

STUDIES

Potential interactive effects between invasive *Lumbricus terrestris* earthworms and the invasive plant *Alliaria petiolata* on a native plant *Podophyllum peltatum* in northeastern Ohio, USA

Colin G. Cope¹, Sarah R. Eysenbach³, Alexandra S. Faidiga¹, Constance E. Hausman³, Juliana S. Medeiros², Jennifer E. Murphy¹ and Jean H. Burns^{*,1}

¹Department of Biology, Case Western Reserve University, Cleveland, OH 44106, USA, ²The Holden Arboretum, 9500 Sperry Road, Kirtland, OH 44094, USA, ³Cleveland Metroparks, 2277 W Ridgewood Drive, Parma, OH 44134, USA

*Corresponding author's e-mail address: jbm122@case.edu

Populations & Communities Chief Editor: Jean Burns

Associate Editor: Colin Orians

Abstract

We test whether the invasive earthworm *Lumbricus terrestris* and leaf litter of the invasive herbaceous plant *Alliaria petiolata* interact to influence the native plant, *Podophyllum peltatum*, using both observational field data and a multi-year experiment. We hypothesized invader interactive effects on the native plant might result from either changes in allelochemical distribution in the soil or nutrient availability mediated by the invasive earthworm pulling leaf litter down into the soil. Within the field data we found that *Alliaria petiolata* presence and higher soil nitrogen correlated with reduced *Podophyllum peltatum* cover, and no evidence for an invader–invader interaction. Within the factorial experiment, we found a super-additive effect of the two invaders on plant biomass only when activated carbon was present. In the absence of activated carbon, there were no differences in *Podophyllum peltatum* biomass across treatments. In the presence of activated carbon, *Podophyllum peltatum* biomass was significantly reduced by the presence of both *Lumbricus terrestris* and *Alliaria petiolata* leaf litter. The absence of an effect of *Alliaria petiolata* leaves without activated carbon, combined with a failure to detect arbuscular mycorrhizal colonization, suggests that indirect effects of allelochemicals on arbuscular mycorrhizal fungi were not the primary driver of treatment responses. Rather direct nutrient availability might influence a potential interaction between these invaders. Leaf nitrogen content was higher and leaf CO₂ concentration was lower in the presence of *Lumbricus terrestris*, but treatment did not influence maximum photosynthetic rate. While the field data do not suggest a negative interaction between these invaders, the experiment suggests that such an interaction is possible with greater environmental stress, such as increasing nitrogen deposition. Further, even plants with rapid physiological responses to increased nitrogen availability may have other physiological limits on growth that prevent them from compensating from the harm caused by multiple invaders.

Keywords: context-dependence; interactive effects; invasion; invasive earthworms; invader impacts; physiology

Received: 4 August 2020; Editorial decision: 7 December 2020; Accepted: 28 December 2020

© The Author(s) 2020. Published by Oxford University Press on behalf of the Annals of Botany Company.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Assessment of effects of invaders have generally focussed on single invasive species, despite the frequent observation of systems with multiple invaders (Kuebbing and Nunez 2015). Interactions between multiple invaders could be additive (e.g. Milton *et al.* 2007; Cushman and Gaffney 2010; Shaben and Myers 2010; Braga *et al.* 2019), sub-additive (Yang *et al.* 2011) or super-additive (Green *et al.* 2011) and mostly focus on plant-plant interactions (Kuebbing and Nunez 2015). We propose that invasive earthworms, like *Lumbricus terrestris* (Lumbricidae), might interact with invasive plants because of their feeding mode of pulling leaf litter down into the soil (Edwards 2004; Fahey *et al.* 2013), potentially redistributing allelochemicals and nutrients produced by invasive plant leaf litter (Fig. 1). For example, *Alliaria petiolata* leaf litter produces allelochemicals that suppress plant mutualists belowground (Cantor *et al.* 2011). Invader interactions belowground might particularly influence plant physiology, because photosynthetic rates are limited by phosphorus availability (Rychter and Rao 2005), and phosphorus uptake is mediated by arbuscular mycorrhizal mutualists in many cases. Photosynthetic rates are also limited by nitrogen availability, which is a component of RuBisCO, the enzyme that fixes carbon in C_3 plants (von Caemmerer and Quick 2000). By partnering field and experimental data, we explore this potential interaction between invasive earthworms and *Alliaria petiolata* leaf litter under realistic field conditions, as well as characterizing physiological mechanisms in a manipulative experiment.

The invasive plant, *Alliaria petiolata*, can directly suppress native plants via competition (Barto and Cipollini 2009), but here we focus on potential effects via leaf litter (Fig. 1). The leaf litter of *Alliaria petiolata* is known to have strong negative effects on native plants, through the production of glucosinolate and flavonoid allelochemicals (Hale and Kalisz 2012) and changes in nutrient cycling (Rodgers *et al.* 2008). *Alliaria petiolata* leaf litter and its chemical leachates can have direct phytotoxic effects on native plants (Fig. 1, arrow 1) (Cipollini *et al.* 2012). In addition, *Alliaria petiolata* is known to suppress arbuscular mycorrhizal fungi (AMF) (Fig. 1, arrow 2) (Hale and Kalisz 2012; Cantor *et al.* 2011; Hale *et al.* 2016) and ectomycorrhizal fungi (Wolfe *et al.* 2008), which help plants acquire nutrients from the soil (Mohammadi *et al.* 2011). When *Alliaria petiolata* allelochemicals kill AMF mutualists, this lowers photosynthetic rates in the native plant *Maianthemum racemosum* (Hale *et al.* 2011, 2016). Isolating effects of allelochemicals is methodologically complex and at times controversial (Lau *et al.* 2008; Weissbuhner and Prati 2009). For example, activated carbon is often used in allelopathy studies to absorb allelochemicals (Callaway and Aschehoug 2000; Prati and Bossdorf 2004), but has multiple effects on the soil, increasing the availability of some nutrients (e.g. nitrate) and decreasing others (e.g. ammonium) (Lau *et al.* 2008). While this method cannot by itself determine whether allelopathy occurs, it does change the context of the soil, allowing us to ask whether soil context might influence *Alliaria petiolata* leaf litter effects. *Alliaria petiolata* leaf litter can also alter nutrient cycling (Fig. 1, arrow 3) (Rodgers *et al.* 2008), potentially increasing nutrient availability to native plants.

Lumbricus terrestris is an invasive earthworm (Eisenhauer *et al.* 2007), which is likely to interact with *Alliaria petiolata*, because it feeds by pulling leaf litter down into the soil (Zicsi *et al.* 2011). *Lumbricus terrestris*' feeding behaviour may break down nutrients in leaves faster than decomposition alone, altering nutrient cycling (Fig. 1, arrow 4) (Costello and Lamberti

2009). Both *Lumbricus terrestris* and *Alliaria petiolata* are native to Europe and were introduced to the USA through European settlers in the 1800s (Nuzzo 1993; Eisenhauer *et al.* 2007) and have become widespread problems throughout much of the Eastern USA, where they often co-occur (Nuzzo *et al.* 2009; Zelles 2012). A meta-analysis has indicated that greater invasive earthworm biomass correlates with greater invasive plant cover, consistent with invasive earthworms facilitating invasive plants (Craven *et al.* 2017). Because of this high degree of co-occurrence, we propose that *Lumbricus terrestris* earthworms may amplify the effects of *Alliaria petiolata* allelopathy through their feeding mechanism of pulling litter into underground burrows. *Lumbricus terrestris* might thus increase the distribution of leaf litter over the roots of native plants, and thus increase the negative allelopathic effects of *Alliaria petiolata* (Fig. 1, arrow 5).

