ECOSYSTEMS

Interactive Effects of White-Tailed Deer, an Invasive Shrub, and Exotic Earthworms on Leaf Litter Decomposition

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ABSTRACT

Herbivore overabundance and species invasions could alter decomposition rates in temperate forests, with consequent effects on carbon sequestration, nutrient retention, and other ecosystem processes. At local scales, herbivores, invasive plants, and soil macroinvertebrates can be important drivers of decomposition, but interactive effects among these different groups are unknown. We tested for the effects of white-tailed deer, Amur honeysuckle, and earthworm activity (manipulated via mesh exclusion) on litter decomposition rates and loss of litter nitrogen (N) in five hardwood forest sites in southwestern Ohio. Each site consisted of a 20 × 20-m deer exclosure paired with a deer access plot; honeysuckle was removed from half of each plot. Effects of earthworm activity were tested using paired litter boxes of fine mesh (0.25 mm; earthworms excluded) or coarse mesh (10 mm; earthworm access). Restriction of earthworm activity in fine mesh treatments slowed litter decomposition and increased retention of N in the litter layer compared to coarse mesh. Deer access interacted with mesh treatments, with faster decomposition occurring in deer access, coarse mesh treatments relative to others. Greater earthworm biomass in deer access plots relative to deer exclosure plots corresponded with more rapid litter decomposition. Honeysuckle presence did not affect litter decomposition, but did increase litter N retention. The interactions between deer and earthworm activity indicate that reductions in deer populations may slow litter decomposition rates, increasing complexity of habitat structure at the soil surface, which relates to habitat for plants and animals.

Key words: earthworms; *Odocoileus virginianus*; *Lonicera maackii*; litter disappearance; temperate deciduous forest; above–belowground interactions.

HIGHLIGHTS

- Earthworm activity accelerated leaf litter decomposition.
- Deer exclosure slowed decomposition by reducing earthworm biomass.
- Honeysuckle had little effect on litter mass loss, but altered litter N dynamics.

INTRODUCTION

Leaf litter decomposition is a critical process in carbon and nutrient cycling in forest ecosystems, providing organic matter and nutrients to soil-dwelling organisms and plants. Broadly, decomposition is influenced by litter chemistry and climate (Aerts 1997), as well as decomposer abundance and community composition (Petersen and Luxton 1982; McGuire and Treseder 2010; Wagg and others 2014). At smaller spatial scales, climate influences become less important while litter quality and biotic controls (for example, herbivory, plant invasion, and decomposers) become more important to variation in rates of decomposition (Chapin and others 2011).

Herbivores, such as ungulates, have strong effects on plant communities that can alter animal communities, microclimate, and ecosystem processes, especially litter decomposition (Rooney and Waller 2003; Wardle and others 2004). Specifically, in deciduous forests of the Eastern United States, overabundance of white-tailed deer (*Odocoileus virginianus*) reduces overall native plant richness, increases success of certain invasive plants, and reduces aboveground plant biomass (Rooney 2009; Averill and others 2018). Preferential deer browse reduces densities of highly palatable species with low carbon/nitrogen (C/N) ratios, whereas increasing densities of unpalatable (high C/N) species, shifting litter chemistry, thereby slowing decomposition (Côté and others 2004; Wardle and others 2004). In contrast to their direct effects on plants and litter composition, deer benefit large-bodied decomposers such as exotic earthworms (Dávalos and others 2015; Mahon and Crist 2019), which accelerate decomposition (Fahey and others 2013b). Deer-mediated changes to plant communities and to the brown food web likely have cascading effects on ecosystem processes, such as decomposition and nutrient cycling (Wardle and others 2004; Harrison and Bardgett 2008; Mahon and Crist 2019).

Invasive plants can influence terrestrial ecosystems, by accelerating N cycling processes, increasing soil carbon and nutrient pools, and altering litter decomposition rates (Vilà and others 2011). In deciduous forests of the Eastern United States, the invasive Amur honeysuckle (*Lonicera maackii*) can create dense thickets in the understory that alter local carbon and nitrogen dynamics (McNeish and McEwan 2016). Honeysuckle litter decomposes and loses nitrogen at faster rates than native leaves (Blair and Stowasser 2009; Trammell and others 2012; Poulette and Arthur 2012), which may advance the timing of nutrient availability (Blair and Stowasser 2009). Honeysuckle effects on native litter decomposition and N recycling are not consistent and have been found to be both positive (Trammell and others 2012; Poulette and Arthur 2012) and negative (Arthur and others 2012). Mixed effects of honeysuckle on litter decomposition could arise from interactions with other organisms, such as dense honeysuckle stands supporting higher densities of white-tailed deer (Peterson 2018) and exotic earthworms (Lloyd and others 2019).

Non-native earthworm invasion into temperate forests alters ecosystem C dynamics by removing the surface organic horizon and increasing organic matter decomposition (Bohlen and others 2004a; Fahey and others 2013b). Studies comparing the presence and absence of exotic earthworms have shown how these earthworms restructure the soil profile and alter C processing (Bohlen and others 2004b; Hale and others 2005; Fahey and others 2013b). Yet, little is known about the effects of variable earthworm densities and community structures on ecosystem processes and properties in areas where exotic earthworm populations have been well established for many years. Further, it is uncertain whether effects of deer and honeysuckle on exotic earthworms (Lloyd and others 2019; Mahon and Crist 2019) correspond to a measurable influence on ecosystem processes, such as decomposition. Interactions among these taxa are expected, as there is broad overlap in their ranges in the Midwestern and Eastern United States.

In the present study, we investigated the direct and interactive effects of white-tailed deer and Amur honeysuckle on microbial and exotic earthworm-mediated leaf litter decomposition and N dynamics in a split-plot design of honeysuckle removal/retention within deer exclosure/access plots. We tested effects of exotic earthworm activity on litter decomposition and N dynamics by using different mesh sizes to restrict access by earthworms. We homogenized leaf litter from deer and honey-

suckle treatments to exclude possible treatment effects on litter chemistry and quantity and to focus on the immediate effects of deer, honeysuckle shrub presence, and exotic earthworm activity. Mixing of litter also allowed us to minimize home-field advantage effects on decomposition rates (Ayres and others 2009).

