 2004, Van der Putten et al. 2013). Increasingly, plants and aboveground herbivores are reported to have broad impacts on soil-dwelling organisms (Van der Putten et al. 2013, Dávalos et al. 2015b). Studies that incorporate both above- and belowground components will improve our understanding of the complex interplays between terrestrial biodiversity and ecosystem processes (Wardle et al. 2004). However, little is known about how these linkages are affected by multiple invasions of

plants and soil invertebrates. Understanding how invasive species change above- and belowground connections is imperative, as most ecosystems are undergoing substantial changes due to multiple invasions by exotic species (Simberloff et al. 2013).

Herbivory by white-tailed deer (*Odocoileus virginianus*) and invasion by exotic species are threats to biodiversity and ecosystem processes in eastern deciduous forests (Côté et al. 2004, Webster et al. 2006). As overabundant generalist herbivores, deer exert pressure on plant communities, which has cascading effects on forest composition and growth (Côté et al. 2004, Allombert et al. 2005). Preferential deer browse reduces diversity, abundance, and recruitment of woody and herbaceous species (Lessard et al. 2012, Shelton et al. 2014, Dávalos et al. 2015a). Moreover, deer browse reduces native plant richness while increasing invasive plant success, which may homogenize plant communities (Rooney 2009, Averill et al. 2018). Conversely, the effects of deer on soil invertebrates are less studied and more equivocal,

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with studies finding negative, positive, and neutral effects of deer activity on invertebrate communities (Wardle et al. 2001, Bressette et al. 2012, Christopher and Cameron 2012). Changes in plant communities, soil organic matter, and nutrient availability associated with deer activity can influence soil invertebrates (Bardgett and Wardle 2003, Heckel et al. 2010).

Invasive plants and soil invertebrates have widespread effects on natural ecosystems by displacing native species, altering nutrient cycling, and modifying abiotic conditions (Simberloff et al. 2013). Exotic earthworms, in particular, accelerate decomposition and rates of nutrient cycling, eliminate the soil organic layer, and mix soil horizons (Bohlen et al. 2004). The effect of exotic earthworms is partially dependent on the relative densities of three main ecological groups: epigeic (litter dwellers), endogeic (soil dwellers), and anecic (deep burrowing surface feeders; Hale et al. 2005, Eisenhauer et al. 2007). Likewise, the invasion of nonnative plants changes the structure and composition of plant and animal communities (Vilà et al. 2011, Pyšek et al. 2012) and rates of ecosystem processes (Vitousek and Walker 1989).

In eastern forests, the invasive understory shrub, *Lonicera maackii* (Amur honeysuckle) broadly overlaps with white-tailed deer and negatively affects native herbaceous plants, shrubs, and saplings (McNeish and McEwan 2016). Further, there is evidence that honeysuckle invasion supports higher deer density in some landscapes (Allan et al. 2010, Martinod and Gorchoy 2017, Peterson 2018). Honeysuckle invasion alters nutrient cycling through physical and biotic effects on decomposition (Trammell et al. 2012, Arthur et al. 2012, Pfeiffer and Gorchoy 2015). Abiotic and biotic changes caused by honeysuckle invasion have marked effects on soil communities. For instance, honeysuckle invasion reduces arbuscular mycorrhizal abundance (Shannon et al. 2014), benefits invasive earthworms (Pipal 2014), and supports distinct microbial communities (Arthur et al. 2012).

The separate, direct effects of white-tailed deer, invasive plants, and invasive earthworms on forest ecosystems are widely studied (Bohlen et al. 2004, Côté et al. 2004, Vilà et al. 2011); however, as most studies have investigated these taxa independently, we know little about interactions among these taxa and their combined effects on ecosystem processes. Although linkages between deer and invasive plants are now documented (Dávalos et al. 2015a, Peebles-Spencer et al. 2017), the three-way interaction among deer, invasive plants, and exotic earthworms is largely unknown. Recent reports found positive effects of deer on exotic earthworms (Rearick et al. 2011, Dávalos et al. 2015b; but see Shelton et al. 2014). Invasive shrub presence may also benefit exotic earthworms (Pipal 2014). Likewise, exotic earthworms may facilitate invasive plants (Nuzzo et al. 2009). Growing evidence suggests complex direct and

indirect interactions involving plants, animals, microorganisms, and soil litter.

