

Effects of earthworms and white-tailed deer on roots, arbuscular mycorrhizae, and forest seedling performance

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Abstract. Changes in understory plant composition and biodiversity declines in northeastern North American forests are widespread. Preserving species and ecosystem function requires appropriate identification and management of important stressors. Coexistence of stressors, among them earthworm invasions and white-tailed deer, makes correct identification of mechanisms that cause diversity declines challenging. We used an established factorial experiment to assess survival and growth of native seedlings (*Actaea pachypoda*, *Aquilegia canadensis*, *Cornus racemosa*, *Quercus rubra*, and *Prenanthes alba*) in response to presence/absence of deer and earthworms. We expected deer and earthworms to reduce seedling survival and biomass, and we evaluated potential pathways to explain this impact (soil N and P concentrations and pools, root architecture, and arbuscular mycorrhizal fungi [AMF] colonization). We developed structural equation models (SEM) to identify specific pathways through which earthworms and deer were impacting plant species with different life histories. Seedling survival was not affected by our treatments nor the plant and soil variables we tested. *Actaea* biomass was smaller in earthworm-invaded plots, and with larger total N pools. In contrast, both deer and earthworm treatments were associated with lower soil nutrient concentrations, and earthworm-invaded plots had smaller N and extractable P pools. *Actaea*, *Cornus*, *Prenanthes*, and *Quercus* seedlings had a lower proportion of fine roots in earthworm-invaded plots, while fine roots in *Aquilegia* made up a higher proportion of the root system. AMF colonization in *Quercus* was reduced in sites colonized by earthworms, but AMF in other species were unaffected. Our SEMs showed high correlation among soil variables, but because we do not know which variables are drivers of this change and which are passengers, we can only conclude that they are changing together as deer and earthworms exert their respective influence. Different plant species responded in idiosyncratic ways to earthworm and deer effects on soil fertility, root architecture and limited effects on AMF colonization. While earthworm and deer-mediated changes to fine roots, soil nutrients, and AMF may lead to changes in plant performance over time, these changes rarely translated to lower plant performance in our seedlings.

Key words: arbuscular mycorrhizal fungi; biodiversity; deer; earthworms; forest understory; invasive species; root architecture; structural equation models.

INTRODUCTION

North American forests are affected by multiple stressors including land use change, pollution, loss of predators, species invasions, and climate change (Wiegmann and Waller 2006, Dávalos et al. 2014). To preserve forest biodiversity and ecosystem function, we must disentangle individual and combined effects of stressors to implement the best conservation and management strategies (Didham et al. 2005, Côté et al. 2016). This can be a

challenge because stressors typically co-occur (Fisichelli et al. 2013, Dávalos et al. 2015a, Simmons et al. 2015) and often involve both above and belowground processes (Kuebbing et al. 2015). In North American forests, white-tailed deer (*Odocoileus virginianus* Zimmermann) and invasive earthworms are two stressors thought to severely affect native understory plant populations (Bohlen et al. 2004a, Frerker et al. 2014). Since the Wisconsin glaciation, earthworms have not been present in northeastern North American forests, but facilitated by human activity, European and Asian species are now spreading to remote areas of the continent (James 1995, Burtelow et al. 1998). White-tailed deer, while native to North America, have grown to historically unprecedented densities (Côté et al. 2004). Notably, it is often

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plant species with similar traits (palatable, highly mycorrhizal, perennial, slow-growing, forest obligates) that are most negatively affected by both deer and earthworms (Gundale 2002, Frelich et al. 2006, Dobson and Blossey 2015), while certain other species, including introduced invasive species, are facilitated (Nuzzo et al. 2015, Craven et al. 2016). Although species most negatively impacted have many shared traits, a mechanism to explain these patterns has not emerged.

The main impact of deer on plant communities is direct consumption of the largest individuals in herbaceous plants that contribute most to the next generation (Waller 2014, Nuzzo et al. 2017). Among indirect effects are impacts on belowground community composition and soil characteristics (Vitousek et al. 2002, Kluber et al. 2012, Shelton et al. 2014, Dávalos et al. 2015*b*); accelerated decomposition by mechanically breaking up litter, increasing leaf contact with soil detritivores (Heckel et al. 2010, Bressette et al. 2012), elevating earthworm abundance (Dávalos et al. 2015*b*), increasing soil compaction, either through trampling or disruption of fine roots and fungal hyphae (Shelton et al. 2014), and browsing and redistribution of nutrients (urine and feces; Eisenhauer 2017). But different studies of ungulate impacts on nutrient dynamics produce variable results, suggesting context-dependent patterns influenced by ungulate density and baseline ecosystem processes.

Earthworms are ecosystem engineers facilitating bacteria over fungi (Dempsey et al. 2011) and their activity leads to physical mixing of litter, priming decomposition of recalcitrant soil organic matter (SOM), and accelerated leaf litter decomposition (Nuzzo et al. 2009, Fahey et al. 2013). Reductions in leaf litter volume lead to cascading fundamental faunal community changes that start with reductions of invertebrate diversity and abundance that serve as prey for salamanders and birds (Maerz et al. 2009, Loss 2012). Accelerated decomposition may ultimately lead to complete loss of organic horizons, causing stressful rooting conditions, including lack of buffering of temperature extremes (Larson et al. 2010). Loss of organic horizons affects soil water dynamics, increasing evaporation rates, amplitude, and frequency of drought at times of low precipitation and erosion and runoff at times of high precipitation (Francis and Fraser 1998, Larson et al. 2010).

Whereas deer preferentially browse larger understory plant individuals, earthworms may consume roots and young seedlings (Fisk et al. 2004, Kirchberger et al. 2015). Estimates from forests in our study region suggest earthworms consume ~14% of fine root biomass annually (Gilbert et al. 2014). Although earthworms can act as dispersal vectors for spores, they consume and physically disrupt fungal hyphal networks, leading to decreases in fungal species diversity, density, and richness (Lawrence et al. 2003, Bohlen et al. 2004*a*, McLean et al. 2006, Paudel et al. 2016). Impacts on fungal communities could be especially problematic in northeastern

North American forests where in response to deep organic horizons, many plant species are highly dependent on mycorrhizal associations and have evolved thick roots with a large cortex to increase colonization (Wang et al. 2017). The combination of stressful growing conditions and direct feeding by earthworms may disproportionately affect fine roots and arbuscular mycorrhizal fungi (AMF), which are vitally important for plant nutrient uptake and water relations (Baylis 1970, Bardgett and van der Putten 2014). However, overall earthworm impacts on soil nutrient concentrations depend on soil type, nutrient identity, sampling depth, and earthworm community composition (Suarez et al. 2004, Hale et al. 2005).

In addition to direct impacts by deer and earthworms, soil nutrients, AMF, and fine roots influence each other. We expect soil P and AMF to be inversely correlated, as plant P status regulates AMF symbiosis, and AMF may in turn affect soil P by solubilizing organic soil P (Bolan 1991, Smith et al. 2011). The effect of N in AMF colonization is more complicated, as plant N deficiency, organic N surplus, and colimitation of N and P have all been shown to promote mycorrhiza formation (Johansen et al. 1994, Olsson et al. 2005, Hodge and Fitter 2010, Bonneau et al. 2013). Soil nutrients and root architecture could covary if plants grow long, thin roots to seek out P-rich microhabitats (Laliberte et al. 2015). Conversely, a thicker root cortex with larger cortical cells might improve growth and pathogen resistance in nutrient-rich soils (Chimungu et al. 2014, Laliberte et al. 2015). Both fine roots and mycorrhizae are important for plant nutrient and water relations, but we expect to see a negative correlation between these two variables due to the evolutionary trade-off between development of root hairs and dependence on AMF (Baylis 1970, Brundrett 2009). If this trade-off occurs as a result of earthworm disruption to AMF mutualisms, it could suggest a plant has the plasticity to grow fine roots to compensate for lower AMF colonization where deer and earthworms create poor conditions for mycorrhiza (Baylis 1970).

Our fundamental understanding of direct deer or earthworm impacts has greatly expanded over the past two decades. However, surprising interactions and multiple, non-mutually exclusive explanations for the impact of these two important stressors have only recently been recognized. For example, high deer populations can facilitate earthworm population increases (Dávalos et al. 2015*b*), leading to accelerated decomposition. It is crucially important to disentangle these impacts in a multiple-stressor investigative framework. Here we incorporate measurements of both direct and indirect effects of deer and earthworms and their interactions on understory seedlings using an established factorial experiment (presence/absence of deer and earthworms) to measure survival of seedlings of five native species (Dobson and Blossey 2015, Dobson et al. 2017).

We modeled seedling performance using structural equation models (SEMs), which are well suited to test multiple, non-mutually exclusive hypotheses for the mechanisms of impact. SEMs can capture compensatory shifts, such as plants relying on fine roots where conditions for AMF colonization are poor (Baylis 1970). We use SEM to assess relative contributions of direct and indirect effects (changes to soil, mycorrhizae and root architecture) of deer and earthworms (Fig. 1). We aim to test whether earthworms and deer reduce seedling survival (1) directly, (2) by decreasing soil nutrient pools, (3) by limiting growth or persistence of fine roots, and (4) by disrupting plant–AMF mutualisms.