Here, we tested the hypothesis that deer, honeysuckle, and exotic earthworms show positive interactions leading to accelerated decomposition rates and loss of litter N. Faster rates of litter decomposition were expected in coarse mesh treatments (earthworm accessible litter) relative to litter in fine mesh treatments and in areas with greater earthworm biomass. As deer exclosure reduces earthworm density and biomass (Mahon and Crist 2019), we predicted deer exclusion would reduce earthworm biomass and slow litter decomposition. As earthworms favor honeysuckle litter (Pipal 2014), we predicted that honeysuckle removal would slow leaf litter decomposition. Further, as dense honeysuckle shrubs may interfere with deer movement and thereby suppress earthworm density, we predict slower litter decomposition under honeysuckle shrubs in deer access plots. We expected similar patterns for loss of litter N.

METHODS

Study Sites

The study was conducted in second growth deciduous forests within the Miami University Natural Areas (a 400 + ha preserve) in southwestern Ohio, USA. The study sites were located on level topographic positions, similar elevations (253 ± 5 m a.s.l.), and similar honeysuckle densities (stem basal area of 0.6–1.6 m^2/ha; Peebles-Spencer and others 2017). The climate is temperate continental with mean annual temperature of 10.8°C and mean annual precipitation of 110 cm. The dominant soil type is a fine, mixed active Hapludalf. The soil profile has minimal to no organic horizon overlying a well-mixed A or A_p horizon. Study sites were mature, closed canopy deciduous forests originating about 60–80 years ago after abandonment from pasture and grazing (Medley and Krisko 2007). Dominant tree species included sugar maple (*Acer saccharum*), oak (*Quercus* spp.), beech (*Fagus grandifolia*), hickory (*Carya* spp.), and elm (*Ulmus* spp.). The invasive Amur honeysuckle dominated the understory of these sites with about 40–50% understory cover. Further details on vegetation in our study sites can be found in Peebles-Spencer and others (2017). Our five sites were located along a natural gradient of exotic earthworm density (Table S1; Mahon and Crist 2019).

Experimental Design

We used a split–split-plot experimental design with the exclusion of deer and removal of honeysuckle at the plot and subplot levels, respectively. At each of the five study sites, two paired 400 m^2 (20 × 20-m) plots located 50–100 m apart were randomly assigned to either deer exclosure or deer access (control); each with a split-plot (20 × 10-m subplots) treatment of honeysuckle removal and retention (control), again randomly assigned within each deer plot (Figure S1). In 2010, 2.5-m high fencing was constructed around the deer exclosures to restrict deer access. Also in 2010, honeysuckle shrubs were cut at the base and Tordon RTU (Dow AgroSciences, Indianapolis, IN) was applied directly to the stem; a second round of honeysuckle removal occurred in 2015 (Peebles-Spencer and others 2017). The experimental deer and honeysuckle treatments (4 deer/honeysuckle treatment subplots × 5 replicate sites = 20 subplots) were further subdivided into mesh treatments with litter boxes of either fine (0.25 mm) or coarse (10.0 mm) mesh openings. Each subplot had 3 pairs of coarse and fine mesh boxes (3 pairs × 2 mesh sizes × 20 subplots = 120 boxes) with pairs of boxes spaced 5 m apart on either side of a line 5 m from the edge of each honeysuckle subplot (Figure S1). We conducted two separate decomposition experiments with the same study design with the first study beginning in the fall of 2015 and the second study beginning in the fall of 2016. The difference between these two studies is in the timing of destructive sampling (described below).

Litter Decomposition

Litter decomposition was measured using unconfined litter samples in 0.5 × 0.5-m litter boxes installed in November 2015 and 2016. Prior to leaf abscission in each year, the O_i horizon was cleared from 1 × 0.5-m areas on which adjacent litter boxes were constructed with landscape edging (12.7 cm height; Master Mark Terrace Board Landscape Edging). We fixed nylon No-See-Um netting (hole size 0.25 mm; American Home and Habitat) beneath the fine mesh box of each pair and nylon mesh (hole size 10 mm; American Home and Habitat) beneath the coarse mesh box of each pair. The coarse mesh was large enough to allow access by nearly all meso- and macroinvertebrates and facilitated recovery of litter during sampling, while the fine mesh allowed access by soil microbes

and microfauna, but restricted access by macroinvertebrates (Chapin and others 2011). We collected all fresh leaf litter from boxes after litterfall, airdried litter, mixed and homogenized litter for uniform initial litter chemistry across all treatments, and allowed the litter to air-dry for 2 weeks in the laboratory. We placed equal masses of the air-dried litter mixture (equal to 80.0 ± 0.88 g oven-dry mass in 2015; 69.9 ± 0.58 g in 2016) in all litterboxes in early December of each year and fixed polypropylene mesh (opening size 1.5 cm; Deer-Block, Easy Gardener) to the top of the boxes.

In the 2016 study, litter boxes were randomly sampled after 100, 200, and 300 days in the field. Little mass was lost over winter in the first 100 days. Therefore, in the 2017 study we adjusted sampling times to 200, 300, and 400 days in the field. On each sampling date, we collected all litter from a randomly chosen pair of litter boxes within each honeysuckle subplot (40 litter boxes destructively sampled at each sampling date), transported litter to the laboratory in plastic bags, removed debris (for example, fine roots, seedlings, and seeds), and oven dried at $60°C$ to constant mass to determine mass loss. In total, 119 litter boxes (1 lost) were collected in 2016 and 116 litter boxes (4 lost) were collected in 2017. In the 2017 study, we homogenized litter and removed small (3.98 ± 1.06 g) subsamples for chemical analyses prior to oven drying. Litter subsamples were frozen at $- 30°C$ until chemical analyses were conducted. Litter subsamples were dried to constant mass at $60°C$, ground to a fine texture with a coffee grinder (Krups), and analyzed for total C and N using a Fisher Scientific Flash 2000 NC Soil Analyzer. Initial litter C concentration was $47.3 \pm 0.7\%$ C, initial N concentration was $1.4 \pm 0.3\%$ N, and initial C/N ratio was 36.44 ± 7.19.