Here we report on a multi-year experimental study to investigate the main and interactive effects of a generalist herbivore and an invasive shrub on standing litter biomass as well as the density, biomass, and community composition of exotic earthworms. We established pairs of experimental (deer enclosure) and control (deer access) plots, then removed or retained honeysuckle within plots using a split-plot design. We hypothesized that deer and honeysuckle would facilitate greater invasive earthworm density and biomass and reduce standing litter biomass. Based on prior studies, we expected lower density and biomass of earthworms in the deer enclosure plots compared to the deer access plots. We also expected a reduction in the density and biomass of earthworms when honeysuckle was removed compared to when it was present. We predicted deer exclusion and honeysuckle removal would have an additive, negative effect on earthworm density and biomass. We expected shifts in earthworm species composition following deer exclusion and honeysuckle removal, but lack information on directionality of change, as few studies have examined the community structure of invasive earthworms. Based on these earthworm predictions, we also predicted an increase in standing litter biomass in the deer enclosure plots and honeysuckle removal subplots. Finally, we expected the effects of our treatments to increase with time since treatments were established.

METHODS

Study sites

The study was conducted in second-growth forests of the Miami University Natural Areas in Butler County, southwestern Ohio, USA. Five study sites (Bachelor Preserve, College Woods, Kramer Woods, Reinhart Preserve, and Western Woods) were separated by >1 km and chosen to have similar upland topographic position and elevation (253 ± 5 m above sea level); mature, closed canopy deciduous forest; and similar, moderate levels of honeysuckle invasion (stem basal area of 0.6–1.6 m²/ha; Peebles-Spencer et al. 2017). Average distance from each site to the forest edge was 152 ± 24 m (mean \pm SE). Agricultural and suburban land uses predominate in the surrounding landscape. Climate of the study area is temperate continental with a mean annual temperature of 11°C (mean temperature of -1°C in January and 23°C in July) and average annual rainfall of 110 cm. Soils are fine, mixed active Hapludalfs. Dominant hardwood species of the study forest include *Acer saccharum* (sugar maple), *Quercus* spp. (oak), *Ulmus* spp. (elm), *Carya* spp. (hickory), and *Fagus grandifolia* (American beech). The understory of each site is dominated by invasive honeysuckle (40–50% understory canopy cover). Detailed information on the herbaceous

plant communities at these sites can be found in Peebles-Spencer et al. (2017).

At each study site, two paired 400 m² (20 × 20 m) plots were randomly assigned to either deer enclosure or deer access; each with a split-plot (20 × 10 m subplots) treatment of honeysuckle removal and control, again randomly assigned within each deer plot. Plot and subplot size were sufficient to detect treatment effects, as similar studies have used smaller plot sizes (Pipal 2014) or have found no effect of plot size on similar endpoints (Dávalos et al. 2015b).

At each site, paired deer plots were located 50–100 m apart. In 2010, 2.5 m high fencing was erected surrounding the deer enclosure plots to restrict deer access and honeysuckle removals were conducted. Shrubs were cut at the base and Tordon RTU (Dow AgroSciences, Indianapolis, Indiana, USA) was applied to the stem to deter regrowth; a second round of honeysuckle removal occurred in 2015 (Peebles-Spencer et al. 2017). The active ingredient in Tordon (picloram) has a soil half-life of 36 d (Lewis et al. 2006), so immediate impacts of pesticide application on soil around treated shrubs in 2010 likely dissipated well before earthworm sampling began in 2013; earthworm sampling occurred prior to reapplication in 2015. While we did not notice non-target plant die back from herbicide application (Peebles-Spencer et al. 2017), we are unable to rule out short term impacts on soil invertebrates. Split-plot experimental designs provide greater statistical power in detecting main subplot effects and interactions of treatments (Dean et al. 2017); as such, honeysuckle effects and interactions between our treatments were more likely to be detected than the main effects of deer. However, a characteristic of split-plot experimental designs is the non-independence of subplots within plots, which must be accounted for with appropriate statistical analyses (Dean et al. 2017).

Earthworm and litter sampling

To examine treatment effects through time, we sampled earthworms in June of 2013–2017. In each sample period, we measured earthworms in three quadrats within each subplot, six quadrats per plot, and 12 quadrats at each site, for a total of 60 samples per period. The quadrat locations were evenly spaced along a line through the center of each subplot at 5-m intervals starting 5 m from the subplot edge to avoid any edge effects from adjacent subplots or areas surrounding plots. However, due to subplot size and orientation, earthworm foraging outside of subplots was possible. We collected samples from the same site on the same day and all five sites were sampled within two weeks. In total, we collected 240 earthworm and leaf litter samples in four of the five years from 2013 to 2017; samples were not collected in June 2014.

We sampled leaf litter and earthworms from square 0.25-m² quadrats. Prior to earthworm extraction, we

collected leaf litter and woody detritus leaving a bare soil surface. We then poured 3.1 L of mustard solution (10 g/L concentration) onto the bare soil to extract earthworms (Lawrence and Bowers 2002). Mustard extraction is a non-destructive method of collection that is sensitive to both soil moisture and earthworm activity (Eisenhauer et al. 2008). We collected earthworms and immediately placed them in 70% ethanol to preserve specimens. In the lab, leaf litter was sorted to remove any woody detritus and sifted with a #4 sieve (4.75-mm openings) and oven dried to constant mass to obtain standing litter biomass (g). We identified earthworm specimens to species when possible (only mature earthworms can be reliably identified to species) and to genus for all individuals. We obtained overall earthworm biomass (wet biomass) for all samples by letting earthworms dry on paper towels and weighing once the ethanol evaporated.