METHODS

Study area

We used an established network of forested long-term research sites in New York State (Bobolink Hill, Connecticut Hill Wildlife Management Area, Hammond Hill State Forest, Ringwood Preserve, and Yellow Barn State Forest) located in the Allegheny section of the Appalachian Plateau at approximately 42° N, 76° W (Dobson and Blossey 2015). Soils are acidic (pH 3.9 – 5.0) Fragiaquepts and Dystrochrepts in the Mardin and Volusia series derived from glacial till, Devonian shale and siltstone (O’Geen et al. 2013). Canopies are dominated by mature *Acer saccharum* Marshall, *Fraxinus americana* L., *Fagus grandifolia* Ehrh., and *Quercus*

rubra L. (leaf area index = 5–7). Although background vegetation within plots was low (likely as a result of deer browse pressure and low light levels), our study species were sporadically present within plots (Table 1). Forest management ranges from some timber harvest (Ringwood) to actively (Connecticut Hill, Yellow Barn) and passively (Hammond Hill, Bobolink Hill) reforested farm and grazing land. Following wide-scale land clearing, deer in the region were functionally absent in the late 19th and early 20th century, but have recolonized and flourished since the mid-1900s (Halls 1984). While we do not know deer abundance or population fluctuations in our forests, deer densities in the Finger Lakes region are estimated to range from 3.6 – 11.6 deer/km², but can reach 22 deer/km² and much larger populations exist in suburbia (Hunn 2007, Boulanger et al. 2014, Russell et al. 2017). Similarly, earthworm invasion histories are unknown, however earthworm-invaded plots at Ringwood, Yellow Barn, and Bobolink Hill retained traces (<5 cm depth) of an organic horizon. Detailed soil, land use history, and environmental variables can be found elsewhere (Dobson and Blossey 2015).

We established long-term plots in summer 2011 creating a 2 × 2 factorial design replicated in each of the five forests (N = 20 plots). Each forest contained an area with an established earthworm invasion and an earthworm-free area (0.5–2 km between locations). Within these areas, we erected a 50 × 50 m deer exclusion plot using 2.5-m-high plastic mesh fence (standard perimeter

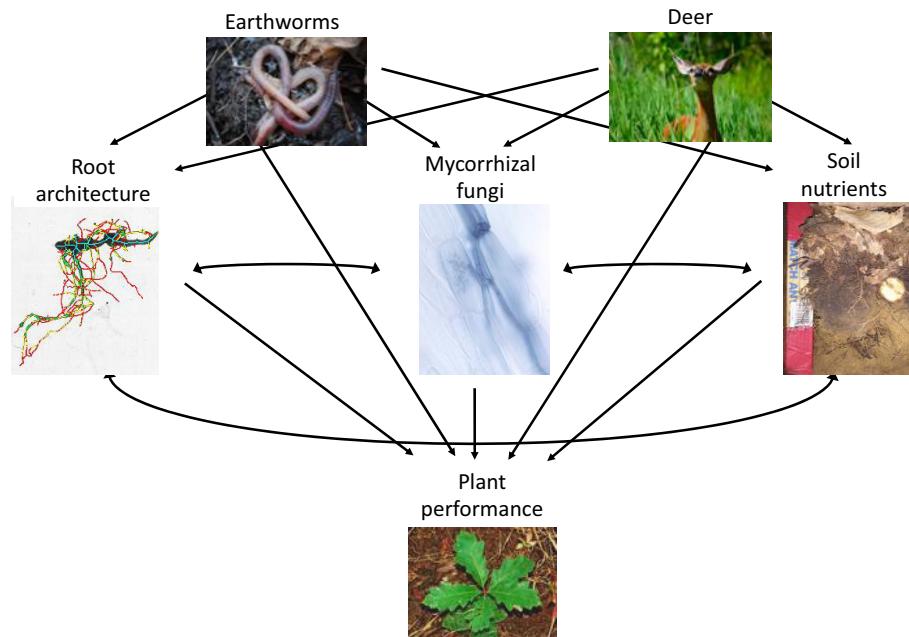


FIG. 1. Conceptual structural equation models of direct (earthworms and deer) and indirect (root architecture, AMF colonization, and soil nutrient) predictors of plant performance. Single-headed arrows reflect causative paths and double-headed arrows reflect covariance (correlated errors). Because mycorrhizal and fine-root variables cannot be measured in plants that did not survive, their effect is only tested in biomass models.

TABLE 1. Traits of each plant species at each site ($n = 5$ sites).

Species	Growth form	Root	Growth rate	Hypothesized effect		Background presence§
				Deer†	Earthworm‡	
<i>Actaea</i>	herb	rhizome	slow	moderately negative	negative	RW
<i>Aquilegia</i>	herb	fibrous	fast	moderately negative	positive	
<i>Cornus</i>	shrub	fibrous, suckering	slow	moderately negative	negative	
<i>Prenanthes</i>	herb	taproot	fast	negative	positive	RW
<i>Quercus</i>	tree	woody taproot	slow	negative	negative	CH, BB, YB, HH

† Based on literature (Jull 2001, Waller and Maas 2013, Shelton et al. 2014, Blossey et al. 2017) and personal observation.

‡ Based on literature (Hale et al. 2006, 2008, Corio et al. 2009, Dobson and Blossey 2015) and personal observation.

§ Background presence at sites. BB, Bobolink Hill; RW, Ringwood Preserve; CH, Connecticut Hill; YB, Yellow Barn; HH, Hammond Hill.

fencing; Deer Busters, Waynesboro, Pennsylvania, USA) held upright by parallel cables secured to trees. Adjacent to the fenced plot, we delineated a control plot where deer had access (for further details, see Dobson and Blossey 2015).

Seedling establishment

We selected five species to represent a breadth of life histories (growth form, mycorrhizal dependence, rooting strategies) and hypothesized responses to deer (Jull 2001, Waller and Maas 2013, Shelton et al. 2014, Blossey et al. 2017), and earthworms (Hale et al. 2006, 2008, Corio et al. 2009, Dobson and Blossey 2015). These plant species represent a small subsample of the species within the larger experiments at this site (Dobson and Blossey 2015). We collected seed from local (within 100 km) sources and germinated seedlings of *Actaea pachypoda* Elliott and *Cornus racemosa* Lam. in summer 2013, and *Aquilegia canadensis* L., *Quercus rubra* L., and *Prenanthes alba* L. in early spring 2014. Seedlings germinated in 2013 were held in a cold frame over the winter. All species support AMF mutualisms, although *Quercus* primarily associates with ectomycorrhizal (EM) fungi (Dickie et al. 2001, Toju et al. 2014). We germinated seedlings in potting soil (BX General Purpose Pro-mix; Premier Brands, Riviere-du-Loup, Quebec, Canada). We selected the most vigorous individuals to transplant into new potting soil in $6 \times 3.7 \times 6$ cm cell packs. *Quercus* were germinated from acorns in the spring directly into 3.8 cm diameter Conetainers (Stuewe and Sons, Corvallis, Oregon, USA). We grew seedlings outdoors under $1.8 \times 1.8 \times 3.6$ m shade tents (Lumite, Alto, Georgia, USA) to protect from deer and elevated off the ground in 2-mm nylon mesh reptarium cages (Reptarium 65 gallon [41 \times 75 \times 70 cm]; Dallas MFG, Dallas, Texas, USA) on a steel mesh table with legs submerged in soapy water to prevent earthworm access.

In May 2014, we rinsed potting soil from roots and planted bare-root seedlings into assigned, randomized planting locations in the forested plots. As we rinsed seedlings, we did not observe any earthworms in potting soil, indicating our setup successfully prevented

earthworm access. Within established paired fenced and unfenced areas at each forest, we delineated a circular plot (7 m in diameter) that was not used in the ongoing experiment. We planted individually marked bare-root seedlings 1 m apart around the perimeter of the plot. Each plot contained three to five individuals of each species due to limited germination in some species. In late August/early September 2014, we carefully extracted seedlings including their root ball using a planting knife (Professional Gardener's Digging Tool; Garret Wade, Cincinnati, Ohio, USA) to retain fine roots. We considered an individual surviving if it was visibly alive above leaf litter. While it is possible some individuals were dormant, we will henceforth refer to the visibly living individuals as surviving. We separated roots from shoots in the field, dried the latter in a greenhouse for two weeks and then determined dry biomass. Due to our destructive sampling of roots for other analyses, we used only the aboveground portion of the dry biomass (which is correlated [$r = 0.4-0.87$] with total root length in all species except *Aquilegia*) in our analyses. Henceforth, we will use biomass to describe dry aboveground biomass. We removed all soil from roots through soaking and cleaning with a paintbrush, weighed them (wet) and immediately transferred roots to 70% ethanol.

Soil sampling

We measured nutrient concentrations (nutrient mass per soil mass) and pools (nutrient mass per volume fine fraction soil) to a depth of 20 cm. We obtained soil subsamples from soil monoliths as detailed by (Dobson et al. 2017). One 15-cm² soil monolith was dug in summer 2016 at a random location in each plot at four forested sites (excluding Connecticut Hill). Monoliths included organic horizons, where present, but excluded Oi litter. Because no samples were excavated from Connecticut Hill, we did not analyze nutrient effects on seedlings from that site. We separated, air-dried, and sieved the A-horizon to <2 mm. We obtained total %N soil concentration from an elemental analyzer through Cornell University's Stable Isotope Laboratory (COIL). For

extractable P concentrations, we suspended 2 g of soil in 20 mL of 1 mol/L ammonium acetate by shaking samples for 1 h and allowing samples to equilibrate for 24 h (Ciesielski et al. 1997, Csuros 2018). We centrifuged soil slurries at 3,000 rpm for 30 min and decanted the extraction. We performed a pseudo-total (henceforth referred to as total) digestion to measure total P concentrations following EPA method 3051A. Total digests did not include dissolved silicates and other refractory compounds. We digested 0.5 g of air-dried material in 5 mL of 8 mol/L reverse aqua regia (9:1, HNO₃:HCl) at 90°C for 45 min on an insulated hot plate in sealed Teflon vials. We analyzed diluted digestate via ICP-OES (SPECTRO Analytical Instruments, Kleve, Germany). For QA/QC, we included a digestion blank for every 25 samples as well as matching standard reference materials for extractable and total P concentrations (Montana Soil 2711 from the National Institute of Standards and Technology, Gaithersburg, Maryland, USA). Relative standard deviations (RSDs) of standard reference materials were <14% for total soil concentrations and <18% for extractable soil concentrations. Blanks had elemental concentrations below detection limit for P (50 µg/L). Total concentration recoveries for Montana Soil SRM were 62–96% for P, likely due to the silicate minerals and other residual compounds insoluble in concentrated HNO₃ and HCl.