Fine mesh litter boxes only permit access by microbes and small microfauna. Therefore, the mass loss from these boxes is a measure of microbial-mediated decomposition. Although macroinvertebrate detritivores other than earthworms were able to access litter in coarse mesh litter boxes, we refer to mass loss in coarse mesh litter boxes as ''earthworm-mediated'' decomposition. Further, in coarse mesh litter boxes (earthworm accessible litter), we do not separate the three main mechanisms of mass loss: ingestion and burial of leaf material, oxidation of organic C to CO_2, and leaching of organic C as DOC. Hence, our measure of mass loss in coarse mesh litter boxes reflects the combined effects of earthworm feeding, vertical transport of litter, earthworm and microbial respiration, other macroinvertebrate activity, and water transport, which may overestimate the role of earthworms in decomposition.

Earthworm Sampling

Earthworms were sampled in late-May to early-June of 2016 and 2017 (results reported in Mahon and Crist 2019). We sampled earthworms using mustard extraction (Lawrence and Bowers 2002) from 0.25 m^2 quadrats located 1 m from paired litter boxes (Figure S1). Earthworms were immediately placed in 70% ethanol and brought to the laboratory for identification and mass determination. Adult earthworms were identified to species, and juveniles were identified to genera. Specimens were placed on paper towels and air-dried before morphotype total wet mass (g) was measured. We collected a single *Aporrectodea tuberculata* specimen, but all other morphospecies were collected two or more times. We collected no native earthworms in our study area; thus, hereafter we use ''earthworms'' and ''exotic earthworms'' synonymously.

Statistical Analyses

When litter decomposition patterns with strong curvature are observed, the single exponential decay rate developed by Olson (1963) can be inappropriate, as the decay rate is assumed constant with simple exponential decay. This assumption is difficult to meet owing to seasonality and to changing chemical quality of the substrate (Chapin and others 2011). Therefore, we fit our data to the traditional Olson single exponential decay and simple linear decay models; we also used the approach of Rovira and Rovira (2010) to compare several possible temporal patterns of decomposition: 1) exponential rate decrease, (2) waveform dynamics (simulating annual cycles), (3) sigmoidal dynamics (simulating a change in decay conditions), and (4) rational-type dynamics (initial rate increase followed by rate decrease). The sigmoidal model was most appropriate based on lowest AIC and highest adjusted R^2 values. Sigmoidal models require four parameters: b gives the shape of the function; c gives the lowermost value of decay; a plus c gives the uppermost value of decay ($r = a + c$); and t_0 gives the time at which r increases from c to $a + c$ (see Rovira and Rovira 2010 for more details). The uppermost value of decay (r) corresponds to the point in the middle of sigmoidal curve, at which the decay rate is highest (see Figure 3B in Rovira and Rovira 2010 and Figure S2 for examples). To aid in parameter convergence and minimize issues of overparameterization due to low temporal sample size, t_0 was set at 0.351 years as

indicated by parameters estimated for the overall model. Parameters a, c, and b were estimated with nonlinear least squares estimation using the Curve Fitting Toolbox in MATLAB (Mathworks Inc., Natick, MA, USA). A total of 40 individual parameter estimates were found for each study (2 mesh treatments × 4 deer/honeysuckle treatment subplots × 5 replicate sites = 40 site-treatment combinations).

All subsequent statistical analyses were conducted in R version 3.5 (R Development Core Team 2018). Where appropriate, we inferred significant differences among treatments when the null hypothesis was rejected with $\alpha < 0.05$. To test effects of our experimental treatments on uppermost decay rate value (r), %N remaining, and C/N ratio, we performed ANOVAs using linear mixed models (*lmer* function in lme4; Bates and others 2015) for a split–split-plot design. The random effects included a random intercept of site, deer plot nested within site, and honeysuckle subplot nested within deer plot (1|Site/Plot/Subplot). Nested random effects incorporate the unstructured covariance in response variables, which accounts for non-independence in response variables among mesh treatments, subplots, plots, and sites (Pinheiro and Bates 2000; Bates and others 2015). Fixed effects included the main and interactive terms of deer, honeysuckle, and mesh treatments. Each sampling period was analyzed separately for litter chemistry endpoints. We refer to results of %N remaining and C/N ratio in the main text, but analyses of litter C/N ratio are presented in Table S2. Significance tests were conducted using the *Anova* function (car package, R; Fox and Weisberg 2011) with type III error as there were unequal sample sizes for nutrient analysis. To test for differences in significant interactions, we conducted post hoc comparisons (*emmeans* function and package, R; Lenth 2018).

To test for potential indirect effects of our treatments on uppermost value of decomposition rates (r), we performed structural equation models (SEM; piecewiseSEM package, R; Lefcheck 2016). SEMs allow for testing direct and indirect causal relationships between variables. Our model included paths linking deer and honeysuckle treatments to earthworm biomass, deer and honeysuckle treatments to decomposition, and earthworm biomass to decomposition. We also tested for differences in relationships between mesh treatments, using multigroup analysis (Lefcheck 2016). In multigroup analyses, significant interactions between paths and mesh treatment were allowed to change across mesh treatments, whereas nonsignificant interactions were conserved and remained constant across mesh treatments.

Finally, to test whether earthworm species composition influenced decomposition, we used non-metric multidimensional scaling (NMDS) ordination on the earthworm community composition (*metaMDS* function, vegan package, Oksanen and others 2018) based on Hellinger-transformed biomass data and Euclidean distances. Following NMDS ordination for each year, we fit the uppermost value of the decomposition rate (r) from litter in coarse mesh litter boxes using generalized additive models with the *ordisurf* function (vegan package, Oksanen and others 2018), which is a test of how well the ordination predicts environmental variables. We pooled earthworm biomass by species across the three quadrat samples in each subplot. As *Octolasion tyrtaeum* was the only *Octolasion* species found in our study, we combined juvenile *Octolasion* and *O. tyrtaeum*. We removed *Aporrectodea tuberculata* from multivariate analyses to reduce the effect of singleton species.