Our aims were to determine whether the invasive earthworm *Lumbricus terrestris* and leaf litter of *Alliaria petiolata* might influence native plant abundance, performance and physiology. We focussed on a common herbaceous plant: *Podophyllum peltatum* (Berberidaceae). We selected native species *Podophyllum peltatum* because it is common in the northeastern USA and co-occurs with the two invaders *Lumbricus terrestris* and *Alliaria petiolata*. In addition, *Podophyllum peltatum* associates with AMF (Soudzilovskaia *et al.* 2020), making it a potential candidate for indirect effects (Fig. 1). First, we utilized a large-scale observational dataset to determine if native *Podophyllum peltatum* abundance correlates with invader abundance in the

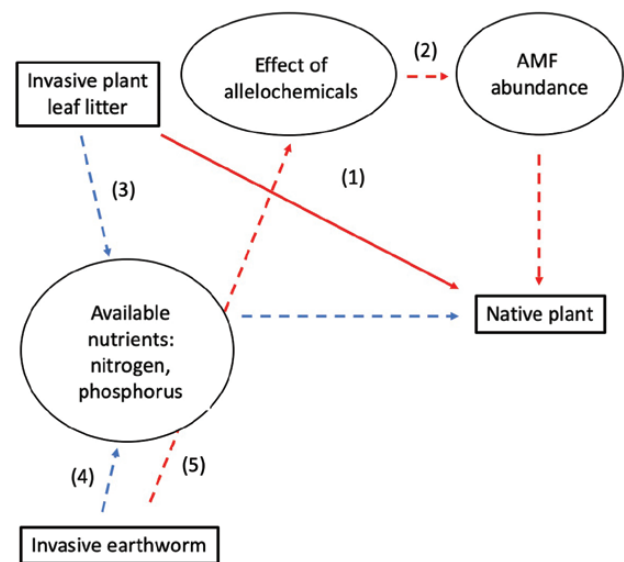


Figure 1. The leaf litter of invasive plants, such as *Alliaria petiolata*, may negatively influence native plant growth via allelochemicals, (1) which may have direct phytotoxic effects on the native plant, or (2) which may suppress plant mutualists, such as arbuscular mycorrhizal fungi (AMF), indirectly suppressing native plants. (3) *Alliaria petiolata* leaf litter can also influence nutrient availability in the soil. Invasive earthworms can also influence native plants by enhancing nutrient (e.g. nitrogen, phosphorus) availability in the soil, which could (4) interact with invader leaf nutrient input (e.g. via changes in litter decomposition), or could (5) shift the distribution or rate of allelochemical input in the soil. Putative direct effects on native plants are shown with solid arrows and indirect with dashed arrows. Putative negative effects are shown in red and positive effects are shown in blue. Circles indicate putative mechanisms that were not directly measured (i.e. 'latent variables' in the language of path analysis). See text in the Introduction for additional details.

field. Because nutrient availability in the soil influences plant physiology, we explored whether soil nutrients and invaders might interact to influence *Podophyllum peltatum* abundance. Second, we conducted a factorial experiment to determine effects of these two invaders on *Podophyllum peltatum* growth and physiology. We predicted that *Lumbricus terrestris* might increase the exposure of *Podophyllum peltatum* to allelochemicals produced by *Alliaria petiolata*, resulting in greater suppression of native plant biomass in the presence of both invaders (super-additive effect). If the interaction between the invasive plant and earthworm is mediated by allelochemicals in the soil, we predict the interaction will be stronger in the absence of activated carbon, which binds allelochemicals (Fig. 1, arrow 5). An interaction only in the presence of activated carbon would be more consistent with nutrient-mediated effects (Fig. 1, arrow 4). Alternatively, *Lumbricus terrestris* might enhance nutrient availability for *Podophyllum peltatum*, leading to greater plant resistance to allelochemicals produced by *Alliaria petiolata* (sub-additive effect). If this is the case, we predict that *Lumbricus terrestris* might enhance plant access to soil nitrogen, increasing the ability of the native plant to fix carbon (greater Ci and greater Amax in the presence of *Lumbricus terrestris*).

Methods

Plant community assessment program—observational data

We utilized a large (400 plots—187 plots met our criteria, see below) plant community dataset (PCAP) collected over a series of 4 years between 2010 and 2013 by Cleveland Metroparks to explore correlates of *Podophyllum peltatum* abundance (percent cover). Cleveland Metroparks encompasses >23 000 acres with 18 reservations that are located within and surrounding the city of Cleveland, OH. The plots were established using the generalized random tessellation stratified design (Stevens and Olsen 2003), providing a spatially balanced random sample. Sampling procedure followed the North Carolina Vegetation Survey method (Peet et al. 2004) and plots were sampled from 11 June to 30 August. While *Podophyllum peltatum* and *Alliaria petiolata* cover may be underestimated towards the end of the sampling season, plots were sampled in a geospatially balanced, stratified random sample to avoid systematic bias (Stevens and Olsen 2003). The plots that were sampled covered a 20 m × 50 m area, in which the percent cover of every plant species was recorded, as well as collecting soil samples for nutrient analysis. The presence or absence of earthworms was recorded by visual assessment, where plots with worms, castings and middens were coded as 'present'. Field crews consisted of 2 teams with typically 4 crew members each. Each visual assessment was the responsibility of 2 crew members. Field crews received 4 weeks of complete protocol training including worm sampling prior to the start of the field season. That training included practice plots and 'flip-flop' assessments to ensure standardized calibration among crew members throughout the season. Because the assessment team dug a soil pit in the middle of the plot, measured leaf litter depth and humus depth at the centre of each intensive module while looking for worm evidence, and carefully removed the leaf litter in search of worm evidence, we are confident that the presence of invasive earthworms was accurately recorded (Loss et al. 2013). Many species of invasive earthworms are thus included in the field assessment, including but not limited to *Lumbricus terrestris*. We collected about 250 mL

of soil for each plot within 10 cm of the soil surface with a soil probe. Soil samples were stored at room temperature for up to 3 months before sending to A&L Great Lakes Laboratories (Fort Wayne, IN). Soil was tested for phosphorus (ppm) and nitrogen (% total). Extractable phosphorus can be broken down into two different forms, phosphorus 1 ('weak Bray') and phosphorus 2 ('strong Bray'). The more commonly used measurement is phosphorus 1, which was developed to correlate with plant-available phosphorus fraction of the soil in slightly acidic soils (Tandon et al. 1967). The phosphorus 1 test corresponds with how much phosphorus is available to plants. The phosphorus 2 test gives total soil phosphorus. If there is a big gap between these two numbers, that means much of the phosphorus in the soil is unavailable to plants.

We analysed only plots with information on the presence or absence of *Alliaria petiolata* and/or earthworms to maintain parallel structure to the potted experiment (discussed subsequently). There were a total of 187 plots that met these criteria. Eighty-seven of these plots had zero percent cover of *Podophyllum peltatum*. We then compared the percent cover of *Podophyllum peltatum*, as well as the percent soil nitrogen and two different measures of inorganic phosphorus in parts per million (ppm) across plots with or without these predictor variables (see analysis below).