Statistical analyses

We conducted analyses on earthworm density, earthworm biomass, and standing litter biomass in response to deer and honeysuckle treatments through time. The R programming language version 3.5 was used for univariate analyses (R Core Team 2018). We used generalized linear mixed effects models (glmmTMB function, glmmTMB package, R; Brooks et al. 2017) with a negative binomial error distribution to test models of earthworm density, and Gaussian error distributions for earthworm biomass and standing litter biomass with treatment and time as predictor variables. Earthworm biomass and standing litter biomass were natural-log transformed prior to analyses. We tested for the fixed effects of deer, honeysuckle, and time as well as their interactions. Models had random intercept terms for sites and for plots nested within sites to reflect the split-plot design of our study. The nested random effects incorporate the unstructured covariance in response variables for all combinations of treatments, plots, and sites (Brooks et al. 2017), which accounts for the dependence in response variables of subplots within plots and plots within sites (Pinheiro and Bates 2000). To account for temporal autocorrelation in responses across years, we also incorporated an autoregressive (ar1) correlation structure to each model, which assumes that repeat observations from the same experimental units are correlated over time (Brooks et al. 2017). Marginal (fixed effects) and conditional (fixed and random effects) R^2 values were calculated using appropriate equations from Nakagawa et al. (2017).

To examine changes of earthworm community composition in response to deer and honeysuckle treatments, we conducted analyses for multivariate location and dispersion using PRIMER-E and PERMANOVA+ (version 6; Anderson 2001). We used permutational multivariate analysis of variance (PERMANOVA) to test for overlap in species composition (multivariate

location; Anderson 2001) among treatments, and analysis of multivariate dispersion to test whether the amount of variation in species composition differed among treatments (Anderson et al. 2006). PERMANOVA and analysis of multivariate dispersion were conducted using 9,999 permutations of the data, with a split-plot and crossed design, respectively. We used nonmetric multidimensional scaling (NMDS) to visualize patterns of species composition among treatments using the metaMDS function in the vegan package of R (Oksanen et al. 2018). All multivariate, community analyses were based on Bray-Curtis distances, using square-root transformed abundance data. We included juvenile earthworms in multivariate analyses, as the species by sample matrix was too sparse without these individuals. We combined juvenile *Octolasion* and *Octolasion tyrtaeum*, as this was the only *Octolasion* species found in our study, and removed singleton earthworms (*Aporrectodea tuberculata*) to reduce the effect of rare species on analyses. We pooled data from the three quadrat samples in each subplot for each site and year; subplots without earthworms were removed from analyses. In total, 72 samples were used in the community analyses.

RESULTS

Earthworm density and biomass

We collected 2,536 individuals of seven nonnative earthworm species in three genera: *Aporrectodea*, *Lumbricus*, and *Octolasion* (Appendix S1: Table S1). Individuals of the genus *Lumbricus* were the most dominant in both density and biomass across sites and years. The anecic *Lumbricus terrestris* was present at low densities (1% of individuals collected) and at only three sites, while the epi-endogeic *Lumbricus rubellus* was the most common adult earthworm collected (13%). Juveniles of the *Lumbricus* genus were dominant across sites representing 55% of all captured earthworms. The endogeic earthworms of the *Aporrectodea* and *Octolasion* genera were present in lower densities across sites (21% and 9%, respectively). *Aporrectodea* consisted of several species: *A. caliginosa*, *A. rosea*, *A. trapezoides*, and *A. tuberculata*. *Octolasion* was represented by a single species, *O. tyrtaeum*. We recorded several native earthworms of the genus *Diplocardia* at Kramer Woods; however, due to their low abundance ($n = 6$) and presence at a single site, they were removed from all analyses.