Root architecture

Where possible, we manually separated individual roots to prevent overlapping segments and captured two-dimensional images of seedling roots in 70% isopropyl alcohol using a photo scanner (Epson Expression 10000XL, 240 dpi; Epson America, Long Beach, California, USA). We measuring length of all roots above and below 0.25 mm diameter (Regent Instruments, Québec, QC, Canada) to assess the proportion very fine roots. We chose the <0.25 mm diameter class to most accurately capture the finest, highest order roots of our 1-yr-old seedlings. Our root architecture variable was the total length of roots <0.25 mm in diameter divided by the total root length of the sample. Although root diameter is a poor predictor of root lifespan or plant growth rate (Smith et al. 2014, Kramer-Walter et al. 2016), it is a reliable general predictor of other root traits associated with soil fertility such as Specific Root Length (SRL) and percent root nitrogen (Kramer-Walter et al. 2016, Wang et al. 2017).

Mycorrhizal colonization

To assess mycorrhizal colonization, we transferred whole roots from smaller seedlings or the lower 5 cm of larger seedlings into 50-mL Falcon tubes with 10% KOH (w/v). Roots varied among species ranging from delicate to woody and pigmented, hence we treated each species differently, heating samples to 80°C for 2 h–7 d and

replacing the KOH solution one to six times. After clearing, we rinsed samples three times in 5% HCl. For heavily pigmented species (*Actaea*, *Cornus*, *Quercus*), we treated samples with 0.5% NH₄OH and 0.5% H₂O₂ for 24 h, followed by a triple wash in 5% HCl. We subsampled roots to confirm sufficient clearing under a dissecting scope (MZ6, Leica Microsystems, Buffalo Grove, Illinois, USA). We stained samples in 5% blue ink (Parker Quink Ink, Atlanta, Georgia, USA) and diluted in 5% acetic acid (Vierheilig et al. 1998) for 48 h. We transferred roots to a 50% glycerol, 50% deionized water solution for destaining for an additional 48 h. Next, we cut ~20 root tips from each individual and trimmed to ~1 cm lengths. We squashed mounted root segments in a row along a microscope slide to quantify endophytic fungi. Using the magnified intersection method (McGonigle et al. 1990), we categorized 50 (*Prenanthes* and *Quercus*, which had limited root tissue) and 100 (all other species) points using a microscope (40–1000× Infinity Plan EPI; Leica Microsystems, Buffalo Grove, Illinois, USA) along root segments as (1) AMF hyphae present, (2) dark septate endophytes (DSE) present, (3) unknown hyphae present, and (4) no fungal colonization. Within samples with AMF hyphae, we quantified presence of arbuscules, vesicles, and hyphal coils (Brundrett et al. 1996). We used the percentage of root length with hyphal coils, arbuscules, or both to represent mycorrhiza (and refer to them henceforth as AMF colonization) because they are the regions of active mutualism (McGonigle et al. 1990). We identified DSE by the presence of melanized microsclerotia (aggregate irregularly lobed hyphae) that do not stain and dark septate hyphae in the host root (Brundrett et al. 1996).

Statistical analysis

To understand mechanisms for deer and earthworm effects on seedling survival, we used structural equation models (SEMs). SEMs are multivariate probabilistic path analyses that can test a suite of interrelated variables in one unified network (Grace 2006). By building models piecewise, we are able to include site as a random effect to prevent pseudoreplication using *piecewiseSEM*, *lme4*, and *lavaan* (Douglas et al. 2015, Lefcheck 2015, Yves et al. 2017, R Core Team 2016). For each component model of each species' SEM, we initially included height of seedlings at time of transplanting, but removed it if it did not lower model Akaike information criterion corrected for sample size (AIC_c) by >2 units. SEMs included deer and earthworms (presence/absence) as exogenous variables on all endogenous variables. For simplicity, implicit in the model is the assumption that deer and earthworms' influence on endogenous variables is unidirectional. Furthermore, although there is evidence that deer exclusion decreases earthworm abundance (Rearick et al. 2011, Shelton et al. 2014, Dávalos et al. 2015b), because deer and earthworms were

categorical variables, we ran our models with the assumption that deer and earthworms are not influencing each other.

We included all pathways including deer and earthworm effects on total N, extractable P, total P, AMF colonization (length/length), initial height/width (depending on species), fine root : total root length), and effect of these parameters on seedling survival. In the rare cases where full models were too poorly fitted to the data ($P < 0.05$) or models failed to converge, we dropped variables from the full model, and compared competing models with AIC_c . We ran separate SEMs for each plant species and assessed covariance (correlated errors) in pathways that we did not manipulate experimentally or build from explicit hypotheses. Models within the SEM were linear mixed models with site as a random effect (allowing only the intercept to vary). However, because colonization of DSE was low, we analyzed presence/absence pooled across all plant species (including site and plant species as random effects) with generalized linear mixed models with Binomial errors.

In all models, we then looked for missing paths using Shipley's test of directed separation (*d-sep*; Shipley 2000). By testing assumptions of no missing relationships among variables, we confirmed all variables are conditionally independent using Fisher's *C* statistic (Shipley 2000). We use Fisher's *C* to obtain model-network *P* values and calculate AIC and AIC_c . *P* values represent the probability that deviations between model predictions and observed data are consistent with the null hypothesis, in other words $P > 0.05$ represents a good model (Lefcheck 2015). We tested for significant paths using unstandardized data, but present regression coefficients standardized by mean and variance for SEMs for comparison (Lefcheck 2015). We calculated all regression coefficients and marginal and conditional R^2 (individual model fits) from residual maximum likelihoods (REML).

In addition to focal models, we tested an alternative set of SEMs with N and P pools (to a 20 cm depth) instead of concentrations and seedling biomass instead of survival (Appendix S1). In addition to SEMs, we used linear mixed models to test whether earthworms and deer had an impact on absolute and relative number of vesicles in root samples. We analyzed each plant species separately, with site as a random and earthworm biomass x fence interaction as a fixed effect. We used R software (version 3.2.4; R Core Team 2016) for all statistical analyses.

RESULTS

Seedling performance

Seedling survival varied greatly among both plots and species, and our models explained 2–23% of the variance (Table 2; Fig. 2; Appendix S1: Tables S25, S9). Neither earthworm nor deer presence impacted

short-term survival of any species. *Actaea* in plots with higher N pools had lower survival (Appendix S1: Fig. S1), but no paths to predict survival were significant in concentration models (Fig. 2). The biomass individual seedlings produced over a single growing season was extremely variable, spanning orders of magnitude even within a species (Table 2). Our models explained 8–74% of the variance in dry aboveground biomass (Table 2; Appendix S1: Tables S1, S17). Initial height at planting did not affect survival or biomass, therefore we removed it from our models. *Actaea* biomass decreased with higher earthworm biomass and total N (Fig. 3, Table 3; Appendix S1: Fig. S2, Tables S2, S18).

Soil nutrient concentrations and pools

Our concentration models explained 18–56% of variance in extractable P, 56–63% in total P and 69–72% in total N. Soil pool models explained 73–76% of variance in extractable P, 36–41% in total P, and 64–70% in total N. Total P concentration decreased with both deer access (survival models, $\beta \approx -1.24$, $SE \approx 0.15$, $P < 0.001$; biomass models, $\beta \approx -0.53$, $SE \approx 0.11$, $P < 0.001$) and earthworm biomass (survival models, $\beta \approx -0.92$, $SE \approx 0.15$, $P < 0.001$; biomass models, $\beta \approx -0.82$, $SE \approx 0.10$, $P < 0.001$), while extractable P concentration was unaffected by either. Total N concentration was 16% lower in deer access plots (survival models, $\beta \approx -0.57$, $SE \approx 0.11$, $P < 0.001$; biomass models, $\beta \approx -1.08$, $SE \approx 0.22$, $P < 0.001$) (Table 4). Soil variables were highly correlated in both concentration (Figs. 2, 3; Appendix S1: Tables S8, S16) and pool models (Appendix S1: Figs. S1, S2; Tables S24, S32). In contrast to concentrations, deer access did not impact soil pools. However, earthworm biomass decreased extractable P (survival models, $\beta \approx -0.81$, $SE \approx 0.11$, $P < 0.001$; biomass models, $\beta \approx 0.58$, $SE \approx 0.1$, $P < 0.001$) and total N pools (survival models, $\beta \approx -0.59$, $SE \approx 0.12$, $P < 0.001$; biomass models, $\beta \approx 0.42$, $SE \approx 0.12$, $P < 0.001$). Parameter estimates differ slightly between species because biomass, AMF, and fine root data were only collected from surviving individuals. Similarly, estimates vary between Gaussian biomass models and Binomial survival models.

Fine roots

Our models explained <1–31% of variability in proportion of fine roots of each species. Earthworm biomass had a positive effect on proportion of fine roots of *Aquilegia* (Figs. 2, 3; Table 3; Appendix S1: Fig. S2, Tables S2, S3, S11, S19, S27), and a negative effect on proportion of fine roots of all four other species in at least one model (Figs. 2, 3; Table 3; Appendix S1). In addition to direct earthworm effects, indirect deer and earthworm influences may be mediated through soil pathways. Although this experiment was not designed

TABLE 2. Number of transplants and range, mean (for parametric data), or median (for non-parametric data) of variables of each plant species at each site ($n = 5$ sites).