Factorial experiment

The experiment was located at Case Western Reserve University's Squire Valleeview Farm ('University Farm' below) in Hunting Valley, OH, USA (41°29' N, 81°25' W) over 2 years (2014–15). The experiment was organized in a 2 × 2 × 2 factorial design, with 6 replicates per experimental treatment, for a total of 48 pots. Each treatment included the presence or absence of the three factors of *Alliaria petiolata*, *Lumbricus terrestris* and activated carbon (Fig. 2). Treatments were randomized within 6 replicate blocks in a common garden. We used 57-L pots (45 cm diameter at top × 42 cm deep). Pots were covered with 30 % shade cloth (Greenhouse Megastore, Danville, IL, USA) to mimic early forest understory conditions in northeastern Ohio. At midday on a sunny day, when ambient PPFD was ~1800, this resulted in an approximate PPFD of 500 under the shade cloth. Plants were kept well-watered throughout the experiment.

On 21 May 2014, 40 L of potting soil (Promix, Quebec, Canada, BX Mycorrhizae general purpose growing medium # 10 381, which uses *Glomus intradices*) were added to each pot, along with a *Podophyllum peltatum* rhizome obtained from Prairie Moon Nursery in Winona, MN and 250 mL of live soil from underneath field *Podophyllum peltatum* to encourage AMF colonization. Initial rhizome size was recorded as length of the rhizome.

Pots with an activated carbon present treatment received 400 g of activated carbon pellets incorporated into the soil evenly distributed through the pots (Lau et al. 2008). We used an amount of activated carbon used in previous experiments (Lau et al. 2008). To ensure that the *Podophyllum peltatum* would receive adequate nutrients, we added 84 g of Nutricote 100-day fertilizer with 18-6-8 N-P-K (#18-6-8NUTRI-100, American Horticultural Supply, Inc.) on 5 June 2014 to all pots, following methods in Hale et al. (2011). By adding a small amount of phosphorus, we created conditions where *Podophyllum peltatum* plants should have maintained their AMF mutualism (Hale et al. 2011). Fertilizer treatments can influence the effect of activated carbon (Lau et al. 2008), so effects of the activated carbon treatment should be interpreted narrowly in this context.

Pots with a *Lumbricus terrestris* present treatment received 16 *Lumbricus terrestris* worms (~100 earthworms per m²). This is

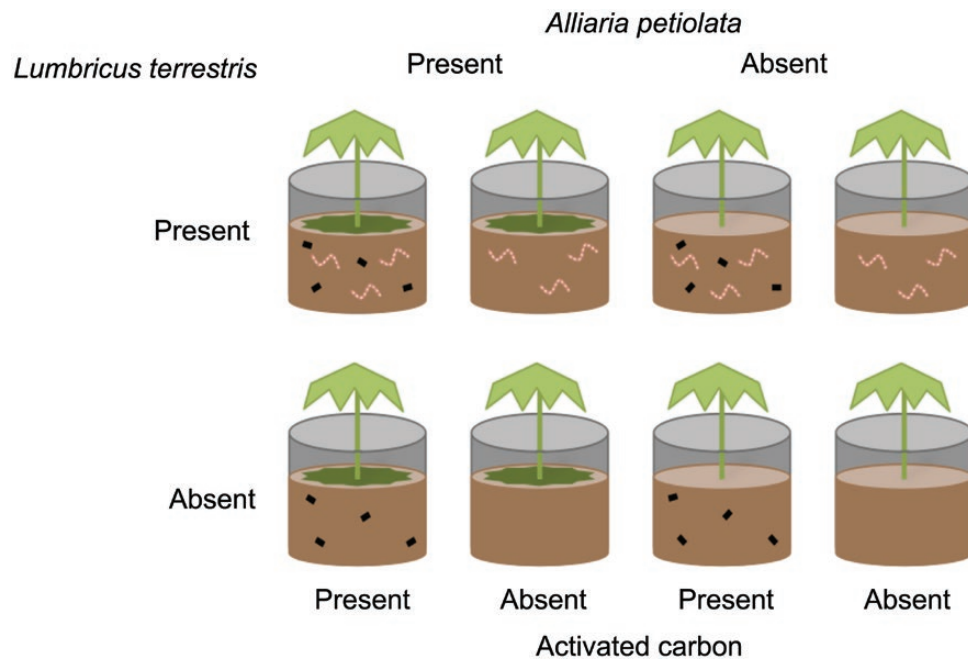


Figure 2. Factorial experimental design assessing the combined effects of invaders on the native plant, *Podophyllum peltatum*. Invasive *Alliaria petiolata* leaves were placed on the soil surface in *Alliaria petiolata* presence treatment, invasive *Lumbricus terrestris* earthworms were placed in pots in the '*Lumbricus terrestris* present' treatment, and activated carbon (black rectangles) was placed in the 'activated carbon present' treatment.

consistent with natural populations, which can have in excess of 100 individuals per square metre (Eisenhauer et al. 2007). We observed earthworms feeding by pulling leaf litter down into the soil, found less leaf cover on top of pots [see Supporting Information—Fig. S1], and observed earthworms and their burrows in '*Lumbricus terrestris* presence' treatments at harvest (C.G. Cope, pers. obs), suggesting that our *Lumbricus terrestris* presence treatment was effective in adding earthworms to the experimental plants and initiating leaf litter feeding, as expected. We observed no earthworms or their burrows in *Lumbricus terrestris* absence treatments.

Pots with the *Alliaria petiolata* present treatment were treated weekly with 100 g of fresh *Alliaria petiolata* leaves from second year (bolting) plants of this biennial, spread on the surface of the soil during *Alliaria petiolata*'s growing season, similar to methods in (Brouwer et al. 2015). This amount allowed the entire surface of the soil within the pot to be covered to provide substantial leaf litter cover for *Lumbricus terrestris* feeding in treatments containing *Lumbricus terrestris* and make sure that the allelochemicals could leach into all of the soil surface (Brouwer et al. 2015). Leaves stayed in place and did not blow away during the experiment, likely because they were wet [see Supporting Information—Fig. S1, C.G. Cope, pers. obs.].

Once *Podophyllum peltatum* began to senesce in the field in mid-July 2014, the pots containing the *Podophyllum peltatum* rhizomes were stored in an uninsulated barn during the winter to prevent the rhizomes from freezing and dying. The pots were then pulled out of storage in early April 2015. Sixteen worms were again added to *Lumbricus terrestris* present pots, and 100 g of *Alliaria petiolata* was again added weekly to *Alliaria petiolata* present pots in the summer of 2015 until 12 June, when the *Podophyllum peltatum* plants were harvested. Roots were washed and biomass was dried in the drying oven for 24 h (leaves) or

72 h (stems, rhizomes, roots and reproductive) at 60 °C, and weighed to 0.01 g.