Mean exotic earthworm density (0.08–30.25 earthworms/0.25 m²) and biomass (0.01–7.77 g/0.25 m²) varied across sites and years (Appendix S1: Table S2). Highest observed densities of earthworms for a single sampling period were located in Reinhart Preserve (363 individuals collected in 2016); while lowest densities were located in Bachelor Preserve (a single individual collected in 2016). These observations held for earthworm biomass as well. While Bachelor Preserve boasted a moderate mean density of earthworms at the start of the

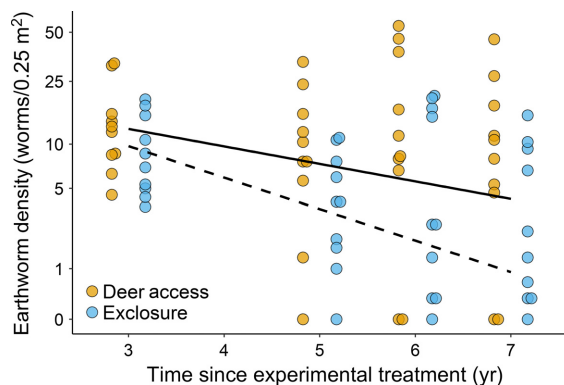


FIG. 1. Relationship between earthworm density over time with and without the fixed effect of deer removal. Yellow circles are deer access plots; blue circles are deer exclusion plots. Points are mean of honeysuckle subplots within deer plots. Lines are the fixed effects of the generalized linear mixed model (solid, deer access plots; dashed, deer exclusion plots). Note y-axis is on a natural-log scale.

study (12 earthworms/0.25 m²), by the end of the study only two individuals were collected at this site (Appendix S1: Table S2).

Deer exclusion did not have an immediate effect on earthworm density ($Z = -0.112$, $P = 0.911$), but deer exclusion significantly reduced earthworm density over time relative to deer access plots ($Z = -2.322$, $P = 0.020$). As a result, there was lower earthworm density in the deer exclusion plots relative to the deer access plots at the end of the study (Fig. 1). We saw a weak trend of decreasing earthworm density through time in deer access plots ($Z = -1.724$, $P = 0.085$), likely due to an overall loss of earthworms at Bachelor Preserve (Appendix S1: Table S2). There was no effect of honeysuckle removal nor an interaction between deer and honeysuckle treatments on earthworm density (Table 1). Random effects of site were important to both overall earthworm density and the effect of deer removal (Table 1; Appendix S1: Fig. S1).

Deer exclusion and honeysuckle removal alone did not affect earthworm biomass (Table 1), but there was a significant interaction between deer exclusion and honeysuckle removal ($Z = -2.024$, $P = 0.043$). In deer access plots, there was a positive effect of honeysuckle removal on earthworm biomass, while in deer exclusion plots, there was a negative effect of honeysuckle removal on earthworm biomass (Fig. 2). There was no significant trend of earthworm biomass over time (Table 1). However, there was a weak negative interaction between time and deer exclusion ($Z = -1.896$, $P = 0.058$), with less earthworm biomass in the deer exclusion plots relative to the deer access plots at the end of the study.

Earthworm community composition

Multivariate analysis indicated a high degree of overlap in earthworm species composition among treatments

TABLE 1. Fixed effects from generalized linear mixed models for the effects of treatments, time, and their interactions on earthworm density, earthworm biomass, and standing litter biomass.

Variable†	Earthworm density				Earthworm biomass				Standing litter biomass			
	Estimate	SE	Z	P	Estimate	SE	Z	P	Estimate	SE	Z	P
Time	-0.240	0.14	-1.724	0.085	-0.041	0.02	-0.508	0.612	-0.215	0.06	-3.508	<0.001
Deer	-0.047	0.42	-0.112	0.911	-0.044	0.26	-0.172	0.863	-0.025	0.16	-0.155	0.877
Honeysuckle	0.050	0.27	0.201	0.841	0.137	0.21	0.656	0.512	-0.112	0.12	-0.933	0.351
Time × Deer	-0.191	0.08	-2.322	0.020	-0.109	0.06	-1.896	0.058	0.141	0.03	4.295	<0.001
Time × Honeysuckle	0.011	0.08	0.148	0.882	-0.016	0.06	-0.277	0.782	0.032	0.03	0.959	0.337
Deer × Honeysuckle	-0.421	0.38	-1.098	0.272	-0.59	0.29	-2.024	0.043	0.075	0.17	0.455	0.649
Time × Deer × Honeysuckle	0.108	0.11	0.963	0.335	0.149	0.08	1.851	0.064	-0.055	0.05	-1.196	0.232
Marginal/ Conditional R ²	0.137/0.854				0.111/0.655				0.295/0.779			

Notes: Estimates, standard error, z value, and P values are given for each response variable. Boldface text indicates coefficients that are significantly different from zero (continuous) or from reference level (categorical) for each endpoint ($P < 0.05$).

† All models contain random intercept terms for sites and for plots nested within sites to reflect the split-plot design of our study and an autoregressive term of time since exclusion of deer and removal of honeysuckle.