Species	No. planted†	Survival‡ (%)		Dry biomass§ (g)		Mycorrhizal colonization (%)¶		Fine/total root (cm/cm)#		DSE (%)	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Median
<i>Actaea</i>	80	50–100	86	0.01–2.19	0.37	6–96	38	0.19–0.55	0.37	0.10	0
<i>Aquilegia</i>	60	0–100	63	0.01–0.76	0.16	0–84	44	0.54–0.98	0.65	0–48	4
<i>Cornus</i>	80	25–100	89	0.03–0.31	0.1	0–68	28	0.31–0.76	0.51	0–42	0
<i>Prenanthes</i>	80	0–100	45	0.01–0.2	0.4	0–96	40	0.19–0.79	0.53	0–8	0
<i>Quercus</i>	100	0–100	58	0.2–1.6	0.68	4–88	37	0.36–0.67	0.52	0–56	16

† Total transplants in the experiment.

‡ Any seedlings present above the leaf litter at the time of the experiment.

§ Dry biomass of aboveground plant tissue.

¶ Percentage of colonization (length/length) by arbuscular fungi. Confirmed by the presence of arbuscules and/or hyphal coils.

Proportion of root length considered fine roots (diameter < 0.25 mm) relative to total root length.

|| Percentage of colonization (length/length) by dark septate endophytes. Confirmed by the presence of melanized microsclerotia.

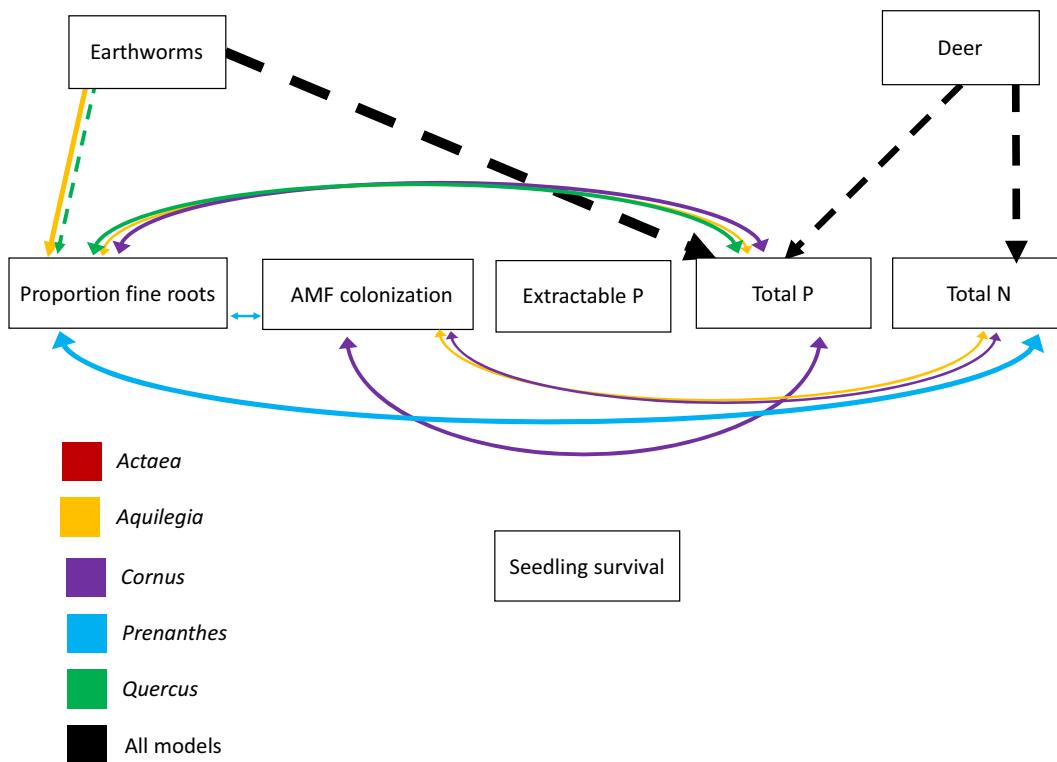


FIG. 2. Piecewise structural equation models of direct (earthworms and deer) and indirect (AMF colonization, proportion of total root length in roots <0.25 mm diameter, concentration of total N, extractable P and total soil P) predictors of seedling survival. Single-headed arrows reflect causative paths and double-headed arrows are covariance (correlated errors). Solid arrows denote significant ($P < 0.05$) positive paths, dotted lines reflect negative paths, and nonsignificant paths are not shown. Arrow weights are proportional to path coefficients standardized by mean and variance. Full model outputs, marginal and conditional R^2 values and random effects are reported in Appendix S1: Tables S9–S16.

to test causality among secondary pathways, errors (covariance) of fine root proportion were positively correlated with total N concentration and AMF colonization in *Prenanthes* (Figs. 2, 3; Appendix S1: Table S2, S5, S13, S21, S29), and total P concentration in *Aquilegia*, *Cornus*, and *Quercus*. Fine root proportions were positively correlated with both total N and extractable P

pools in *Actaea*, *Cornus*, and *Quercus* (Appendix S1: Figs. S1, S2).

AMF

Our models explained 3–32% of variance in AMF colonization. Earthworm biomass was associated with lower

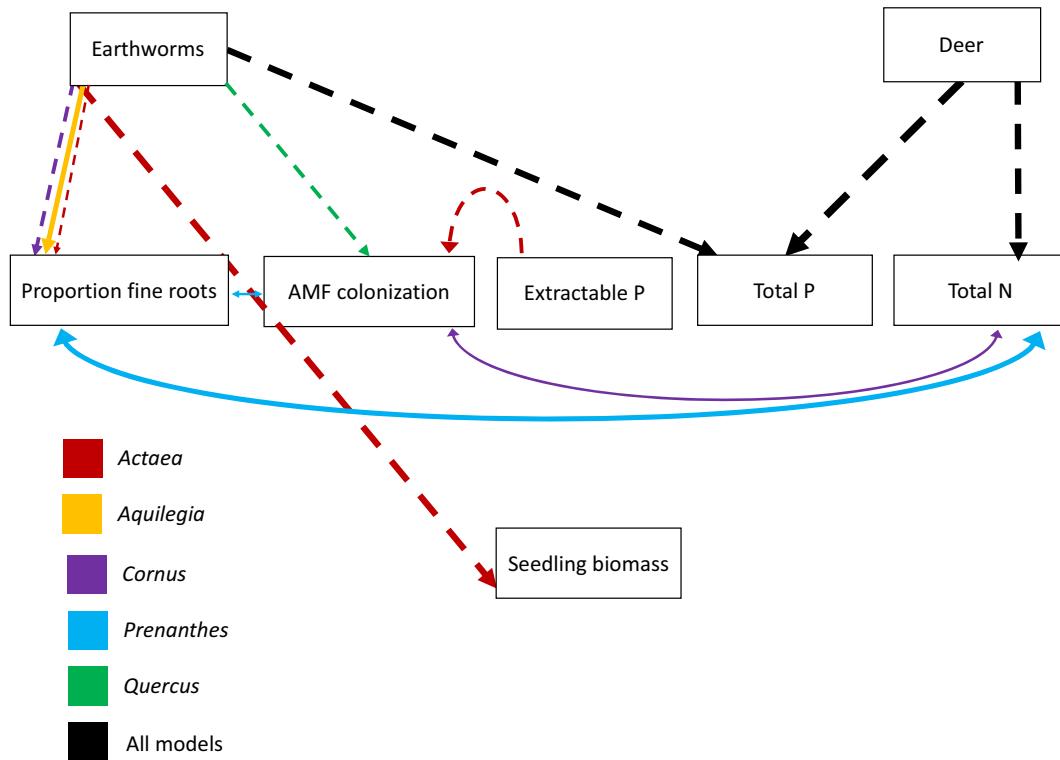


FIG. 3. Piecewise structural equation models of direct (earthworms and deer) and indirect (AMF colonization, proportion of total root length in roots <0.25 mm diameter, concentration of total N, extractable P, and total soil P) predictors of seedling biomass. See Fig. 2 for description of paths. Arrow weights are proportional to path coefficients standardized by mean and variance. Full model outputs, marginal and conditional R^2 values, and random effects are reported in Appendix S1: Tables S1–S8.

AMF colonization of *Quercus* roots (Fig. 3; Appendix S1: Table S2, S6, S14, S22, S30). Deer did not directly affect mycorrhizal colonization, but may have had indirect effects through changes to soil variables. AMF colonization was positively correlated with total N concentration in *Aquilegia* and *Cornus* and N pools in *Actaea*, *Aquilegia*, and *Cornus* (Figs. 2, 3; Appendix S1: Figs. S1, S2). In addition, AMF colonization was positively correlated with total P concentration in *Cornus* and extractable P in *Aquilegia* and *Cornus* (Fig. 2; Appendix S1: Table S16). To have a converging model for *Actaea* biomass, we included extractable P concentration as an additional predictor for AMF colonization, with higher extractable P concentrations being associated with lower AMF colonization (Fig. 3; Appendix S1: Tables S2, S10, S18, S26). Neither earthworm biomass nor deer significantly impacted absolute vesicle abundance nor vesicle abundance relative to total mycorrhizal colonization in any species (data not shown).