Plant nutrient content and physiology

In order to assess the photosynthetic rate of *Podophyllum peltatum* across experimental treatments, we used an LI-6400XT portable photosynthesis system. Ambient conditions were mostly sunny with an average photosynthetic photon flux density (PPFD) of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and air temperature of ≈ 21 °C. Conditions within the chamber were maintained at a flow rate of 400 $\mu\text{mol s}^{-1}$, a CO_2 concentration of 400 $\mu\text{mol mol}^{-1}$, a leaf temperature of 21.4 °C, and between 40 and 50 % relative humidity. PPFD was set to 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for all individuals measured. Based on previous data (not presented), this light level represented saturation for plants without inducing photoinhibition. Once plants stabilized in the chamber we logged 3 data points per plant on 23 May 2015 between 11:00 AM and 1:00 PM before rates began to lose stability.

In order to assess leaf nutrient content, we followed the methods described in Khasanova et al. (2013) for CN analysis. Plant biomass was washed with DI water and oven-dried at 65 °C. Subsamples of dried plant tissue were ground with a ball mill, then 2 ± 0.1 μg samples were weighed for total N and C concentration by micro Dumas combustion on a CN analyzer (Costech Analytical, Valencia, CA).

To determine AMF colonization, we stained roots collected from the *Podophyllum peltatum* plants at the end of the experiment. This procedure followed root staining protocol put forth by McGonigle and colleagues where roots were cleared for 20 min at 121 °C in a 10 % KOH solution, and stained in Chlorazol Black E (McGonigle et al. 1990). Following staining we examined the stained roots under a microscope [digital camera (Model #C-3040; Olympus America Inc., Melville, NY, USA) attached to a microscope (Model #BH-2;

Olympus Corporation, Lake Success, NY, USA)] at $\times 40$ and $\times 100$ magnification. We found few traces of any AMF colonization on any of the 47 roots examined (one plant died before the end of the experiment). Seven out of 47 pots had some signs of colonization and there was no apparent pattern with respect to treatment. We also performed DNA extractions on both ground and unground roots, and used polymerase chain reaction (PCR) using the AMG1F and AM1 primers to amplify any AMF DNA found within the *Podophyllum peltatum* roots. We then used gel electrophoresis to determine whether there was AMF colonization of our samples. There were no visible bands on any of the 41 roots examined (a few roots did not yield strong DNA extractions), indicating that the *Podophyllum peltatum* roots had no detectable AMF DNA present.

Statistical analysis

Plant community assessment program—observational data. To ensure the predictors in the model below were statistically independent, we tested whether soil nutrients (% soil nitrogen, phosphorus 1, phosphorus 2) predicted *Alliaria petiolata* percent cover in the observational data set. We used three separate linear models with log transformed *Alliaria petiolata* percent cover—with a small constant (0.001) added before transformation to retain zero values in the data—as a function of log transformed % soil nitrogen, phosphorus 1 or phosphorus 2. This is important because *Alliaria petiolata* may directly influence soil nutrient availability (Rodgers et al. 2008). Further, we also explored whether *Alliaria petiolata* presence, invasive earthworm presence or their interaction predicted soil nutrient availability for % soil nitrogen, phosphorus 1 and phosphorus 2 in separate linear models. Soil nitrogen and phosphorus were log transformed to normalize the data. Because we used observational data, the ‘design’ of this analysis was unbalanced. Therefore, we used ‘type II’ sums of squares (Herr 1986), using the drop1 function in R.

In addition, we conducted a contingency table analysis and found that the presence of one invader did not influence the presence of the other ($\chi^2 = 1.53$, $df = 1$, $P = 0.22$). Thus, we treated the presence of *Alliaria petiolata* and earthworms as independent variables in the following analysis. We used the presence/absence of *Alliaria petiolata* (rather than percent cover) for parallel structure with the experimental design, described below. Next we modelled percent cover of *Podophyllum peltatum* as a response variable in a linear model with a Gaussian error distribution and log transformed percent cover, with a small constant added (0.001) to prevent zero values of percent cover from being dropped from the model. We included predictors: *Alliaria petiolata* presence, earthworm presence, soil nitrogen and all of their interactions. Because our analyses indicated that soil phosphorus was not independent of earthworms, we did this analysis only for soil nitrogen [see Supporting

Information—Table S1]. We used stepAIC to select a minimal model (Venables and Ripley 2002) and we used the drop1 function in R to test for significance.

Factorial experiment. We fit a linear model with *Podophyllum peltatum* final dry biomass as a response variable and *Alliaria petiolata*, *Lumbricus terrestris* and activated carbon treatments, and their interactions as predictors. We used log-transformed final biomass and a Gaussian error distribution. We then conducted planned contrasts within both the *Lumbricus terrestris* and *Alliaria petiolata* treatments. We explored a model with initial rhizome size (length) as a covariate. Initial size was not predictive of final mass after 2 years in the pot experiment ($F_{1,38} = 0.11$, $P = 0.74$), and was, therefore, not included in the final models. Block was not significant in initial models ($F_{1,38} = 0.71$, $P = 0.48$), and was not included in final models.

For our physiological measures, C_i and A_{max} , we modelled untransformed variables as a function of *Alliaria petiolata*, *Lumbricus terrestris* and activated carbon treatments, and their interactions as predictors. Then we used model selection by AIC, using the function stepAIC in the MASS package (Venables and Ripley 2002), to select a minimal model.

All datasets were examined for potential outliers as well as tested for normality. Q-Q norm and residual vs. fitted plots were examined for each model to check model assumptions (Bolker et al. 2008). All model assumptions were met. All analyses were conducted in the R statistics program (version 3.4.3, R Core Development Team 2014).

Results

Plant community assessment program observational data

There was no evidence in the observational data for a direct interaction between invasive earthworm presence and *Alliaria petiolata* on cover of the native plant, *Podophyllum peltatum* (Table 1). The presence of *Alliaria petiolata* and high concentrations of soil nitrogen interacted in a model of *Podophyllum peltatum* cover, with the least *Podophyllum peltatum* cover in *Alliaria petiolata* sites with high soil nitrogen (Table 1, see Supporting Information—Fig. S2). Soil phosphorus predicted *Alliaria petiolata* abundance across the field plots [see Supporting Information—Table S1], indicating that *Alliaria petiolata* and soil phosphorus could not be used as independent predictors of *Podophyllum peltatum* cover. Thus, we included only soil nitrogen as a predictor in models of *Podophyllum peltatum* cover (Table 1).

We also asked whether the presence of these invasive species predicted soil nitrogen or phosphorus. The presence of earthworms predicted lower total soil nitrogen [see Supporting

Table 1. Observational field data, modelling the interaction of invasive *Alliaria petiolata*, invasive earthworm presence and soil nitrogen on the percent cover of native *Podophyllum peltatum**.

	DF	Deviance	AIC	F-value	P-value
<i>Alliaria petiolata</i> presence/absence	1	1726	962.25	2.46	0.12
earthworm presence/absence	1	1713	960.88	1.14	0.29
Total Soil Nitrogen (%)	1	1745	964.41	4.56	0.03
<i>Alliaria petiolata</i> \times earthworm	1	1715	961.17	1.41	0.24
<i>Alliaria petiolata</i> \times Nitrogen	1	1742	964.10	4.27	0.04*
earthworm \times Nitrogen	1	1712	960.76	1.02	0.31
<i>Alliaria petiolata</i> \times earthworm \times Nitrogen	1	1724	962.03	2.25	0.14

*An alternative model excluding zero values for percent cover of *Podophyllum peltatum* found only a marginally significant interaction here ($F_{1,373} = 2.98$, $P = 0.087$).