RESULTS

Decomposition

The upper most values of decomposition rate, r, were similar across years and ranged from 0.00 to 5.42 in 2016 and 0.23 to 3.59 in 2017. Mass loss followed the same pattern as the sigmoidal decay function and final mass remaining followed the treatment pattern of the uppermost value of decomposition rate, r (Figure S2, Figure S3). In both years of the study, fastest mean decomposition rate was found in deer access, honeysuckle removed, coarse mesh treatments (Table 1). Much of the variation in decomposition rates was explained by the mixed models, with most due to random effects of site (2016 Marginal $R^2 = 0.15$, Conditional $R^2 = 0.77$; 2017 Marginal $R^2 = 0.36$, Conditional $R^2 = 0.66$). In both years of the study, mesh size had a strong effect on decomposition rate (Table 2, Figure 1); litter in coarse mesh treatments (where large-bodied earthworms had access) decayed 58% and 67% faster than litter in fine mesh treatments in 2016 and 2017, respectively. In both years of the study, there were no significant direct main effects of deer or honeysuckle treatments on the uppermost decomposition rate (Table 2). There was a significant interaction between deer and mesh treatments in 2017 ($F_{1,16} = 7.249$, $p = 0.016$; Table 2; Figure 1B). Specifically, in deer access plots, leaf litter decomposition rates were two times

Table 1. Mean Maximum Decay Rates (r), Percent Nitrogen (N) Remaining, and Percent Carbon (C) Remaining After 400 days in the Field for Litter Boxes Located Within Deer and Honeysuckle Treatment Combinations

	Deer treatment	Honeysuckle treatment	Mesh treatment	Max. decay rate r (1/y)	%N remaining	%C remaining
2016	Access	Retained	Coarse	1.50 (0.43)	–	–
	Access	Retained	Fine	1.20 (0.33)	–	–
	Access	Removed	Coarse	2.36 (0.93)	–	–
	Access	Removed	Fine	1.14 (0.48)	–	–
	Exclosure	Retained	Coarse	1.50 (0.50)	–	–
	Exclosure	Retained	Fine	0.96 (0.32)	–	–
	Exclosure	Removed	Coarse	1.17 (0.27)	–	–
	Exclosure	Removed	Fine	0.84 (0.17)	–	–
2017	Access	Retained	Coarse	1.97 (0.37)	41.0 (9.9)	24.6 (3.6)
	Access	Retained	Fine	0.76 (0.19)	72.0 (11.8)	43.6 (5.9)
	Access	Removed	Coarse	2.07 (0.49)	16.7 (7.1)	11.4 (4.2)
	Access	Removed	Fine	1.19 (0.10)	39.2 (7.4)	26.9 (3.9)
	Exclosure	Retained	Coarse	1.54 (0.21)	36.1 (10.4)	19.0 (4.0)
	Exclosure	Retained	Fine	1.04 (0.15)	60.2 (11.5)	36.6 (5.5)
	Exclosure	Removed	Coarse	1.08 (0.19)	52.4 (12.7)	35.4 (10.3)
	Exclosure	Removed	Fine	1.00 (0.25)	81.4 (12.8)	47.4 (8.1)

Values are treatment mean with standard error in parentheses.

Table 2. Split–Split-Plot ANOVA Results for Maximum Decay, r, for Each Year of the Study

Source	df1	df2	F-value	p value
2016				
Deer	1	4	1.524	0.285
HS	1	8	0.110	0.749
Mesh	**1**	**16**	**11.616**	**0.004**
Deer:HS	1	8	1.448	0.263
Deer:Mesh	1	16	0.845	0.372
HS:Mesh	1	16	0.997	0.333
Deer:HS:Mesh	1	16	2.528	0.131
2017				
Deer	1	4	2.807	0.169
HS	1	8	0.003	0.961
Mesh	**1**	**16**	**22.961**	**< 0.001**
Deer:HS	1	8	3.418	0.102
Deer:Mesh	**1**	**16**	**7.249**	**0.016**
HS:Mesh	1	16	1.757	0.204
Deer:HS:Mesh	1	16	0.019	0.892

Significant treatment effects ($p < 0.05$) are bolded.

greater in coarse mesh treatments than in fine mesh treatments, while in deer exclosure plots, there was no difference between mesh treatments (Figure 1B). There were no other significant interactions among treatments in either year of the study (Table 2).

Structural equation models (SEMs) tested whether deer and honeysuckle effects on decomposi-tion rates were mediated through differences in earthworm biomass; SEMs indicated strong positive controls of earthworm biomass on decomposition rates in both years (Figure 2). The positive rela-tionship between earthworm biomass and decom-position rates was stronger in coarse mesh treatments (coef.: 0.75) compared to fine mesh treatments (coef.: 0.58) in 2016 (Figure 2A). The significant, positive relationship between earth-worm biomass and decomposition rates in fine mesh treatments suggests either that microbial decomposition increases with greater earthworm biomass or that some earthworms were able to access the leaf litter in fine mesh treatments. Conversely, in 2017, there were strong positive effects of earthworm biomass on decomposition in coarse mesh treatments (coef.: 0.67), but non-significant negative effects of earthworm biomass on decomposition in fine mesh treatments (coef.: − 0.29; Figures 2B, 3). In both years, deer exclo-sure indirectly slowed decomposition via reduc-tions in earthworm biomass (Figure 2). Multigroup analysis indicated differences in strength and direction of the relationship between deer and honeysuckle treatments and decomposition rates across mesh treatments in 2017 only, but these relationships were weak and not significant across mesh treatments (Figure 2B).