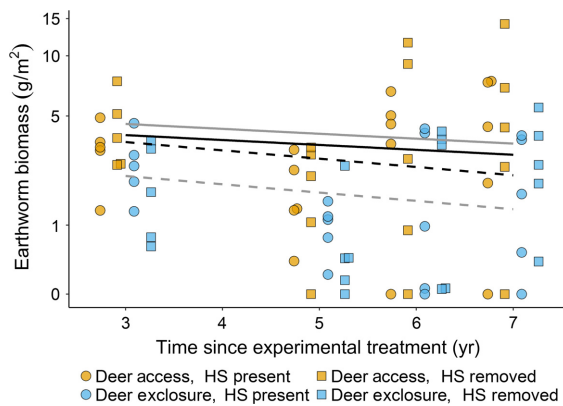


FIG. 2. Relationship between earthworm biomass over time as it varied with deer and honeysuckle (HS) removals. Subplot treatments are deer access, honeysuckle present (black solid line; yellow circles); deer access, honeysuckle removal (gray solid line; yellow squares); deer exclusion, honeysuckle present (black dashed line; blue circles); and deer exclusion, honeysuckle removed (grey dashed line; blue squares). Points are mean of honeysuckle subplots within deer plots. Note y-axis is on a natural-log scale.

TABLE 2. Split-plot PERMANOVA results for the effect of site, deer exclusion, honeysuckle removal, and deer × honeysuckle treatment interaction on earthworm community composition over the entire sampling period.

Source	df	SS	MS	Pseudo F	P
Site	4	3,151	787.9	3.276	0.0614
Deer	1	732	731.7	3.043	0.0897
Whole-plot error	4	962	240.5		
Whole-plot total	9	4,845			
Honeysuckle	1	243	243	0.818	0.533
Deer × Honeysuckle	1	171	171	0.576	0.684
Sub-plot error	8	2,376	297		
Sub-plot total	19	11,999			

(NMDS, $k = 3$, final stress = 0.103; Table 2; Fig. 3). There was strong clustering of sample scores among the deer access plots, which formed a subset of the variation in species composition found among the deer exclusion plots (Fig. 3A). This was supported by analysis of multivariate dispersion, which showed significantly greater variation in composition in deer exclusion plots relative to deer access plots (Table 3). We saw no effect of honeysuckle removal on the composition of earthworm communities as determined by multivariate location (Table 2) or dispersion (Table 3). There was a strong interaction between deer exclusion and honeysuckle removal on multivariate dispersion (Table 3). In deer access plots, honeysuckle removal resulted in lower multivariate dispersion, while in deer exclusion plots, honeysuckle removal resulted in higher multivariate dispersion. Locations of species scores in multivariate space indicated endogeous species were ubiquitous in both deer access and exclusion plots, while *Lumbricus* species tended to have higher relative abundances in deer exclusion plots (Fig. 3B).

Standing litter biomass

Standing litter biomass ($\text{g}/0.25 \text{ m}^2$) varied across sites and years (Appendix S1: Table S2), with the highest mean standing litter biomass in 2013 ($112.95 \text{ g}/0.25 \text{ m}^2$) and lowest in 2015 ($50.95 \text{ g}/0.25 \text{ m}^2$). Kramer Woods had the highest standing litter biomass across years ($92.34 \text{ g}/0.25 \text{ m}^2$), while Reinhart Preserve had the lowest ($55.66 \text{ g}/0.25 \text{ m}^2$). Deer exclusion did not have an immediate effect on standing litter biomass ($Z = -0.155$, $P = 0.877$). There was a reduction in standing litter biomass in deer access plots through time ($Z = 3.508$, $P < 0.001$), but there was a divergence in the amount of standing litter biomass between deer access and deer exclusion plots ($Z = 4.295$, $P < 0.001$). There was not a significant decrease in the amount of standing litter