Information—Table S2, Fig. S3a]. We also found that *Alliaria petiolata* and earthworm presence interacted in a model of phosphorus 1 in the soil, with the lowest phosphorus available when *Alliaria petiolata* was absent and earthworms were present [see **Supporting Information—Fig. S3b**]. Phosphorus 2 availability was not correlated with these invaders [see **Supporting Information—Table S2**].

Factorial experiment

Overall, we found some evidence for a potential interaction between the invasive earthworm, *Lumbricus terrestris* and *Alliaria petiolata* leaf litter presence, though there was no direct evidence that this interaction was driven by allelopathy. We observed no colonization of *Podophyllum peltatum* roots by AMF in any experimental treatment, either via staining or qPCR. We found that the total biomass of *Podophyllum peltatum* was significantly reduced when *Lumbricus terrestris* earthworms, *Alliaria petiolata* and activated carbon were present (Table 2, Fig. 3), contrary to the prediction of an allelopathy effect in the absence of activated carbon. Contrasts showed a significant negative effect of *Alliaria petiolata* on *Podophyllum peltatum* biomass when both *Lumbricus terrestris* and activated carbon were present ($P = 0.007$, see **Supporting Information—Table S3, Fig. 3**). Further we found a marginally significant effect of *Lumbricus terrestris* on *Podophyllum peltatum* biomass in the presence of *Alliaria petiolata* ($P = 0.07$, see **Supporting Information—Table S4, Fig. 3**).

Podophyllum peltatum leaf nutrient content and internal CO_2 concentration were both influenced by experimental treatments. We found leaf nitrogen content was significantly increased in the presence of *Lumbricus terrestris* (Fig. 4a). There was also a significant decrease of internal CO_2 concentration (C_i) within the leaves of *Podophyllum peltatum* when in the presence of *Lumbricus terrestris* (Table 3, Fig. 4b). There was a marginally significant reduction in photosynthetic rate when both *Alliaria petiolata* and activated carbon were present (Table 4), and *Alliaria petiolata* alone resulted in only a 1 % reduction in photosynthetic rate. Further, there was no effect of *Lumbricus terrestris* on photosynthetic rate (Table 4).

Discussion

Because relatively few studies have examined effects of multiple invaders on native plants, whether such relationships are largely additive, or sub- or super-additive is still not fully understood (Braga et al. 2018). We find evidence consistent with the possibility of super-additive effects (Braga et al. 2018), but only in some experimental contexts. The interaction only in the presence of activated carbon, combined with the lack of AMF colonization in any experimental treatment, suggests that the experimental interaction between *Alliaria petiolata* and *Lumbricus terrestris* was not due to an indirect effect of allelochemicals

on AMF (Fig. 1, arrow 2), contrary to indirect effects of *Alliaria petiolata* allelochemical via AMF in other systems (Hale et al. 2016). Our experimental plants may have lacked AMF colonization because they were large healthy rhizomes at the beginning of the experiment, though this is speculative. Although *Podophyllum peltatum* has not been reported in the field to have facultative AMF association (Soudzilovskaia et al. 2020), the ability to ‘cut off’ mycorrhizal partners is well-described and common in many plant species (Johnson et al. 1997). In the field, *Podophyllum peltatum* cover was lowest at sites that had both greater soil nitrogen and *Alliaria petiolata* present, and the presence of earthworms reduces soil nitrogen content in the field and increases leaf nitrogen content in the experiment. If earthworms speed up nutrient cycling (Costello and Lamberti 2009), there might be less nitrogen in the soil and more within plant tissues. This increase in nutrients within the plant might reduce some of the harmful effects *Alliaria petiolata* has on *Podophyllum peltatum*, though our survey data suggest that such effects are likely weak. In addition, like others before us, we found no evidence for a correlation between earthworms and garlic mustard in the field (Zelles 2012), [however, see Nuzzo et al. (2009)], inconsistent with an ‘invasion meltdown’ hypothesis of these two invaders facilitating one another (Simberloff and Von Holle 1999).

Alliaria petiolata has the potential for both direct phytotoxic effects (Cipollini et al. 2012; Cipollini and Bohrer 2016) and indirect effects via AMF mutualists (Cipollini and Bohrer 2016; Hale et al. 2016). However, there is also mixed evidence that *Alliaria petiolata* has any direct effects on abundance in the field (Davis et al. 2015). Contrary to some previous work (Davis et al. 2015), we find a strong negative effect of *Alliaria petiolata* presence on native *Podophyllum peltatum* abundance in the field, when soil nitrogen levels are low, consistent with either competition or allelopathy (Cipollini and Cipollini 2016). Our results are consistent with studies of the demography of native plant *Trillium erectum*, whose population growth rate is suppressed by *Alliaria petiolata* in the field (Bialic-Murphy et al. 2020). Generally, more work is needed to disentangle direct and indirect effects of invaders like *Alliaria petiolata* on native plants (Cipollini and Cipollini 2016). Our work contributes to this line of research by suggesting that soil nutrient-mediated effects may be as or more important than allelopathy, at least for this native plant (Fig. 1), and that these effects can occur even in the absence of AMF mutualists and may depend on nutrient availability in the soil.

Both our experiment and field observational data show that changes in nutrient acquisition or soil conditions can influence the strength or presence of the interaction between these invaders. While adding activated carbon to our experiment was intended to neutralize allelochemicals from *Alliaria petiolata*, effects of activated carbon on soil nutrient availability and plant

Table 2. Total plant biomass of native *Podophyllum peltatum* in response to experimental treatments (see contrasts in Supporting Information—Tables S3 and S4).

	DF	Deviance	AIC	F-value	P-value
<i>Alliaria petiolata</i>	1	4090.8	359.30	1.03	0.32
Activated carbon (AC)	1	3989.6	358.12	0.04	0.85
<i>Lumbricus terrestris</i>	1	4103.5	359.44	1.15	0.29
<i>Alliaria petiolata</i> × AC	1	4053.9	358.87	0.67	0.42
<i>Alliaria petiolata</i> × <i>Lumbricus terrestris</i>	1	4253.1	361.13	2.62	0.11
AC × <i>Lumbricus terrestris</i>	1	4226.2	360.83	2.35	0.13
<i>Alliaria petiolata</i> × AC × <i>Lumbricus terrestris</i>	1	4695.3	365.78	6.94	0.01