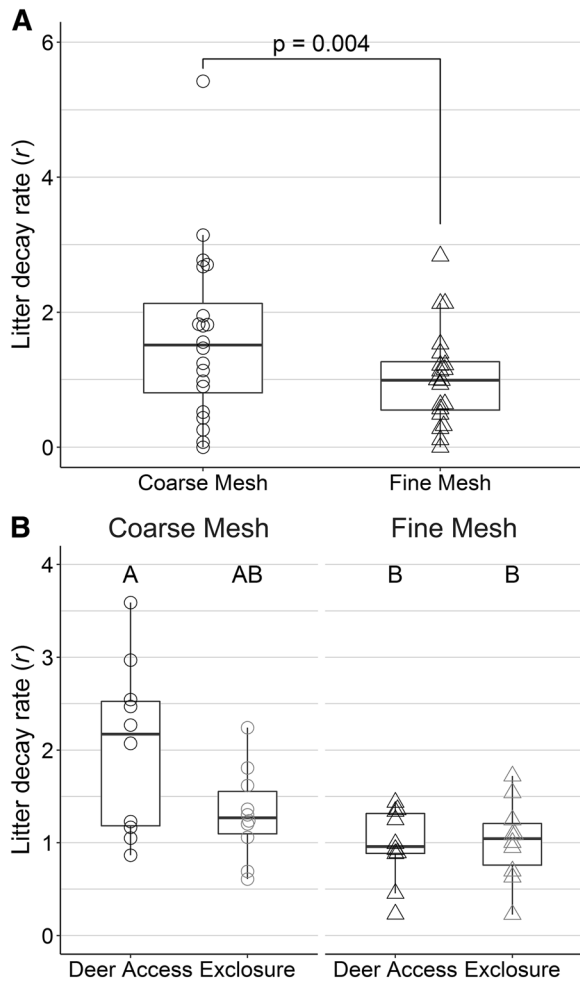Multivariate analyses indicated a strong rela-tionship between earthworm species composition

Figure 1. Uppermost value of litter decay rates (r) across 5 sites over 300 days in 2016 (**A**) and 400 days in 2017 (**B**). Circles are coarse mesh treatments, triangles are fine mesh treatments. In **B** black symbols are deer access, gray are deer exclosures. In **B**, same letters indicate no significant difference between treatments.

Figure 2. Structural equation models (SEMs) for the uppermost value of decay (r) for 2016 (**A**) and 2017 (**B**). Significant paths are solid lines, nonsignificant paths are dashed. Positive relationships are in black, negative relationships are in grey. Standardized coefficients are presented. Honeysuckle removal and deer exclosure paths are in comparison with honeysuckle presence and deer access, respectively.

and uppermost decomposition rates in 2016 (Dev. explained = 52.8%, $p < 0.001$) and a weak relationship in 2017 (Dev. explained = 26.5%, $p = 0.091$; Figure 4). Species scores of earthworms based on biomass were associated with moderate to high rates of decomposition in both years (higher decomposition values on right side of ordinations; Figure 4). Consistent between years, subplots with little or no earthworm biomass corresponded to subplots with slow rates of decomposition (Figure 4). Locations of earthworm species scores relative to decomposition rate (r) were consistent across years (Figure 4). In both years, the biomass of the endogeic earthworms (*Octolasion tyrtaeum* and *Aporrectodea* spp.) was associated with faster litter decomposition (Figure 4). Species scores for biomass of *Lumbricus terrestris* were consistent be-
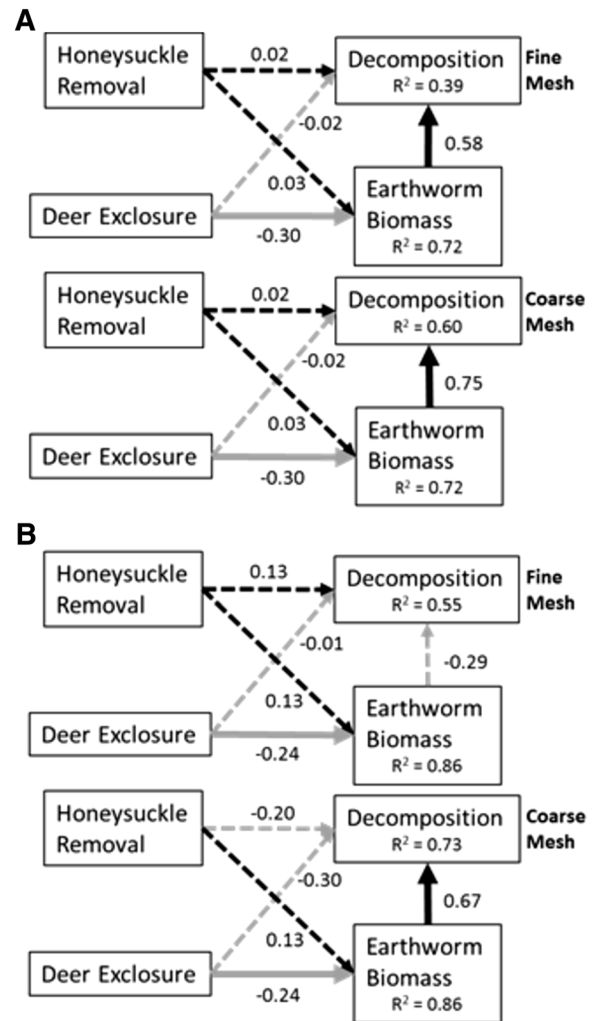
tween years and were associated with moderate decomposition rates (Figure 4). Conversely, species scores for biomass of *Lumbricus* juveniles and *Lumbricus rubellus* were not consistent between years, but were found in areas of moderate to high decomposition rates (Figure 4).