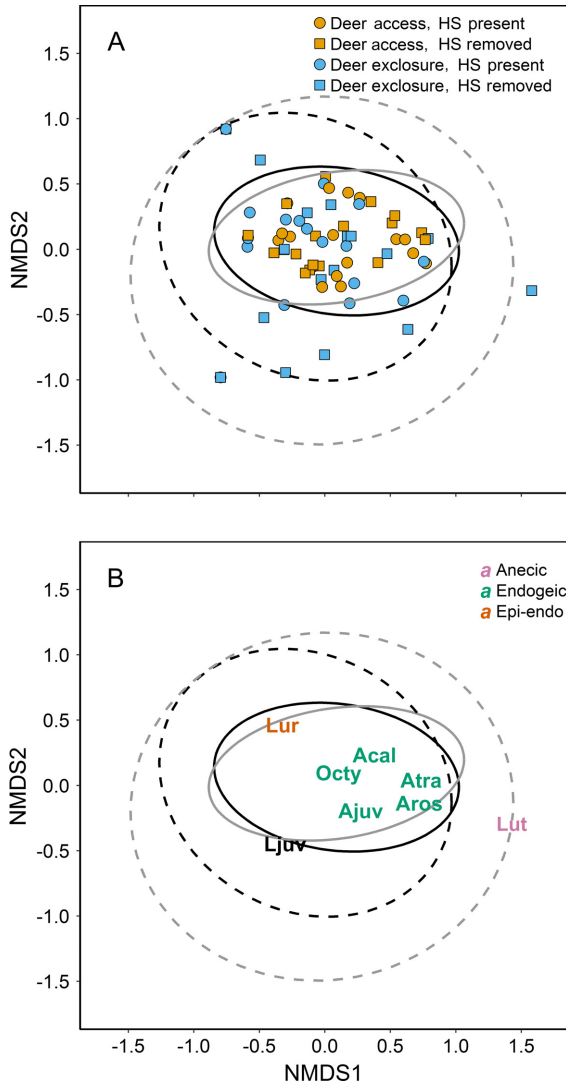


FIG. 3. Nonmetric multidimensional scaling (NMDS) ordination of the earthworm community composition ($n = 72$). (A) Site scores with 95% CIs of subplot treatments: deer access, honeysuckle present (black solid ellipse, yellow circles); deer access, honeysuckle removal (gray solid ellipse, yellow squares); deer exclusion, honeysuckle present (black dashed ellipse, blue circles); and deer exclusion, honeysuckle removed (gray dashed ellipse, blue squares) treatments. (B) Species scores are represented by their ecotype (purple, anecic; green, endogeic; orange, epi-endogeic). Acal, *Aporrectodea caliginosa*; Aros, *Aporrectodea rosea*; Atra, *Aporrectodea trapezoides*; Ajuv, *Aporrectodea juveniles*; Lur, *Lumbricus rubellus*; Lut, *Lumbricus terrestris*; Ljuv, *Lumbricus juveniles*; Octy, *Octolasion tyraeum*. Note that ecotype for *Lumbricus juveniles* cannot be determined; thus, they are not colored by a given ecotype.

biomass through time in deer exclusion plots ($Z = -1.218$, $P = 0.223$ [comparison to zero slope]). By the end of the study, there was $\sim 50 \text{ g}/0.25 \text{ m}^2$ less litter biomass in deer access plots relative to deer exclusion plots (Fig. 4). There was no effect of honeysuckle removal nor an interaction between treatments on standing litter biomass (Table 1).

TABLE 3. Results for the effect of site, deer exclusion, honeysuckle removal, and deer \times honeysuckle treatment interaction on earthworm community dispersion over the entire sampling period.

Source	df	F	P
Site	4,67	3.689	0.034
Deer	1,70	14.255	0.001
Honeysuckle	1,70	1.132	0.379
Deer \times Honeysuckle	3,68	6.466	0.003

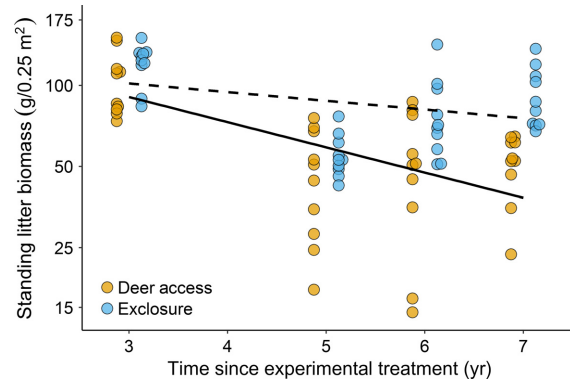


FIG. 4. Relationship between standing litter biomass and time for deer access and exclusion treatments. Yellow circles are deer access plots; blue circles are deer exclusion plots. Points are mean of honeysuckle subplots within deer plots. Lines are the fixed effects of the generalized linear mixed model (solid, deer access plots; dashed, deer exclusion plots). Note y-axis is on a natural-log scale.

DISCUSSION

The results supported our predictions that deer exclusion would reduce density of earthworms and increase standing litter biomass. Moreover, these effects became stronger with time since deer exclusion. However, we saw no support for our prediction of lower earthworm biomass in response to deer exclusion. Our findings of declining earthworm populations in response to deer exclusion support the findings of Rearick et al. (2011) and Dávalos et al. (2015b). Together, these studies suggest white-tailed deer activity facilitates increased densities of exotic earthworms, which may lead to reductions of leaf litter biomass in eastern deciduous forests of North America. Shifts in leaf litter biomass are likely to influence the retention of soil C and N, but further work is needed to document the fate of litter C and N in this context. Our study is the first to show that deer effects on earthworm density are also accompanied by a shift in earthworm community structure, potentially narrowing the range of species, burrowing habits, and their vertical redistribution of soil organic matter. We found no support for our predictions that honeysuckle removal would decrease earthworm density and increase standing litter

biomass. However, the effect of honeysuckle removal on earthworm biomass was dependent on the exclusion of deer. We saw no indication of honeysuckle effects increasing with time, but our ability to detect these effects may have been hindered by the size and orientation of our subplots. Building upon evidence from previous studies, our results demonstrate that large herbivores have non-consumptive effects on soil invertebrates, and potentially the larger decomposer food web, which lead to changes in the soil organic matter (Bardgett and Wardle 2003, Lessard et al. 2012, Dávalos et al. 2015b).