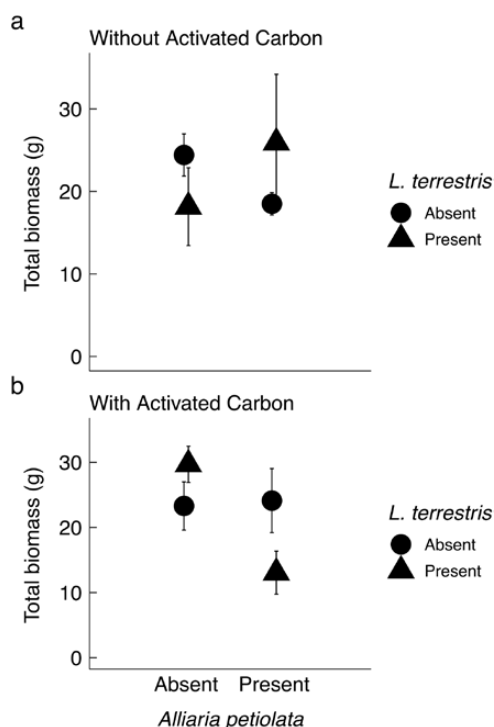


Figure 3. The effect of invasive *Lumbricus terrestris* earthworms and invasive *Alliaria petiolata* leaf litter on native *Podophyllum peltatum* total biomass in a factorial experiment. (a) In the absence of activated carbon, there were no differences in *Podophyllum peltatum* biomass across treatments. (b) In the presence of activated carbon, *Podophyllum peltatum* biomass was significantly reduced by the presence of both earthworms and *Alliaria petiolata* [see Supporting Information—Tables S3 and S4]. Means \pm 1 SE.

performance are complex (Lau et al. 2008), and activated carbon may have acted as an additional stressor to the native plant—e.g. through reduced ammonium availability (Lau et al. 2008) or changes to the soil microbial community (Weissshuhn and Prati 2009). Too much nitrogen can lead to excess leaf production at the cost of root production, leaving plants vulnerable to drought (Templer 2013). Stress caused by activated carbon in this experiment may have caused *Podophyllum peltatum* to be more vulnerable to the harmful effects of the worms and *Alliaria petiolata*, which resulted in reduced *Podophyllum peltatum* biomass; however, the mechanisms that govern this effect are unknown. In the field study, both earthworms and *Alliaria petiolata* were a significant function of soil nutrients. This suggests that significant decreases in *Podophyllum peltatum* cover or biomass due to earthworm and *Alliaria petiolata* invasion may be context-dependent and influenced by nutrient availability within the soil.

Plant responses to multiple invaders are likely to be mediated by plant physiology, as others have seen for responses to single invaders, like *Alliaria petiolata* (Brouwer et al. 2015; Hale et al. 2016). In prior studies on native herbaceous perennial *Maianthemum racemosum*, *Alliaria petiolata* has been shown to reduce carbon storage by suppressing fungal mutualists (Brouwer et al. 2015), reducing the native plant's photosynthetic rate (Hale et al. 2016). We found no AMF colonization in our experimental plants, and no direct effects of *Alliaria petiolata* on native plant physiology. Here, we found that the internal CO_2 concentration in the leaves was reduced in the presence of the invasive earthworm *Lumbricus terrestris*. This suggests that *Lumbricus terrestris* alters plant physiology by changing nutrient availability. If these invasive earthworms increase nitrogen availability to the plant, this could influence *Podophyllum peltatum* production or activation of

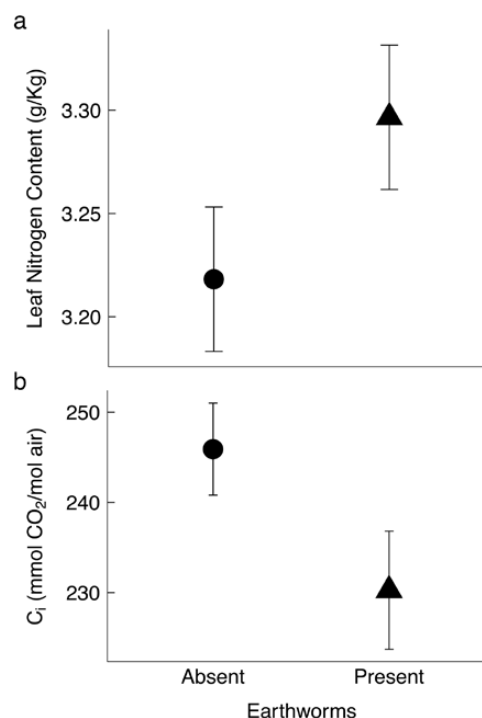


Figure 4. (a) Leaf nitrogen content of *Podophyllum peltatum* was greater in the presence of invasive *Lumbricus terrestris* earthworms ($F_{1,1} = 4.16$, $P = 0.05$) in a pot experiment. (b) Internal leaf CO_2 concentration (C_i) of *Podophyllum peltatum* was reduced when earthworms were present (Table 3). Means \pm 1 SE.

RuBisCO, the enzyme used to fix CO_2 in the leaf (Spreitzer and Salvucci 2002). The higher leaf nitrogen content in the presence of *Lumbricus terrestris* is consistent with this explanation and the observation that *Lumbricus terrestris* enhances soil nutrient cycling (Costello and Lamberti 2009). This could increase the rate of CO_2 usage, decreasing internal CO_2 concentrations (C_i). Therefore, if stomatal conductance stays the same, lower C_i could indicate greater carbon drawdown within the leaf. This makes sense if earthworms like *Lumbricus terrestris* make nitrogen more available to the plants, increasing leaf RuBisCO content and thus drawdown capacity. However, this faster C_i drawdown does not lead to greater net photosynthetic rates. A possible reason for this could be that photosynthetic rates are limited not just by RuBisCO availability, but also by the rate sugars move through the plant. If there is a backup of sugars near the photosynthetic machinery, there could be a reduction in photosynthetic rates (Brodribb et al. 2007). In sum, *Lumbricus terrestris* directly impacts *Podophyllum peltatum* physiology (lower CO_2 concentration in the leaf), but does not lead to improvements in plant performance (e.g. greater maximum photosynthetic rate or plant biomass).

Greater nutrient cycling by invasive earthworms like *Lumbricus terrestris* (Costello and Lamberti 2009) combined with *Alliaria petiolata* leaves (Rodgers et al. 2008), harms the native plant *Podophyllum peltatum* in the absence of AMF, at least under some soil conditions. Additional experiments with non-*Alliaria petiolata* leaf litter controls might help distinguish between allelopathy and nutrient effects as mechanisms (Fig. 1) (Hale et al. 2011). While our observational data in the field suggests that these two invaders might not be interacting currently to suppress *Podophyllum peltatum* cover, our experiment suggests that such interactions are likely, given sufficient stress on native plants. As environmental stressors, such as nutrient deposition (Fowler et al. 2013) and climate change (IPCC 2014), increase, the potential for such negative super-additive effects between invaders may also increase

Table 3. C_i , the intercellular CO_2 concentration in leaves, as a function of experimental treatments on native plant *Podophyllum peltatum*. Model was chosen by AIC.

	DF	Deviance	AIC	F-value	P-value
<i>Alliaria petiolata</i>	1	26 255	430.31	0.13	0.72
Activated carbon (AC)	1	26 339	430.45	0.25	0.62
<i>Lumbricus terrestris</i>	1	30 764	437.44	6.51	0.01
<i>Alliaria petiolata</i> × AC	1	26 372	430.51	0.30	0.59
<i>Alliaria petiolata</i> × <i>Lumbricus terrestris</i>	1	27 984	433.18	2.58	0.12
AC × <i>Lumbricus terrestris</i>	1	27 782	432.85	2.29	0.14
<i>Alliaria petiolata</i> × AC × <i>Lumbricus terrestris</i>	1	27 562	432.49	1.98	0.17

Table 4. *Podophyllum peltatum* photosynthesis rate (A_{max}) as a function of *Alliaria petiolata* and activated carbon experimental treatments. Minimal model chosen by AIC. Earthworm presence/absence was dropped from the model as this treatment had no effect on photosynthetic rate.