DSE

Our model for the probability of DSE colonization explained 65% of the variance (Fig. 4). To have a converging model, we dropped fine root paths and plant species identity from the DSE analysis. The only

significant predictor of DSE was total N ($\beta = 0.69$, $SE = 0.26$, $P = 0.007$), which was in turn negatively affected by deer ($\beta = -0.63$, $SE = 0.03$, $P < 0.001$), suggesting a negative indirect effect. Earthworms did not have a direct or indirect effect on DSE.

DISCUSSION

Deer and earthworm effects were ubiquitous in our plots; however, these changes did not impact seedling survival through our hypothesized pathways (Fig. 1). We found no direct deer browse impacts, likely because our transplants did not grow to sufficient size to become attractive as deer browse targets. Instead of discovering dominant mechanisms of how deer and earthworms may impact native plants, we found diverse species-specific and idiosyncratic responses (with large intraspecific variation) that defy attempts at easy generalizations. The strongest impacts of both deer and earthworms resulted in changes in soil nutrients. Excluding deer over a six-year period led to substantial localized increases in both total N and P concentrations (mass of a nutrient per mass of soil) while earthworm presence was associated with marked declines in total soil P concentrations as well as extractable P pools (mass of nutrient per area of soil to a depth of 20 cm).

TABLE 3. All significant paths from all models tested in this experiment. Correlated errors and full models reported in Appendix S1.

Plant, nutrient metric, and seedling metric	Response variable	Predictor variables	Estimate	SE	<i>P</i>
<i>Actaea</i>					
Concentration					
Biomass	biomass	earthworm	-1.014	0.41	0.022
Biomass	mycorrhizae	extractable P	-0.63	0.22	0.008
Biomass	fine root	earthworm	-0.41	0.23	0.078
Biomass	total N	deer	-1.08	0.22	<0.001
Biomass	total P	deer	-1.24	0.15	<0.001
Biomass	total P	earthworm	-0.92	0.15	<0.001
Survival	total N	deer	-0.57	0.11	<0.001
Survival	total P	deer	-0.53	0.11	<0.001
Survival	total P	earthworm	-0.82	0.1	<0.001
Pools					
Biomass	biomass	total N	-1.29	0.52	0.02
Biomass	total N	earthworm	-0.42	0.10	<0.001
Biomass	extractable P	earthworm	-0.58	0.10	<0.001
Survival	total N	earthworm	-0.59	0.12	<0.001
Survival	extractable P	earthworm	-0.81	0.10	<0.001
<i>Aquilegia</i>					
Concentration					
Biomass	fine root	earthworm	0.75	0.29	0.014
Biomass	total N	deer	-1.07	0.25	<0.001
Biomass	total P	deer	-1.24	0.18	<0.001
Biomass	total P	earthworm	-0.92	0.18	<0.001
Survival	total N	deer	-0.57	0.11	<0.001
Survival	total P	deer	-0.53	0.11	<0.001
Survival	total P	earthworm	-0.82	0.1	<0.001
Pools					
Biomass	fine root	earthworm	0.40	0.16	0.03
Biomass	total N	earthworm	-0.42	0.12	<0.001
Biomass	extractable P	earthworm	-0.58	0.11	<0.001
Survival	survival	total P	-0.10	0.05	0.05
Survival	total N	earthworm	-0.58	0.14	<0.001
Survival	extractable P	earthworm	-0.80	0.12	<0.001
<i>Cornus</i>					
Concentration					
Biomass	fine root	earthworm	-0.56	0.22	0.013
Biomass	total N	deer	-1.08	0.22	<0.001
Biomass	total P	deer	-1.24	0.15	<0.001
Biomass	total P	earthworm	-0.92	0.15	<0.001
Survival	fine root	deer	-0.56	0.22	0.01
Survival	total N	deer	-1.08	0.22	<0.001
Survival	total P	deer	-1.24	0.15	<0.001
Survival	total P	earthworm	-0.92	0.15	<0.001
Pools					
Biomass	total N	earthworm	-0.42	0.10	<0.001
Biomass	extractable P	earthworm	-0.58	0.10	<0.001
Survival	survival	total P	-0.10	0.05	0.049
Survival	fine roots	earthworm	-0.33	0.11	<0.001
Survival	total N	earthworm	-0.58	0.14	<0.001
Survival	extractable P	earthworm	-0.80	0.12	<0.001
<i>Prenanthes</i>					
Concentration					
Biomass	total N	deer	-1.08	0.22	<0.001
Biomass	total P	deer	-1.24	0.15	<0.001
Biomass	total P	earthworm	-0.92	0.15	<0.001
Survival	total N	deer	-1.08	0.22	<0.001

TABLE 3. Continued

Plant, nutrient metric, and seedling metric	Response variable	Predictor variables	Estimate	SE	<i>P</i>
Survival	total P	deer	-1.24	0.15	<0.001
Survival	total P	earthworm	-0.92	0.15	<0.001
Pools					
Biomass	total N	earthworm	-0.42	0.09	<0.001
Biomass	extractable P	earthworm	-0.58	0.08	<0.001
Survival	total N	earthworm	-0.58	0.14	<0.001
Survival	extractable P	earthworm	-0.80	0.12	<0.001
<i>Quercus</i>					
Concentration					
Biomass	mycorrhizae	earthworm	-0.54	0.26	0.046
Biomass	total N	deer	-1.09	0.19	<0.001
Biomass	total P	deer	-1.25	0.14	<0.001
Biomass	total P	earthworm	-0.92	0.14	<0.001
Survival	total N	deer	-1.08	0.22	<0.001
Survival	total P	deer	-1.24	0.15	<0.001
Survival	total P	earthworm	-0.92	0.15	<0.001
Pools					
Biomass	total N	earthworm	-0.42	0.09	<0.001
Biomass	extractable P	earthworm	-0.58	0.08	<0.001
Survival	fine roots	earthworm	-0.30	0.12	0.01
Survival	total N	earthworm	-0.59	0.12	<0.001
Survival	extractable P	earthworm	-0.81	0.10	<0.001

TABLE 4. Mean earthworm biomass ($n = 5$ sites), nutrient concentrations ($n = 4$ sites), and pools ($n = 4$ sites) within treatments.

Treatment	Dry earthworm biomass (g/m ²)	Concentrations (w/w)			Pools (0–20 cm)		
		Extractable P (mg/kg)	Total P (mg/kg)	Total N	Extractable P (mg/m ²)	Total P (mg/m ²)	Total N (mg/m ²)
-Worm -Deer	0.02	1.88	1.07	0.38	1.63	1	0.4
-Worm +Deer	0.01	2.21	0.85	0.41	1.5	1.35	10.24
+Worm -Deer	2.65	0.12	0.76	0.46	0.36	1.01	1.73
+Worm +Deer	1.57	0.07	0.54	0.33	0.38	0.85	1.48

Despite these profound impacts, declines in nutrient concentrations and pools did not significantly affect short-term survival of any of the five native plant species used in our experiment.

Loss of topsoil P following earthworm invasions was previously reported (Paré and Bernier 1989, Resner et al. 2015), but it is not universal (Suarez et al. 2004, Hale et al. 2008). We did observe near-complete elimination of the organic horizon due to earthworm activities, and decreased total N pools at our sites. However, total N concentrations in the A-horizon were unaffected, suggesting earthworms are consuming the forest floor, but not selectively removing N-rich organic matter. Previous studies found variable effects of earthworm invasion on forest soil N, potentially as a function of invasion history (Bohlen et al. 2004b, Marhan and Scheu 2006). In recent earthworm invasion and pot experiments, nutrient enrichment was associated with accelerated decomposition, while in forests with established earthworm

invasions nutrients are lost through leaching and erosion (Hale et al. 2008, Resner et al. 2015, Dobson et al. 2017). This process was evident at our research sites, where following large rain events, earthworm-invaded plots were visibly eroded, as mobile top soil accumulated downhill. These impacts may become more important as invasive earthworm populations expand globally (Hendrix et al. 2008) and frequency of extreme rain events increases (Donat et al. 2016, Pfahl et al. 2017).

The increase in total N and P concentrations in areas where deer are excluded by fences suggests a novel and poorly recognized ability of deer to either deplete nutrients or modify their distribution on the landscape. Murray et al. (2013) reported higher levels of available ammonia in deer exclosures, positing that deer increase nitrogen heterogeneity through browsing vegetation and excretion of nitrogenous wastes in small, concentrated patches that vary seasonally. Other experiments using exclosures report variable impacts of deer on soil