We observed large variation in earthworm density and biomass among sites and through time (Appendix S1: Fig. S1), indicating high spatial and temporal heterogeneity of soil organisms within a relatively uniform forest type, likely related to varying soil environments. Local densities of earthworms are known to be driven by soil pH, litter chemistry, soil moisture, and time since invasion (Hale et al. 2005, Reich et al. 2005, Eisenhauer et al. 2008). We did not measure these properties, but they likely varied somewhat across sites, even though we controlled for topographic position (uplands) and stand age (60–80 yr old). All sites also had a similar land-use history of pasture and grazing (Medley and Krisko 2007). Nonnative earthworms likely invaded these soils prior to forest regrowth, but exact invasion dates for these sites are unknown. Nonetheless, earthworms currently represent a large pool of belowground biomass (~150 kg/ha) in our study area, indicating that this taxon plays an integral role in belowground processes and soil turnover (Bohlen et al. 2004). The relatively low density of earthworms in 2015 compared to other years was likely due to an early season drought, which negatively affect earthworm populations (Eisenhauer et al. 2008). However, this period of drought appeared to have little effect on overall earthworm persistence, as earthworm density rebounded in 2016. The rapid decrease in earthworms at Bachelor Preserve may have been caused by drought, disease, or parasitism (Curry 1998), and exemplifies strong temporal variation in earthworm density and their effects on soil processes. Despite the variability in random site effects, however, the overall fixed negative effect of deer exclusion on earthworm density grew larger over time.

Deer

While the effect of deer exclusion on earthworm density increased with time, there was no main effect of deer exclusion on earthworm biomass. This suggests there was an increase in mean earthworm size in the deer exclusion plots (0.44 ± 0.08 g) compared to the deer access plots (0.34 ± 0.04 g). There are two possible mechanisms for this result: increased juvenile earthworm recruitment in the deer access plots relative to the deer exclusion plots, or a shift in species composition from smaller, endogeic earthworms (*Aporrectodea* and

Octolasion) to larger earthworms (*Lumbricus*) in the deer exclusion plots. However, processes influencing earthworm recruitment may occur at spatial scales larger than the experimental plots. There was no obvious difference in relative density of juveniles between deer exclusion and access plots (data not presented), but the relative ratios of *Lumbricus* individuals to endogeic individuals was higher in the deer exclusion plots (2.8) than in the access plots (2.4). This is supported by ordination where *Lumbricus* species fell outside of the multivariate space represented by deer access (Fig. 3). Moreover, homogenization of earthworm species composition in the deer access plots indicates that deer preferentially benefit the endogeic species. Increased multivariate dispersion of earthworm species composition in response to deer exclusion is likely related to the larger standing crop of leaf litter in the deer exclusion plots. A larger standing crop of leaf litter would provide more benefit for *Lumbricus* individuals than endogeic individuals, as it represents habitat for *L. rubellus* and a food resource for *L. rubellus* and *L. terrestris*. The shift in relative abundance of endogeic species in the presence of deer may have additive or synergistic effects on soil compaction in these areas, as deer compact soil through trampling (Frerker et al. 2014) and endogeic species increase bulk density (Hale et al. 2005). Such increased soil compaction would likely further alter plant and soil invertebrate communities (Bressette et al. 2012, Frerker et al. 2014).

The effect of deer on invasive earthworms may be mediated through direct or indirect biotic and abiotic interactions, though mechanisms remain elusive. Deer-mediated alterations to soil chemistry and physical properties may aid exotic earthworm growth and reproduction (Heckel et al. 2010, Bressette et al. 2012, Dávalos et al. 2015b). Deer pellets may also benefit earthworms by providing an accessible, nutritious, and abundant resource (Rearick et al. 2011). This is consistent with studies showing earthworm preference for deer pellets over leaf litter (Karberg and Lilleskov 2009, Rearick et al. 2011). Preliminary results from small-scale experiments at our study sites suggest supplemental deer pellets increase earthworm densities but artificial trampling has no effect (M. B. Mahon, *unpublished data*). Disentangling abiotic and biotic controls of deer on exotic earthworms needs further study.