	DF	Deviance	AIC	F-value	P-value
<i>Alliaria petiolata</i>	1	0.75	184.97	1.23	0.27
Activated carbon (AC)	1	0.71	184.85	1.12	0.30
<i>Alliaria petiolata</i> × AC	1	1.07	187.05	3.23	0.08

(Mooney and Hobbs 2000). We suggest that field experiments that manipulate multiple stressors simultaneously, including nutrient deposition and multiple invaders, are needed to fully understand multi-invader interactions and their context-dependence. Our data suggests that even plants with positive physiological responses, like increased CO_2 drawdown in the leaf with increased nitrogen availability, are not able to compensate for the harm caused by multiple invaders.

Supporting Information

The following additional information is available in the online version of this article—

Table S1. Three separate linear models testing the effects of soil nutrients on the percent cover of *Alliaria petiolata* in observational field data.

Table S2. The interaction between invasive *Alliaria petiolata* and invasive earthworms on soil nitrogen, phosphorus 1 and phosphorus 2 in observational field data.

Table S3. Contrasts for *Alliaria petiolata* effect, contrasting *Alliaria petiolata* presence to *Alliaria petiolata* absence treatments, either in the presence or absence of *Lumbricus terrestris* earthworms and activated carbon (AC) in the soil.

Table S4. Contrasts for earthworm effect, contrasting earthworm presence to earthworm absence treatments, either in the presence or absence of *Alliaria petiolata* and activated carbon in the soil.

Figure S1. Experimental *Podophyllum peltatum* plants, in pots with *Alliaria petiolata* leaves added.

Figure S2. The interaction between soil nitrogen and *Alliaria petiolata* presence correlated with *Podophyllum peltatum* cover in the 187 observational field plots (Table 1).

Figure S3. Supplemental field data.

Sources of Funding

This research was funded by National Science Foundation (DEB 1 250 170 to J.H.B.), The Ohio Invasive Plant Council (to C.G.C.), Case Western Reserve University's (CWRU) SOURCE fellowship and CWRU's Department of Biology SPUR program (A.S.F.).

Authors' contributions

Conceptualization, C.G.C. and J.H.B.; methodology, C.G.C., J.E.M., J.S.M. and J.H.B.; formal analysis, C.G.C. and J.H.B.; data curation, C.G.C., C.E.H., J.H.B. and S.R.E.; writing—original draft preparation, C.G.C. and A.S.F.; writing—review and editing, C.G.C., J.E.M. and J.H.B.; supervision, J.H.B.; funding acquisition, C.G.C. and J.H.B.

Conflicts of Interest

The authors declare no conflict of interests.

Acknowledgements

We would like to thank Squire Valleevue and Valley Ridge Farms for providing the space and assistance necessary to perform this experiment, especially C. Bond, S. Brown and A. Locci. We would also like to thank the Holden Arboretum for use of their lab space and Li-Cor and D. Burke for discussion of the ideas presented within this paper. We also thank Cleveland Metroparks for access to their PCAP dataset. Thanks also to Rebecca Drenovsky at John Carroll University for the lab space to do the nutrient analyses and help with the analysis of the nutrient data. We thank Colin Orians and two anonymous reviewers for comments that improved the manuscript.

Data availability

Data are available at the Open Science Framework. Doi: 10.17605/OSF.IO/FTBYG

Code availability

DOI 10.17605/OSF.IO/FTBYG

References

- Barto EK, Cipollini D. 2009. Density-dependent phytotoxicity of *impatiens pallida* plants exposed to extracts of *Alliaria petiolata*. *Journal of Chemical Ecology* 35:495–504.