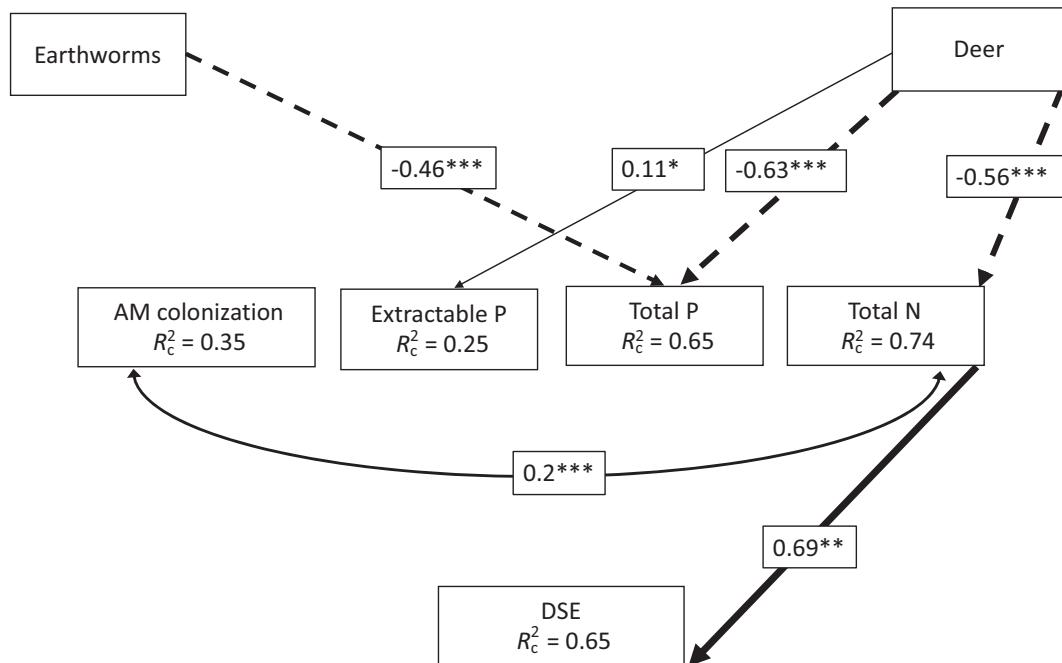


FIG. 4. Piecewise structural equation models of direct (earthworms and deer) and indirect (AMF colonization, total N, concentration of total soil P) predictors of dark septate endophyte (DSE) colonization of roots. DSE observations from all species are pooled, and arrow weights are proportional to path coefficients, standardized by mean and variance. See Fig. 2 for description of paths. Model: Fisher's $C = 58.801$; $P < 0.001$; $AIC_c = 102.584$. Full model outputs, marginal and conditional R^2 values, and random effects are reported in Appendix S1: Tables S33–S36. Paths: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

nutrients (Bressette et al. 2012, Shelton et al. 2014), and the increasing spatial heterogeneity of nutrients in a landscape due to deer activity could explain these discrepancies. Unfortunately, our limited spatial and temporal soil sampling design is unable to provide insights into these patterns, and we cannot exclude the possibility that deer caused net declines in A-horizon N. Additional detailed studies are required to investigate these important nutrient redistribution pathways in forests that have received scant attention.

We expected AMF colonization to be an important explanatory variable, but based on our results we need to reject this path as a dominant driver of seedling biomass. We could not test the impact of AMF on survival because it could only be measured in surviving individuals. AMF colonization was extremely variable even within species. Our results agree with those of Shelton et al. (2014), who saw no change in AMF colonization after 2–7 yr of deer exclusion in a forest in Indiana. Although earthworm biomass was associated with lower AMF colonization in *Quercus*, which translated to lower survival, AMF are secondary to ectomycorrhizal mutualisms in this genus. Our data question the importance of earthworms in affecting native plant survival through consumption and disruption of AMF–plant mutualisms, at least in the short term (Gundale 2002, Lawrence et al. 2003, McLean et al. 2006, Paudel et al. 2016). Earthworms may be preferentially consuming AMF-colonized roots (de Novais et al. 2019), consuming fungal hyphae

or modifying fungal microhabitat, but neither AMF colonization (measured as the percentage of root length with hyphal coils and/or arbuscules) nor mutualism productivity (relative abundance of arbuscules: vesicles) influenced biomass of experimental transplants. Our results concur with other reports that suggest limited earthworm-associated declines in AMF colonization in both forest and grassland species (Eisenhauer et al. 2009, Wurst and Rillig 2011, Yang et al. 2015). However, since our experiment focused on young seedlings, we cannot reject the possibility that this is an important pathway for older, reproductive individuals.

While we did not find strong effects of earthworms or deer on AMF colonization, our transplanted seedlings showed extremely variable AMF colonization patterns, suggesting existence of other important variables that we did not capture. DSE were not excluded nor facilitated by AMF, but appear to respond to the same forces. DSE associations with plants range from mutualistic to parasitic, depending on growing conditions, host plant species and fungal species or genotype (Jumpponen 2001). While not directly affected by deer or earthworms as we expected, colonization by both DSE and AMF was higher with increased total soil N. Nitrogen can influence how DSE interact with plants, with higher soil N causing DSE to affect plant growth and P acquisition similar to mycorrhiza (Jumpponen et al. 1998). Therefore, in addition to influencing DSE colonization through changes to soil nutrient pools and

concentrations, deer and earthworms may modify the activity of DSE.

Our results suggest an enormous ability of plants to adapt their resource capture strategies to local growing conditions without paying survival or growth penalties. We studied transplant survival and growth for a single season and prolonged earthworm invasion or deer browse pressure may cause important survival or growth differences in the future; only long-term data sets and assessments can provide a true assessment. All five of our study species showed changes to root architecture in response to earthworms in at least one of our SEMs. In four of five species, the proportion of fine roots (<0.25 mm) was lower for plants growing with earthworms, while the opposite was true of *Aquilegia*. While we do not have sufficient phylogenetic replication to confirm the role of life history, this result potentially supports the hypothesis that responses of native plants to invasive earthworms may depend on plant traits (Cameron et al. 2014). It is possible that slow-growing species such as *Actaea*, *Cornus*, and *Quercus* respond differently to physical disturbances of earthworms, or that earthworms are consuming more fine roots than the plants can replace (Fisk et al. 2004, Gilbert et al. 2014, Paudel et al. 2016). These species may have less plasticity to respond to stressors with alternative root architecture strategies and foraging behavior (Cameron et al. 2014, Liu et al. 2015). Conversely, fine root growth of fast-growing species such as *Aquilegia* may be stimulated either by earthworm herbivory or rapid mineralization of soil nutrients. While it is likely plant (and particularly root) traits influence a plants' ability to respond to earthworm activity (Cameron et al. 2014), changes to root architecture in response to presence of earthworms and deer did not affect plant biomass. This leads us to reject hypothesis 4 and conclude that either variability of field conditions overwhelms seedling responses in the short term, or changes in fine roots represent plastic responses, allowing plants to buffer themselves from spatial, temporal, and seasonal changes to their habitat.

We tested a limited number of species but already found diverse responses that defy our attempts at generalizations. Instead, plants represent a spectrum of syndromes including those that can take advantage of soil modifications, forage precisely, and withstand root herbivory, and those that cannot (Cameron et al. 2014). While earthworms and deer decreased nutrient pools and concentrations, we found equally strong coping mechanisms that allowed individual seedlings to persist and grow under these different circumstances. Our experiments took place in areas with established earthworm populations and a long-term influence of high deer populations. We cannot exclude the possibility that dramatic effects have occurred in the past that have resulted in sorting of local communities, disappearance of many individuals rooted in the extensive leaf litter, or rapid evolutionary responses to changed conditions

(Gundale 2002, Wiegmann and Waller 2006, Johnson et al. 2015). Given the considerable variation in responses within a species and within a plot, capturing multiple time steps in a larger pool of plants with similar growth syndromes would further clarify the mechanisms we tested. Still, our results point to the ability for individuals, at least of the species we tested, to continue to persist under these changed conditions. Our experimental species continue to be present and common in our region, and other species that have become rare or been locally extirpated may respond very differently. Furthermore, overall consequences for plant performance may become more apparent as the plant grows and matures. We need more extensive long-term studies with more manipulated variables to assess these possibilities. For example, when considering N and P pools (but not concentrations), we observed many correlations among AMF, root architecture, and soil nutrient variables. This suggests that these plants are not compensating for lower nutrient pools by recruiting more AMF or growing more fine roots to seek out P-rich microhabitats. Instead, this likely reflects the cascade of effects on plants growing in an organic horizon diminished by earthworm activity. However, as we did not modify these variables directly, the drivers of this pattern remain unknown.

By limiting the magnitude of the most destructive disturbances (i.e., erosion of earthworm-modified soils in extreme weather events or deer browse of the most productive plants), we may be able to minimize loss of understory plant diversity. While this study focuses on North American forests, invading or rapidly growing deer and earthworm populations threaten temperate ecosystems globally (Hendrix et al. 2008, Seki et al. 2014, Shelton et al. 2014). Therefore, it is vitally important to assess these impacts over extended time periods, often decades, to better capture and understand deer and earthworm impacts on abiotic ecosystem conditions and their biotic communities. Mechanistic studies testing multiple paths of stressor impacts on forest understories can ultimately prioritize practices that will be the most effective in realizing management and conservation goals.