Litter Chemistry

Mean litter N remaining ranged from 16.7 to 81.4% after 400 days in the field in 2017 (Table 1). Patterns of N dynamics were similar to patterns of mass loss (Figure 5; Figure S3). Mesh treatment had strong effects on litter N remaining across sampling dates
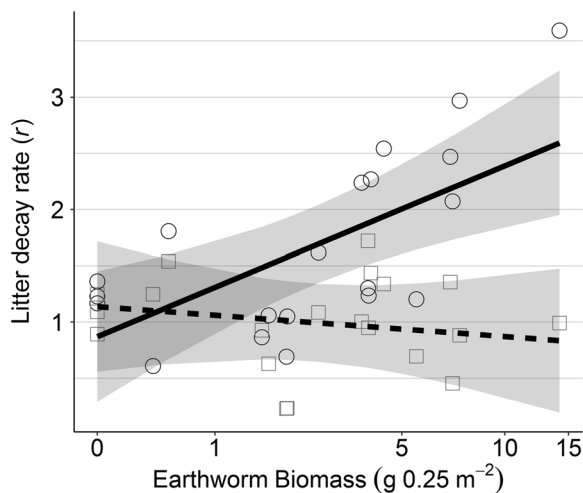
Figure 3. Relationship between earthworm biomass, mesh treatment and maximum decay rate (*r*) in 2017. Lines are fixed effects from a generalized linear mixed model. Circles and solid line are coarse mesh treatments; squares and dashed line are fine mesh treatments. There was a positive relationship between earthworm biomass and decay rate in coarse mesh treatments, but a weak negative relationship between earthworm biomass and decay rate in fine mesh treatments. *Note*: *x*-axis is natural log-transformed.

(Table 3, Figure 5). Litter N in coarse mesh treatments (where large-bodied earthworms had access) declined by about 50% between 200 and 300 days in the field, whereas litter N in fine mesh boxes declined by a similar amount between 300 and 400 days in the field (Figure 5). Remaining litter N was 42% lower in coarse mesh treatments compared to fine mesh treatments after 400 days in the field. There were no main deer or honeysuckle treatment effects on litter N remaining across sampling dates (Table 3). After 300 days in the field, there was a significant interaction between deer and mesh treatments on litter N remaining ($F_{1,15.2} = 6.673$, $p = 0.021$). Litter N remaining was greater in coarse mesh treatments compared to fine mesh treatments in deer access plots ($T_{16.1} = 4.755$, $p < 0.001$), but there was no difference in litter N remaining between mesh treatments in deer exclosure plots ($T_{14.4} = 1.352$, $p = 0.197$; Figure 5). After 400 days in the field, there was a significant interaction between deer and honeysuckle treatments on litter N remaining ($F_{1,7.5} = 8.073$, $p = 0.023$). In deer access plots, honeysuckle removal weakly reduced percent litter N remaining ($T_{7.8} = 2.156$, $p = 0.064$), whereas in deer exclosure plots, honeysuckle removal weakly increased percent litter N remaining ($T_{7.1} = 1.852$, $p = 0.106$; Figure 5).
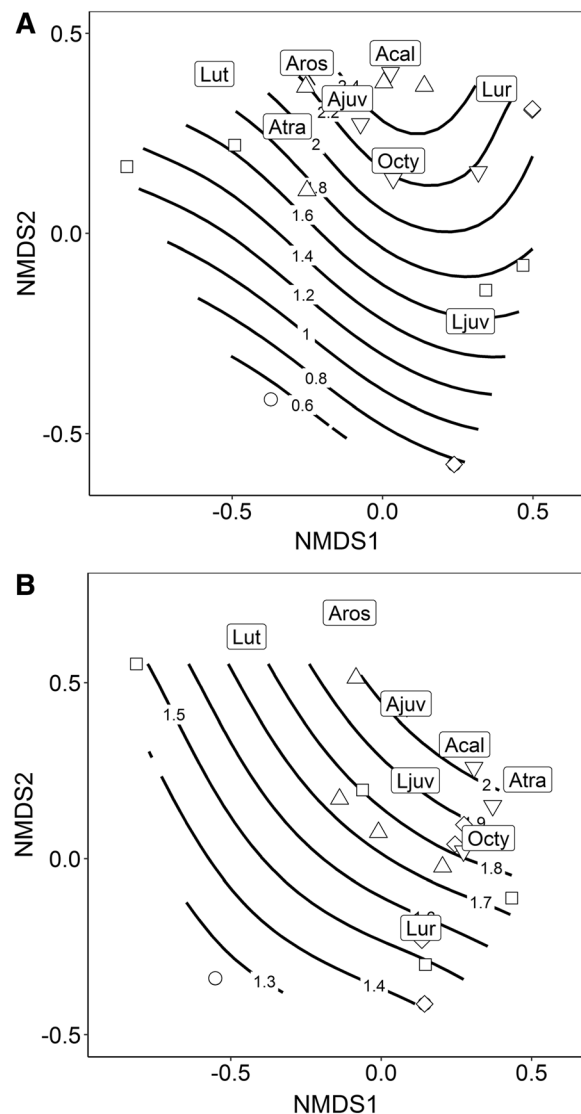

Figure 4. Non-metric multidimensional scaling (NMDS) ordination of exotic earthworm community based on species variation in earthworm biomass found in 2016 (**A**) and 2017 (**B**). Points are subplots and contours show relationships between ordination and decomposition rates (*r*), as determined by the *ordisurf* function, *vegan* package, R. Shapes of points represent sites (circle—Bachelor; diamond—Kramer; triangle—Reinhart; square—College; inverted triangle—Western). Species scores are Acal: *Aporrectodea caligonosa*, Aros: *Aporrectodea rosea*, Atra: *Aporrectodea trapezoides*, Ajuv: *Aporrectodea* juveniles Lur: *Lumbricus rubellus*, Lut: *Lumbricus terrestris*, Ljuv: *Lumbricus* juveniles, Octy: *Octolasion tyrtaeum*.