- Bialic-Murphy L, Brouwer NL, Kalisz S. 2020. Direct effects of a non-native invader erode native plant fitness in the forest understory. *Journal of Ecology* 108:189–198.
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS. 2008. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution* 24:127–135.
- Braga RR, Gómez-Aparicio L, Heger T, Vitule JRS, Jeschke JM. 2018. Structuring evidence for invasional meltdown: broad support but with biases and gaps. *Biological Invasions* 20:923–936.
- Braga RR, Ribeiro VM, Padial AA, Thomaz SM, Affonso IP, Wojciechowski J, dos Santos Ribas LG, Cunha ER, Tiburcio VG. 2019. Invasional meltdown: an experimental test and a framework to distinguish synergistic, additive, and antagonistic effects. *Hydrobiologia* 847: 1603–1618.
- Brodribb TJ, Feild TS, Jordan GJ. 2007. Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology* 144:1890–1898.
- Brouwer NL, Hale AN, Kalisz S. 2015. Mutualism-disrupting allelopathic invader drives carbon stress and vital rate decline in a forest perennial herb. *Aob Plants* 7:plv014.
- Callaway RM, Aschehoug ET. 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science (New York, N.Y.)* 290:521–523.
- Cantor A, Hale A, Aaron J, Traw MB, Kalisz S. 2011. Low allelochemical concentrations detected in garlic mustard-invaded forest soils inhibit fungal growth and AMF spore germination. *Biological Invasions* 13:3015–3025.
- Cipollini K, Bohrer MG. 2016. Comparison of allelopathic effects of five invasive species on two native species. *Journal of the Torrey Botanical Society* 143:427–436.
- Cipollini D, Cipollini K. 2016. A review of garlic mustard (*Alliaria petiolata*, Brassicaceae) as an allelopathic plant. *Journal of the Torrey Botanical Society* 143:339–348.
- Cipollini K, Titus K, Wagner C. 2012. Allelopathic effects of invasive species (*Alliaria petiolata*, *Lonicera maackii*, *Ranunculus ficaria*) in the Midwestern United States. *Allelopathy Journal* 29:63–76.
- Costello DM, Lamberti GA. 2009. Biological and physical effects of non-native earthworms on nitrogen cycling in riparian soils. *Soil Biology & Biochemistry* 41:2230–2235.
- Craven D, Thakur MP, Cameron EK, Frelich LE, Beauséjour R, Blair RB, Blossey B, Burtis J, Choi A, Dávalos A, Fahey TJ, Fisichelli NA, Gibson K, Handa IT, Hopfensperger K, Loss SR, Nuzzo V, Maerz JC, Sackett T, Scharenbroch BC, Smith SM, Vellend M, Umek LG, Eisenhauer N. 2017. 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.
- Cushman JH, Gaffney KA. 2010. Community-level consequences of invasion: impacts of exotic clonal plants on riparian vegetation. *Biological Invasions* 12:2765–2776.
- Davis MA, Anderson MD, Bock-Brownstein L, Staudenmaier A, Suliteanu M, Wareham A, Dosch JJ. 2015. Little evidence of native and non-native species influencing one another's abundance and distribution in the herb layer of an oak woodland. *Journal of Vegetation Science* 26:1005–1012.
- Edwards CA. 2004. *Earthworm ecology*. Boca Raton, FL: CRC Press.
- Eisenhauer N, Partsch S, Parkinson D, Scheu S. 2007. Invasion of a deciduous forest by earthworms: changes in soil chemistry, microflora, microarthropods and vegetation. *Soil Biology and Biochemistry* 39:1099–1110.
- Fahey TJ, Yavitt JB, Sherman RE, Maerz JC, Groffman PM, Fisk MC, Bohlen PJ. 2013. Earthworm effects on the incorporation of litter C and N into soil organic matter in a sugar maple forest. *Ecological Applications: a Publication of the Ecological Society of America* 23:1185–1201.
- Fowler D, Coyle M, Skiba U, Sutton MA, Cape JN, Reis S, Sheppard LJ, Jenkins A, Grizzetti B, Galloway JN, Vitousek P, Leach A, Bouwman AF, Butterbach-Bahl K, Dentener F, Stevenson D, Amann M, Voss M. 2013. The global nitrogen cycle in the twenty-first century. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 368:20130164.
- Green PT, O'Dowd DJ, Abbott KL, Jeffery M, Retallick K, Mac Nally R. 2011. Invasional meltdown: invader-invader mutualism facilitates a secondary invasion. *Ecology* 92:1758–1768.
- Hale AN, Kalisz S. 2012. Perspectives on allelopathic disruption of plant mutualisms: a framework for individual- and population-level fitness consequences. *Plant Ecology* 213:1991–2006.
- Hale AN, Lapointe L, Kalisz S. 2016. Invader disruption of belowground plant mutualisms reduces carbon acquisition and alters allocation patterns in a native forest herb. *The New Phytologist* 209:542–549.
- Hale AN, Tonsor SJ, Kalisz S. 2011. Testing the mutualism disruption hypothesis: physiological mechanisms for invasion of intact perennial plant communities. *Ecosphere* 2:15.
- Herr DG. 1986. On the History of ANOVA in unbalanced, factorial designs: the first 30 years. *The American Statistician* 40:265–270.
- IPCC CWT. 2014. Climate change 2014: synthesis report. In: Core Writing Team, Pachauri RK, Meyer LA, eds. *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Geneva, Switzerland: IPCC.
- Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* 135:575–586.
- Khasanova A, James JJ, Drenovsky RE. 2013. Impacts of drought on plant water relations and nitrogen nutrition in dryland perennial grasses. *Plant and Soil* 372:541–552.
- Kuebbing SE, Nuñez MA. 2015. Negative, neutral, and positive interactions among nonnative plants: patterns, processes, and management implications. *Global Change Biology* 21:926–934.
- Lau JA, Puliafico KP, Kopshever JA, Steltzer H, Jarvis EP, Schwarzländer M, Strauss SY, Hufbauer RA. 2008. Inference of allelopathy is complicated by effects of activated carbon on plant growth. *The New Phytologist* 178:412–423.
- Loss SR, Hueffmeier RM, Hale CM, Host GE, Sjerven G, Frelich LE. 2013. Earthworm Invasions in Northern Hardwood forests: a rapid assessment method. *Natural Areas Journal* 33:21–30.
- McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990. A new method which gives and objective-measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytologist* 115: 495–501.
- Milton SJ, Wilson JR, Richardson DM, Seymour CL, Dean WRJ, Iponga DM, Proches S. 2007. Invasive alien plants infiltrate bird-mediated shrub nucleation processes in arid Savanna. *Journal of Ecology* 95:648–661.
- Mohammadi K, Khalesro S, Sohrabi Y, Heidari G. 2011. A review: beneficial effects of the mycorrhizal fungi for plant growth. *Journal of Applied Environmental and Biological Sciences* 1:310–319.
- Mooney H, Hobbs R. 2000. *Invasive species in a changing world*. Washington, D.C.: Island Press.
- Nuzzo VA. 1993. Distribution and spread of the invasive biennial *Alliaria petiolata* (Bieb. [Cavara and Grande]) in North America. In: McKnight BL, ed. *Biological pollution: control and impact of invasive exotic species*. Indianapolis: Indiana Academy of Science.
- Nuzzo VA, Maerz JC, Blossey B. 2009. Earthworm invasion as the driving force behind plant invasion and community change in northeastern North American forests. *Conservation Biology: the Journal of the Society for Conservation Biology* 23:966–974.
- Peet RK, Wentworth TR, Schafale MP, Weakley AS. 2004. *Carolina vegetation survey database. version 3.0 ed*. Chapel Hill: North Carolina Botanical Garden.
- Prati D, Bossdorf O. 2004. Allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae). *American Journal of Botany* 91:285–288.
- R Core Development Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rodgers VL, Wolfe BE, Werden LK, Finzi AC. 2008. The invasive species *Alliaria petiolata* (garlic mustard) increases soil nutrient availability in northern hardwood-conifer forests. *Oecologia* 157:459–471.
- Rychter AM, Rao IM. 2005. Role of phosphorus in photosynthetic carbon metabolism. In: Pessarakli M, ed. *Handbook of photosynthesis*, 2nd edn. Boca Raton: CRC Press.
- Shaben J, Myers JH. 2010. Relationships between Scotch broom (*Cytisus scoparius*), soil nutrients, and plant diversity in the Garry oak savannah ecosystem. *Plant Ecology* 207:81–91.
- Simberloff D, Von Holle B. 1999. Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions* 1:21–32.
- Soudzilovskaia NA, Vaessen S, Barcelo M, He J, Rahimlou S, Abarenkov K, Brundrett MC, Gomes S, Merckx V, Martinez-Suz L, Tedersoo L. 2020. Taxon occurrence data for the FungalRoot database. In: Pluto F, ed. *Occurrence dataset Pluto F*. doi:10.15468/a7ujmj.

- Spreitzer RJ, Salvucci ME. 2002. Rubisco: structure, regulatory interactions, and possibilities for a better enzyme. *Annual Review of Plant Biology* 53:449–475.
- Stevens, DL, Jr. Olsen AR. 2003. Variance estimation for spatially balanced samples of environmental resources. *Environmetrics* 14:593–610.
- Tandon HLS, Motto HL, Kurtz LT. 1967. Composition of dilute-acid-fluoride (Bray P1) extracts of Illinois soils. *Soil Science Society of America Proceedings* 31:168–171.
- Templer P. 2013. Limits on carbon uptake by plants. *Nature Climate Change* 3:184–185.
- Venables WN, Ripley BD. 2002. *Modern Applied Statistics with S*. New York: Springer.
- von Caemmerer S, Quick WP. 2000. Rubisco: physiology in vivo. In: Leegood RC, Sharkey TD, von Caemmerer S, eds. *Photosynthesis: physiology and metabolism*. The Netherlands: Kluwer Academic Publishers.
- Weissshuhn K, Prati D. 2009. Activated carbon may have undesired side effects for testing allelopathy in invasive plants. *Basic and Applied Ecology* 10:500–507.
- Wolfe BE, Rodgers VL, Stinson KA, Pringle A. 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. *Journal of Ecology* 96:777–783.
- Yang S, Ferrari MJ, Shea K. 2011. Pollinator behavior mediates negative interactions between two congeneric invasive plant species. *The American Naturalist* 177:110–118.
- Zelles AM. 2012. Examining the relationship between garlic mustard (*Alliaria petiolata*) and European earthworms. CORE Scholar, Masters of Science, Wright State University.
- Zicsi A, Szlavecz K, Csuzdic C. 2011. Leaf litter acceptance and cast deposition by peregrine and endemic European lumbricids (Oligochaeta: Lumbricidae). *Pedobiologia* 54S:S145–S152.