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LITERATURE CITED

- Bardgett, R. D., and W. H. van der Putten. 2014. Belowground biodiversity and ecosystem functioning. *Nature* 515:505–511.
- Baylis, G. T. S. 1970. Root hairs and phycomycetous mycorrhizas in phosphorus deficient soil. *Plant and Soil* 33:713–716.
- Blossey, B., A. Dávalos, and V. Nuzzo. 2017. An indicator approach to capture impacts of white-tailed deer and other ungulates in the presence of multiple associated stressors. *AoB Plants* 9:1–16.
- Bohlen, P. J., P. M. Groffman, T. J. Fahey, M. C. Fisk, E. Suarez, D. M. Pelletier, and R. T. Fahey. 2004a. Ecosystem consequences of exotic earthworm invasion of north temperate forests. *Ecosystems* 7:1–12.
- Bohlen, P. J., D. M. Pelletier, P. M. Groffman, T. J. Fahey, and M. C. Fisk. 2004b. Influence of earthworm invasion on redistribution and retention of soil carbon and nitrogen in northern temperate forests. *Ecosystems* 7:13–27.
- Bolan, N. S. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil* 134:189–207.
- Bonneau, L., S. Hugué, D. Wipf, N. Pauly, and H. Truong. 2013. Combined phosphate and nitrogen limitation generates a nutrient stress transcriptome favorable for arbuscular mycorrhizal symbiosis in *Medicago truncatula*. *New Phytologist* 199:188–202.
- Boulanger, J. R., P. D. Curtis and B. Blossey. 2014. An integrated approach for managing white-tailed deer in suburban environments: the Cornell University study. Cornell University Cooperative Extension and the Northeast Wildlife Damage Research and Outreach Cooperative, Ithaca, New York, USA. http://wildlifecontrol.info/deer/Documents/IDRM_12-5-2014.pdf
- Bressette, J. W., H. Beck, and V. B. Beauchamp. 2012. Beyond the browse line: complex cascade effects mediated by white-tailed deer. *Oikos* 121:1749–1760.
- Brundrett, M. C. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: Understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil* 320:37–77.
- Brundrett, M. C., N. Bougheer, B. Dell, T. Grove, and N. Malajczuk. 1996. Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agricultural Research, Canberra, Australian Capital Territory, Australia.
- Burlingame, A. E., P. J. Bohlen, and P. M. Groffman. 1998. Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. *Applied Soil Ecology* 9:197–202.
- Cameron, E. K., J. F. Cahill, and E. M. Bayne. 2014. Root foraging influences plant growth responses to earthworm foraging. *PLoS ONE* 9:e108873.
- Chimungu, J. G., K. M. Brown, and J. P. Lynch. 2014. Large root cortical cell size improves drought tolerance in maize. *Plant Physiology* 166:2166–2178.
- Ciesielski, H., T. Sterckeman, M. Santerne, and J. Willery. 1997. A comparison between three methods for the determination of cation exchange capacity and exchangeable cations in soils. *Agronomie* 17:9–16.
- Corio, K., A. Wolf, M. Draney, and G. Fewless. 2009. Exotic earthworms of Great Lakes forests: A search for indicator plant species in maple forests. *Forest Ecology and Management* 258:1059–1066.
- Côté, S. D., T. P. Rooney, J.-P. Tremblay, C. Dussault, and D. M. Waller. 2004. Ecological impacts of deer overabundance. *Annual Review of Ecology, Evolution & Systematics* 35:113–147.
- Côté, I. M., E. S. Darling, and C. J. Brown. 2016. Interactions among ecosystem stressors and their importance in conservation. *Proceedings of the Royal Society B* 283:20152592.
- Craven, D., et al. 2016. The unseen invaders: introduced earthworms as drivers of change in plant communities in North American forests (a meta-analysis). *Global Change Biology* 23:1065–1074.
- Csuros, M. 2018. Cation Exchange Capacity. Pages 313–315 in A. G. Norman, editor. *Environmental sampling and analysis*. American Society of Agronomy, Soil Science Society of America, Madison, Wisconsin, USA.
- Dávalos, A., V. Nuzzo, and B. Blossey. 2014. Demographic responses of rare forest plants to multiple stressors: the role of deer, invasive species and nutrients. *Journal of Ecology* 102:1222–1233.
- Dávalos, A., V. Nuzzo, and B. Blossey. 2015a. Single and interactive effects of deer and earthworms on non-native plants. *Forest Ecology and Management* 351:28–35.
- Dávalos, A., E. Simpson, V. Nuzzo, and B. Blossey. 2015b. Non-consumptive effects of native deer on introduced earthworm abundance. *Ecosystems* 18:1029–1042.
- Dempsey, M. A., M. C. Fisk, and T. J. Fahey. 2011. Earthworms increase the ratio of bacteria to fungi in northern hardwood forest soils, primarily by eliminating the organic horizon. *Soil Biology & Biochemistry* 43:2135–2141.
- Dickie, I. A., R. T. Koide, and A. C. Fayish. 2001. Vesicular-arbuscular mycorrhizal infection of *Quercus rubra* seedlings. *New Phytologist* 151:257–264.
- Didham, R. K., J. M. Tylianakis, M. A. Hutchison, R. M. Ewers, and N. J. Gemmill. 2005. Are invasive species the drivers of ecological change? *Trends in Ecology & Evolution* 20:470–474.
- Dobson, A. M., and B. Blossey. 2015. Earthworm invasion, white-tailed deer and seedling establishment in deciduous forests of north-eastern North America. *Journal of Ecology* 103:153–164.
- Dobson, A. M., B. Blossey, and J. B. Richardson. 2017. Invasive earthworms change nutrient availability and uptake by forest understory plants. *Plant and Soil* 421:175–190.
- Donat, M. G., A. L. Lowry, L. V. Alexander, P. A. O’Gorman, and N. Maher. 2016. More extreme precipitation in the world’s dry and wet regions. *Nature Climate Change* 6:508–513.
- Douglas, B., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1–48.
- Eisenhauer, N. 2017. Consumers control carbon. *Nature Ecology and Evolution* 1:1596–1597.
- Eisenhauer, N., S. König, A. C. W. Sabais, C. Renker, F. Buscot, and S. Scheu. 2009. Impacts of earthworms and arbuscular mycorrhizal fungi (*Glomus intraradices*) on plant performance are not interrelated. *Soil Biology & Biochemistry* 41:561–567.
- Fahey, T. J., J. B. Yavitt, R. E. Sherman, J. C. Maerz, P. M. Groffman, M. C. Fisk, and P. J. Bohlen. 2013. Earthworm effects on the incorporation of litter C and N into soil organic matter in a sugar maple forest. *Ecological Applications* 23:1185–1201.
- Fischelli, N., L. Frelich, P. Reich, and N. Eisenhauer. 2013. Linking direct and indirect pathways mediating earthworms, deer, and understory composition in Great Lakes forests. *Biological Invasions* 15:1057–1066.
- Fisk, M. G. C., T. J. Fahey, P. M. Groffman, and P. J. Bohlen. 2004. Earthworm invasion, fine-root distributions, and soil respiration in north temperate forests. *Ecosystems* 7:55–62.

- Francis, G. S., and P. M. Fraser. 1998. The effects of three earthworm species on soil macroporosity and hydraulic conductivity. *Applied Soil Ecology* 10:11–19.
- Frelich, L. E., C. M. Hale, S. Scheu, A. R. Holdsworth, L. Heneghan, P. J. Bohlen, and P. B. Reich. 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. *Biological Invasions* 8:1235–1245.
- Frerker, K., A. Sabo, and D. Waller. 2014. Long-term regional shifts in plant community composition are largely explained by local deer impact experiments. *PLoS ONE* 9:e115843.
- Gilbert, K. J., T. J. Fahey, J. C. Maerz, R. E. Sherman, P. Bohlen, J. J. Dombroskie, P. M. Groffman, and J. B. Yavitt. 2014. Exploring carbon flow through the root channel in a temperate forest soil food web. *Soil Biology and Biochemistry* 76:45–52.
- Grace, J. B. 2006. *Structural equation modeling and natural systems*. Cambridge University Press, Cambridge, UK.
- Gundale, M. J. 2002. Influence of exotic earthworms on the soil organic horizon and the rare fern *Botrychium mormo*. *Conservation Biology* 16:1555–1561.
- Hale, C. M., L. E. Frelich, P. B. Reich, and J. Pastor. 2005. Effects of European earthworm invasion on soil characteristics in northern hardwood forests of Minnesota, USA. *Ecosystems* 8:911–927.
- Hale, C. M., L. E. Frelich, and P. B. Reich. 2006. Changes in hardwood forest understory plant communities in response to European earthworm invasions. *Ecology* 87:1637–1649.
- Hale, C. M., L. E. Frelich, P. B. Reich, and J. Pastor. 2008. Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: a mesocosm study. *Oecologia* 155:509–518.
- Halls, L. K. 1984. *White-tailed deer: ecology and management*. Stackpole Books, Harrisburg, Pennsylvania, USA.
- Heckel, C. D., N. A. Bourg, W. J. McShea, and S. Kalisz. 2010. Nonconsumptive effects of a generalist ungulate herbivore drive decline of unpalatable forest herbs. *Ecology* 91:319–326.
- Hendrix, P. F., M. A. Callahan, J. M. Drake, C.-Y. Huang, S. W. James, B. A. Snyder, and W. Zhang. 2008. Pandora's Box contained bait: the global problem of introduced earthworms. *Annual Review of Ecology, Evolution, and Systematics* 39:593–613.
- Hodge, A., and A. H. Fitter. 2010. Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. *Proceedings of the National Academy of Sciences USA* 107:13754–13759.
- Hunn, J. R. 2007. Retention of logging debris to reduce deer browsing and promote forest regeneration. Thesis. Cornell University, Ithaca, New York, USA.
- James, S. W. 1995. Systematics, biogeography, and ecology of Nearctic earthworms from eastern, central, southern and southwestern United States. Pages 29–52 in P. F. Hendrix, editor. *Earthworm ecology and biogeography in North America*. Lewis, Boca Raton, Florida, USA.
- Johansen, A., I. Jakobsen, and E. S. Jensen. 1994. Hyphal N transport by a vesicular-arbuscular mycorrhizal fungus associated with cucumber grown at three nitrogen levels. *Plant and Soil* 160:1–9.
- Johnson, M. T. J., K. A. Thompson, and H. S. Saini. 2015. Plant evolution in the urban jungle. *American Journal of Botany* 102:1951–1953.
- Jull, L. G. 2001. *Plants not favored by deer*. Cooperative Extension Publishing, Madison, Wisconsin, USA.
- Jumpponen, A. 2001. Dark septate endophytes—are they mycorrhizal? *Mycorrhiza* 11:207–211.
- Jumpponen, A., K. G. Mattson, and J. M. Trappe. 1998. Mycorrhizal functioning of *Phialocephala fortinii* with *Pinus contorta* on glacier forefront soil: interactions with soil nitrogen and organic matter. *Mycorrhiza* 7:261–265.
- Kirchberger, J., N. Eisenhauer, W. W. Weisser, and M. Türke. 2015. Experimental evaluation of herbivory on live plant seedlings by the earthworm *Lumbricus terrestris* L. in the presence and absence of soil surface litter. *PLoS ONE* 10:e0123465.
- Kluber, L. A., S. R. Carrino-kyker, K. P. Coyle, J. L. Deforest, C. R. Hewins, A. N. Shaw, K. A. Smemo, and D. J. Burke. 2012. Mycorrhizal response to experimental pH and P manipulation in acidic hardwood forests. *PLoS ONE* 7:e48946.
- Kramer-Walter, K. R., P. J. Bellingham, T. R. Millar, R. D. Smissen, S. J. Richardson, and D. C. Laughlin. 2016. Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. *Journal of Ecology* 104:1311–1313.
- Kuebbing, S. E., A. T. Classen, N. J. Sanders, and D. Simberloff. 2015. Above- and below-ground effects of plant diversity depend on species origin: An experimental test with multiple invaders. *New Phytologist* 208:727–735.
- Libalbert, E., H. Lambers, T. I. Burgess, and S. J. Wright. 2015. Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. *New Phytologist* 206:507–521.
- Larson, E. R., K. F. Kipfmüller, C. M. Hale, L. E. Frelich, and P. B. Reich. 2010. Tree rings detect earthworm invasions and their effects in northern Hardwood forests. *Biological Invasions* 12:1053–1066.
- Lawrence, B., M. C. Fisk, T. J. Fahey, and E. R. Suarez. 2003. Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (*Acer saccharum*). *New Phytologist* 157:145–153.
- Lefcheck, J. S. 2015. PIECEWISE SEM: piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution* 7:573–579.
- Liu, B., H. Li, B. Zhu, R. T. Koide, D. M. Eissenstat, and D. Guo. 2015. Complementarity in nutrient foraging strategies of absorptive fine roots and arbuscular mycorrhizal fungi across 14 coexisting subtropical tree species. *New Phytologist* 208:125–136.
- Loss, S. R. 2012. Nesting density of hermit thrushes in a remnant invasive earthworm-free portion of a Wisconsin hardwood forest. *Wilson Journal of Ornithology* 124:375–379.
- Maerz, J. C., V. A. Nuzzo, and B. Blossey. 2009. Declines in woodland salamander abundance associated with non-native earthworm and plant invasions. *Conservation Biology* 23:975–981.
- Marhan, S., and S. Scheu. 2006. Mixing of different mineral soil layers by endogeic earthworms affects carbon and nitrogen mineralization. *Biology and Fertility of Soils* 42:308–314.
- McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 115:495–501.
- McLean, M. A., S. Migge-Kleian, and D. Parkinson. 2006. Earthworm invasions of ecosystems devoid of earthworms: effects on soil microbes. *Biological Invasions* 8:1257–1273.
- Murray, B. D., C. R. Webster, and J. K. Bump. 2013. Broadening the ecological context of ungulate–ecosystem interactions: the importance of space, seasonality, and nitrogen. *Ecology* 94:1317–1326.
- de Novais, C. B., J. R. de Oliveira, J. O. Siqueira, S. M. de Faria, E. M. R. da Silva, A. M. Aquino, and O. J. Saggin Júnior. 2019. Trophic relationships between the earthworm *Pon-toscolex corethrurus* and three tropical arbuscular mycorrhizal fungal species. *Applied Soil Ecology* 135:9–15.