The divergence of standing litter biomass between deer treatments through time is likely due to differences in decomposition rates in the plots. Deer facilitate decomposition through several mechanisms: direct consumption of leaf litter (Johnson et al. 1995), trampling (Bressette et al. 2012), or beneficial effects on microbes, microfauna, and earthworms (Wardle et al. 2001, Dávalos et al. 2015b, this study). Differences between deer access and exclusion plots due to litter trapped in the fencing are unlikely, as we observed no obvious accumulation of leaves on either side of the fencing. Differences in litter due to changes in herbaceous cover

between deer treatments (Peebles-Spencer et al. 2017) were also minimal, as litter from mature trees is the majority of standing litter biomass in temperate forests (Gosz et al. 1972). While we did not measure litter fall within the plots, the random assignment of treatments and close proximity of plots minimized systematic differences in litter fall. Instead, decomposition rates are likely slower when deer are excluded. Preliminary results of decomposition rates in the same experimental plots indicate both direct and indirect effects of deer on decomposition (M. B. Mahon and T. O. Crist, *unpublished data*). Therefore, microbial-mediated effects of deer on decomposition warrant further attention.

Honeysuckle

The lack of honeysuckle removal on earthworms in our study differs from the results of Madritch and Lindroth (2009) and Pipal (2014), which showed that honeysuckle removal decreased earthworm abundance. These effects are linked to changes in microclimate (cooler, moister conditions under honeysuckle shrubs; Trammell et al. 2012, Pipal 2014) and higher quality litter (lower C:N; Lobe et al. 2014). One possible explanation for our contradictory results may be that earthworms sampled in honeysuckle removal subplots may have foraged on leaf litter in honeysuckle presence subplots, but resided in soils in honeysuckle removal subplots; thereby minimizing effects of honeysuckle removal. An alternative explanation may be differing levels of shrub invasion and dominance among study locations (Trammell et al. 2012) as well as differences in earthworm community composition. *L. rubellus* and juvenile *Lumbricus* were dominant in our soils, whereas *Aporrectodea* and *L. terrestris* dominated soils in Pipal (2014). We hypothesize earthworm responses to invasive shrub removal depend upon functional group dominance at a given site such that strong negative effects of invasive shrub removal occur when endogeic and anecic species are dominant.

Similarly, standing litter biomass did not differ between honeysuckle removal and control subplots, which is supported by previous studies that have found no effect of honeysuckle shrubs on decomposition rates of leaf litter (Madritch and Lindroth 2009; but see Arthur et al. 2012). These mixed effects may be due to differences in honeysuckle densities among these studies (Trammell et al. 2012). At our closed-canopy forest sites, honeysuckle cover was 40–50% before honeysuckle removal and these shrubs were typically <1.5 m in size (Peebles-Spencer et al. 2017); the effects of honeysuckle removal on earthworm and litter biomass may be greater in sites with larger shrubs or greater understory cover. As honeysuckle litter decomposes rapidly (Arthur et al. 2012), we likely missed the influence of honeysuckle litter at the time of sampling by early summer. Since we found little effect of honeysuckle treatment on earthworms, there were likely weak corresponding effects on

the decomposer community. However, due to the possibility for earthworms to forage outside of subplots, our ability to detect honeysuckle removal effects on earthworm density and biomass was limited.

Interactions

While we expected additive, negative effects of deer exclusion and honeysuckle removals on earthworms, we found no support for additive nor synergistic effects of removals. However, there was an interactive effect on earthworm biomass, such that when deer were excluded earthworm biomass was greater with honeysuckle present, but this effect became more variable toward the end of the study (Fig. 2). The effects of deer on earthworms were much stronger and more consistent over time than the effects of honeysuckle removal. Overall, our results support the findings of Christopher and Cameron (2012) and Peebles-Spencer et al. (2017), which found no synergistic effects of honeysuckle and deer on litter depth or bare ground, respectively.

CONCLUSIONS

White-tailed deer, invasive plants, and invasive earthworms are major drivers of native plant decline and changes in ecosystem processes (Côté et al. 2004, Hale et al. 2006, McNeish and McEwan 2016). Our study shows that white-tailed deer and invasive earthworms are highly linked, supporting the findings of Dávalos et al. (2015b). A possible reciprocal relationship may occur between white-tailed deer and invasive earthworms in which earthworms reduce leaf litter and expose seedlings to deer browse. The recent study of relationships among aboveground herbivory, invasive species, and decomposers (both micro- and macroorganisms) has highlighted the complexities of ecosystem functions and the need for multiple, complementary approaches when attempting to understand these relationships. Building on the findings of Dávalos et al. (2015b), our results suggest that it is difficult to parse effects of deer from those of earthworms in enclosure studies, as these effects are likely interrelated. Finally, our findings that earthworms continued to decline and increased litter biomass were stable over five years of deer exclusion suggest that these impacts are reversible with long-term intervention. Reducing deer densities will likely reduce pressures of deer browse and invasive earthworms on native plants, invertebrates, and ecosystem processes.

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