Litter in coarse mesh treatments had a higher C/N ratio than litter in fine mesh treatments after 300 days in the field ($F_{1,15.1} = 6.543$, $p = 0.022$), indicating deviation of N and C dynamics in later stages of litter decomposition. C/N ratios were not
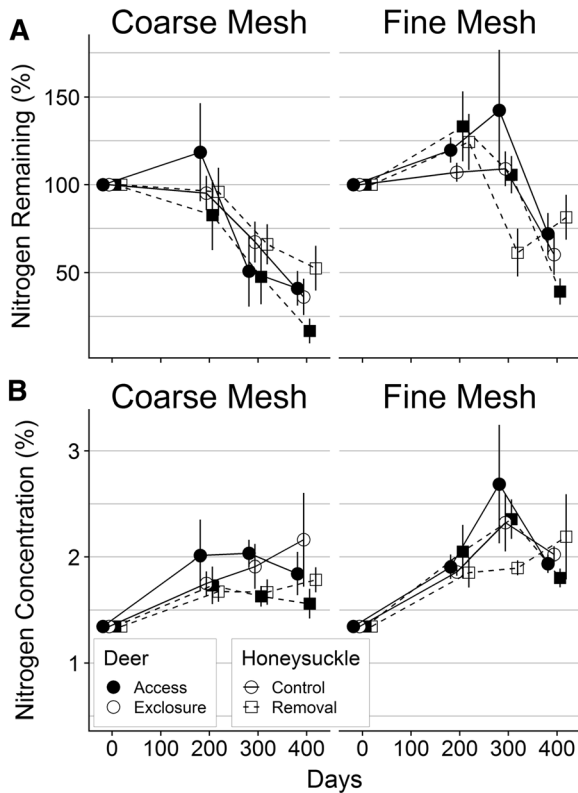
Figure 5. Nitrogen dynamics of mixed litter over 400 days and across treatment combinations for 2017. **A** Litter N mass remaining (percentage of initial litter N remaining). **B** N concentration of litter. Error bars represent standard errors.

Table 3. Split–Split Plot ANOVA Results for Percent Litter N Remaining

Source	df1	df2	*F*-value	*p*-value
200 days				
Deer	1	4.00	0.599	0.482
HS	1	8.00	0.011	0.918
Mesh	**1**	**16.00**	**5.086**	**0.038**
Deer:HS	1	8.00	0.979	0.351
Deer:Mesh	1	16.00	0.079	0.782
HS:Mesh	1	16.00	2.608	0.126
Deer:HS:Mesh	1	16.00	0.649	0.432
300 days				
Deer	1	3.94	0.593	0.485
HS	*1*	*7.85*	*4.805*	*0.060*
Mesh	**1**	**15.24**	**19.483**	**< 0.001**
Deer:HS	1	7.85	0.050	0.829
Deer:Mesh	**1**	**15.24**	**6.673**	**0.021**
HS:Mesh	*1*	*15.22*	*3.883*	*0.067*
Deer:HS:Mesh	1	15.22	0.102	0.754
400 days				
Deer	1	3.90	2.246	0.210
HS	1	7.48	0.134	0.724
Mesh	**1**	**15.00**	**26.962**	**< 0.001**
Deer:HS	**1**	**7.48**	**8.073**	**0.023**
Deer:Mesh	1	15.00	0.000	0.984
HS:Mesh	1	15.00	0.031	0.863
Deer:HS:Mesh	1	15.00	0.419	0.527

Deer refers to deer treatment, HS refers to honeysuckle treatment, and mesh refers to mesh size treatment. Significant treatment effects (p < 0.05) are bolded; marginally significant effects (p < 0.10) are italicized.

different between mesh treatments at any other sampling date (Table S2). There were no main deer or honeysuckle treatment effects nor interactions among treatments on litter C/N ratio across sampling dates (Table S2).

DISCUSSION

The goal of our study was to determine the main and interactive effects of deer, honeysuckle, and exotic earthworms on aboveground leaf litter decomposition in eastern deciduous forest. We found sharply increased rates of litter decomposition in coarse mesh treatments (earthworm accessible litter) compared to fine mesh treatments in both years of our study, supporting our hypothesis that macroinvertebrates (mainly, but not solely, earthworms) facilitate leaf litter decomposition in eastern deciduous forests. Structural equation models showed a positive relationship between earthworm biomass and maximum decomposition rate, *r*, indicating that earthworm activity represents the majority of macroinvertebrate activity

associated with mass loss in our system. Further, these results suggest variable earthworm activities influence ecosystem processes in areas where exotic earthworm populations have been established for many years. Structural equation models also indicate strong indirect effects of deer on decomposition rates, mediated through earthworm biomass, supporting our hypothesis that deer alter decomposition rates through changes to the decomposer community. In both years of our study, multivariate analyses indicated correlation between earthworm species composition and decomposition rates, suggesting that earthworm composition based on biomass may be key in generalizing effects of earthworm invasion on ecosystem processes.

We found no support for our hypothesis that honeysuckle removal would accelerate litter decomposition; in fact, we saw little influence of honeysuckle on decomposition. There were no differences in litter decomposition rates in fine mesh treatments across deer and honeysuckle treatments, indicating that deer and honeysuckle

have little effect on microbial-mediated litter decomposition. Differences in treatment patterns between the two consecutive studies are likely due to sampling error, differences in weather between the 2 years of the studies, and study duration; however, it is beyond the scope of this study to determine which of these factors induced differences between consecutive years of this study.

Earthworm Impacts on Litter Decomposition

As we predicted, we found a strong effect of mesh size on litter decomposition and N dynamics in our system, indicating earthworms accelerate decomposition, which is consistent with previous studies on earthworm presence/absence (Suárez and others 2006; Holdsworth and others 2008; Fahey and others 2013b). Mass loss in coarse mesh litter boxes was the result of the combined activity of micro- and macroinvertebrate decomposers (for example, insects, isopods, millipedes, and earthworms) with a body width of 10 mm or less. As studies using various mesh sizes indiscriminately exclude and include all macroinvertebrates, the role of earthworms in terrestrial decomposition may be overestimated. Although we did not quantify the density nor diversity of other soil fauna in this study, a meta-analysis by Ferlian and others (2018) indicates substantial reduction in the density and diversity of soil fauna following earthworm invasion. Thus, it would be expected that the activities of other macroinvertebrates would be reduced in our study system. Further, our structural equation models showed a strong relationship between earthworm biomass and litter decomposition, suggesting earthworm activity represents the majority of macroinvertebrate activity associated with mass loss in our system.

Our study is among the first to show that earthworm effects on ecosystem processes in areas with established exotic earthworm populations are dependent on earthworm density/biomass. The positive relationship between earthworm biomass and litter decomposition is consistent with the findings of Suárez and others (2006) and Heneghan and others (2007) who found greater litter mass loss associated with greater earthworm density. Although we did not explicitly follow the fates of litter C after being mixed into soil, Fahey and others (2013b) found no accumulation of litter-derived C in earthworm-invaded soils. Our results then suggest earthworm effects on C dynamics vary on a landscape level, relating to variation in earthworm density and community structure.