- Nuzzo, V. A., J. C. Maerz, and B. Blossey. 2009. Earthworm invasion as the driving force behind plant invasion and community change in northeastern North American forests. *Conservation Biology* 23:966–974.
- Nuzzo, V., A. Davalos, and B. Blossey. 2015. Invasive earthworms shape forest seed bank composition. *Diversity and Distributions* 21:560–570.
- Nuzzo, V., A. Davalos, and B. Blossey. 2017. Assessing plant community composition fails to capture impacts of white-tailed deer on native and invasive plant species. *AoB Plants* 9:plx026.
- O'Geen, A. T., D. E. Beaudette, and M. Walkinshaw. 2013. SoilWeb: a delivery system for USDA-NRCS Soil Survey. University of California, Davis, California, USA.
- Olsson, P. A., S. H. Burleigh, and I. M. Van Aarle. 2005. The influence of external nitrogen on carbon allocation to *Glomus intraradices* in monoxenic arbuscular mycorrhiza. *New Phytologist* 168:677–686.
- Paré, D., and B. Bernier. 1989. Origin of the phosphorus deficiency observed in declining sugar maple stands in the Quebec Appalachians. *Canadian Journal of Forest Research* 19:24–34.
- Paudel, S., T. Longcore, B. MacDonald, M. K. McCormick, K. Szlavetz, G. W. Wilson, and S. R. Loss. 2016. Belowground interactions with aboveground consequences: Invasive earthworms and arbuscular mycorrhizal fungi. *Ecology* 97:605–614.
- Pfahl, S., P. A. O'Gorman, and E. M. Fischer. 2017. Understanding the regional pattern of projected future changes in extreme precipitation. *Nature Climate Change* 7:423–427.
- R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Rearick, D., L. Kintz, K. L. Burke, and T. S. Ransom. 2011. Effects of white-tailed deer on the native earthworm, *Eisenoides carolinensis*, in the southern Appalachian Mountains, USA. *Pedobiologia* 54:S173–S180.
- Resner, K., K. Yoo, S. D. Sebestyen, A. Aufdenkampe, C. M. Hale, A. Lyttle, and A. Blum. 2015. Invasive earthworms deplete key soil inorganic nutrients (Ca, Mg, K, and P) in a northern hardwood forest. *Ecosystems* 18:89–102.
- Russell, M. B., C. W. Woodall, K. M. Potter, B. F. Walters, G. M. Domke, and C. M. Oswalt. 2017. Interactions between white-tailed deer density and the composition of forest understories in the northern United States. *Forest Ecology and Management* 384:26–33.
- Seki, Y., K. Okuda, and M. Koganezawa. 2014. Indirect effects of sika deer on Japanese badgers. *Mammal Study* 39:201–208.
- Shelton, A. L., J. A. Henning, P. Schultz, and K. Clay. 2014. Effects of abundant white-tailed deer on vegetation, animals, mycorrhizal fungi, and soils. *Forest Ecology and Management* 320:39–49.
- Shipley, B. 2000. A new inferential test for path models based on directed acyclic graphs. *Structural Equation Modeling: A Multidisciplinary Journal* 7:206–218.
- Simmons, W., A. Dávalos, and B. Blossey. 2015. Forest successional history and earthworm legacy affect earthworm survival and performance. *Pedobiologia* 58:153–164.
- Smith, S. E., I. Jakobsen, M. Grønlund, and F. A. Smith. 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiology* 156:1050–1057.
- Smith, M. S., J. D. Fridley, M. Goebel, and T. L. Bauerle. 2014. Links between belowground and aboveground resource-related traits reveal species growth strategies that promote invasive advantages. *PLoS ONE* 9:e104189.
- Suarez, E. R., D. M. Pelletier, T. J. Fahey, P. M. Groffman, P. J. Bohlen, and M. C. Fisk. 2004. Effects of exotic earthworms on soil phosphorus cycling in two broadleaf temperate forests. *Ecosystems* 7:28–44.
- Toju, H., H. Sato, and A. S. Tanabe. 2014. Diversity and spatial structure of belowground plant–fungal symbiosis in a mixed subtropical forest of ectomycorrhizal and arbuscular mycorrhizal plants. *PLoS ONE* 9:e86566.
- Vierheilig, H., A. P. Coughlan, U. R. S. Wyss, and Y. Piché. 1998. Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi. *Applied and Environmental Microbiology* 64:5004–5007.
- Vitousek, P. M., et al. 2002. Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* 57/58:1–45.
- Waller, D. M. 2014. Effects of deer on forest herb layers. Pages 369–399 in F. S. Gilliam and M. R. Roberts, editors. *The herbaceous layer in forests of eastern North America*. Second edition. Oxford University Press, New York, New York, USA.
- Waller, D. M., and L. I. Maas. 2013. Do white-tailed deer and the exotic plant garlic mustard interact to affect the growth and persistence of native forest plants? *Forest Ecology and Management* 304:296–302.
- Wang, R., Q. Wang, N. Zhao, Z. Xu, X. Zhu, C. Jiao, G. Yu, and N. He. 2017. Different phylogenetic and environmental controls of first-order root morphological and nutrient traits: evidence of multidimensional root traits. *Community Ecology* 32:29–39.
- Wiegmann, S. M., and D. M. Waller. 2006. Fifty years of change in northern upland forest understories: Identity and traits of “winner” and “loser” plant species. *Biological Conservation* 129:109–123.
- Wurst, S., and M. C. Rillig. 2011. Additive effects of functionally dissimilar above- and belowground organisms on a grassland plant community. *Journal of Plant Ecology* 4:221–227.
- Yang, N., K. Schützenmeister, D. Grubert, H. F. Jungkunst, D. Gansert, S. Scheu, A. Polle, and R. Pena. 2015. Impacts of earthworms on nitrogen acquisition from leaf litter by arbuscular mycorrhizal ash and ectomycorrhizal beech trees. *Environmental and Experimental Botany* 120:1–7.
- Yves, A., D. Oberski, J. Byrnes, L. Vanbrabant, V. Savalei, E. Merkle, M. Hallquist, M. Chow, T. Jorgensen, and M. Y. R. Yvesrosseelugentbe. 2017. Package *lavaan*. <http://www.jstatsoft.org/v48/i02/>

SUPPORTING INFORMATION

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DATA AVAILABILITY

Data and code are available on Zenodo: <https://doi.org/10.5281/zenodo.3459794>