Further work is needed to understand landscape-scale relationships among earthworm functional groups, species composition, and ecosystem processes in areas with established exotic earthworm populations.

We also found a correlation between the species composition of earthworms based on biomass and decomposition rates, which is consistent with previous studies that have found influence of earthworm community structure on ecosystem processes along an invasion gradient (Hale and others 2005; Suárez and others 2006; Chang and others 2016). Our results, together with Suárez and others (2006) and Chang and others (2016), suggest the controls of earthworm community on decomposition are related to the biomass of individual species. Surprisingly, while studies have shown the anecic *L. terrestris* removes litter at a greater rate than earthworms in other functional groups (Suárez and others 2006), ordinations indicated that species scores of *L. terrestris* were associated with moderate–fast decomposition rates rather than areas with fastest decomposition. An explanation for this may be that the mesh size used in the coarse mesh treatments allowed access by most *L. terrestris*, but excluded larger *L. terrestris* individuals. Species scores of several endogeic species (*O. tyrtaeum* and *Aporrectodea* spp.) were consistently found in areas with faster decomposition. This is likely an artifact of the earthworm invasion severity captured across sites, as endogeic species are typically restricted to areas where the forest floor has been depleted and incorporated into the mineral soil (strong earthworm invasion severity; Hendrix and others 1999). The spatial variation in the biomass of earthworm species and their combined effects on decomposition rates found in our study have not been widely tested in previous studies. Further research should investigate the relationships between earthworm community composition and leaf litter decomposition in other systems.

The strong effects of earthworms on decomposition corresponded to changes in litter N dynamics. Litter in fine mesh treatments had greater litter N and lower litter C/N ratios relative to litter in coarse mesh treatments. As native earthworms were not present in our study soils, these results suggest exotic earthworms alter nitrogen dynamics, by transferring litter N into mineral soil about 100 days sooner than via microbial pathways alone. Our results are consistent with Fahey and others (2013a), who found that the transfer of litter N to mineral soil was twice as rapid in areas with earthworms compared to those without earthworms. Hence, exotic earthworms reduce the N

sink provided by litter on the soil surface, but whether this N sink persists in the soil depends on the fate of ingested and soil-mixed litter material (Fahey and others 2013a).

Deer Impacts on Litter Decomposition

We found faster litter decomposition and litter N loss in deer access plots compared to deer exclosures in coarse mesh treatments, but similar litter decomposition and litter N loss between deer access and deer exclosure plots in fine mesh treatments. This pattern indicates strong effects of deer on macroinvertebrate-mediated decomposition but not microbial-mediated decomposition. This is supported by SEMs, which indicated strong indirect effects of deer on decomposition, mediated through changes to earthworm biomass. These results suggest that the interaction between deer and earthworms may accelerate C loss more than the effects of earthworms alone. Our findings of faster decomposition in deer access plots consistent with previous studies that have found more leaf litter in deer exclosures relative to deer access plots (Bressette and others 2012; Mahon and Crist 2019). There was some evidence that deer facilitated N immobilization, but litter N concentration was not different between deer access and exclosure plots through time.

Previous studies linked ungulate-mediated changes in decomposition to browse-mediated changes in plant communities and litter chemistry (Wardle and others 2002). In forest ecosystems, preferential deer browse reduces palatable species (high N) and simultaneously increases unpalatable species (low N), thereby altering chemistry of litter entering the system and decelerating decomposition and N cycling (Wardle and others 2002; Côté and others 2004; Wardle and others 2004). Though we did not explicitly test for this hypothesis, we provide evidence for strong controls of deer on decomposition rates through non-consumptive (brown food web) pathways that may outweigh consumptive effects, at least over shorter time scales. Future work should investigate the relative contribution of consumptive and non-consumptive pathways by which ungulates alter ecosystem processes.

Honeysuckle Impacts on Litter Decomposition

We found little effect of honeysuckle removal on litter decomposition rates; these results are consistent with previous studies that found no effect of honeysuckle shrubs on decomposition rates (Madritch and Lindroth 2009; Pipal 2014; but see Arthur and others 2012). The mixed results of studies investigating decomposition rates in response to honeysuckle presence is likely due to differences in honeysuckle densities (Trammell and others 2012), exotic earthworm densities (Pipal 2014), and microbial communities (Arthur and others 2012) among study locations. Previous studies have typically used leaf litter from a single species rather than mixed litter (Arthur and others 2012; Pipal 2014), which may further cloud generalizable patterns.

The effects of honeysuckle on N dynamics were weaker than earthworm or deer effects and were dependent on deer treatment. We found no evidence for this interactive effect on C/N ratios, suggesting this interaction is likely following patterns of mass loss and C dynamics. Thus, while we did not see a strong interaction between deer and honeysuckle on decomposition rates, patterns of remaining litter N indicate effects of honeysuckle on litter decomposition and N dynamics is dependent on deer and earthworms. Further work is needed to understand whether landscape-level relationships among honeysuckle, deer, and earthworms (Peterson 2018; Lloyd and others 2019) influence landscape variability of ecosystem processes.

CONCLUSIONS

White-tailed deer, invasive plants, and invasive earthworms are major drivers of change in deciduous forests of the Eastern and Northern United States (Bohlen and others 2004b; Côté and others 2004; Vilà and others 2011). Our results indicate strong interactions between white-tailed deer and non-native earthworms on leaf litter decomposition. We also found weaker effects of honeysuckle on N cycling. Earthworm species composition and total biomass of earthworms present in an area are important regulators of decomposition in eastern deciduous forests. Earthworm-mediated increased litter decomposition and reduced litter biomass likely have cascading effects forest ecosystems, including reduced habitat for ground- and litter-dwelling invertebrates (Ferlain and others 2018; Mahon and others 2019), higher recruitment for invasive species (Belote and Jones 2009), and greater effects of deer browse on herbaceous plants (Frelich and others 2006).

Compliance with Ethical Standards

Conflict of interest The authors declare they have no conflict of interest.

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