



Plant invader alters soil food web via changes to fungal resources

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Abstract

While aboveground impacts of invasive plants are well documented, their influence on soil food webs remains less understood. Previous research has revealed that bottom-up forces are widespread in soil food webs of woodlands. Thus, an invasive plant that negatively impacts the base of the food web will likely decrease primary consumers as well as their predators. We examined how a North American plant invader, garlic mustard (*Alliaria petiolata*), affects arthropod primary (springtails and oribatid mites) and secondary (predaceous mites) consumers of the soil food web via changes to fungal resources. We measured the abundances of plants, soil fungi, fungivores, and predators in garlic mustard-invaded and uninvaded 1-m² plots in five Midwestern USA woodlands. We then conducted a mesocosm (0.25-m² plots) experiment to tease apart the direct and indirect effects of garlic mustard by manipulating plant identity (garlic mustard vs. native plant), soil history (invaded vs. uninvaded), and fungicide application (fungicide vs. no fungicide). Our first study revealed that plots without garlic mustard had 2.8 and 1.4 × more fungi and fungivores, respectively. Predator densities did not differ. Fungal composition and structural equation modeling (SEM) revealed the garlic mustard effects on fungivores were correlated with fungal declines. The mesocosm experiment confirmed that the impacts were indirect, as fungicide plots harbored similar fungivore densities, whereas fungivore densities differed according to plant identity and soil history in the fungicide-free plots. Our results reveal that by altering soil fungal abundance, an invasive plant can indirectly affect primary consumers in soil food webs, but this indirect effect does not influence predators.

Keywords Arthropods · Food webs · Fungi · Garlic mustard · Invasive plants

Introduction

Invasive plants create a wide range of novel interactions with native species when they invade an ecosystem. Most documented interactions are direct, but invasive plants can also indirectly influence native communities by altering

intermediary species in terrestrial food webs (Lau 2013). Altered indirect effects underpin some of the most significant changes that biological invasions can have on invaded ecosystems, with repercussions for diversity and community composition (Vilá et al. 2011). For example, many plant invaders indirectly affect arthropod herbivores and their predators by reducing resource quality of the invaded habitat by displacing native vegetation (Gerber et al. 2008; Bezemer et al. 2014). Invasive plants can also increase top-down pressure of predators on prey by providing more structural habitat, e.g., for web-building spiders (Pearson 2009), thereby limiting herbivore damage. Improved mechanistic understanding of how such indirect effects propagate through food webs will strengthen management efforts to minimize the adverse impacts of invasive plants on terrestrial communities.

Invasive plants likely impose substantial direct and indirect effects on the soil system, which provides habitat for numerous organisms and is critical in carbon storage and nutrient cycling (Coleman and Crossley 2003). As one of

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the most diverse networks in terrestrial ecosystems (Bardgett 2005), the soil food web includes microbes (bacteria and fungi) and micro-arthropods such as mites (Acari) and springtails (Collembola). Soil microbes are involved in regulating nutrient cycling and soil formation (Coleman and Crossley 2003), and as primary decomposers, are critical basal organisms in the soil food web (Bardgett and Wardle 2010). Soil micro-arthropods feed extensively on fungi and detritus, thus directly and indirectly influencing rates of decomposition and nutrient cycling (Ayres et al. 2009). Fungivorous micro-arthropods, because of their small size and high abundance, are major prey for predators such as mesostigmatid mites (Coleman and Hendrix 2000; Moore et al. 2003).

Invasive plants can indirectly affect soil food webs by excreting toxic allelochemicals, thereby changing the abundance and diversity of soil microbes. For instance, high densities of the aggressive North American plant invader *Centaurea maculosa* (spotted knapweed) can decrease soil fungi by releasing toxic chemicals (Ridenour and Callaway 2001). Similarly, in mesocosm experiments, the invasive *Carduus nutans* (musk thistle) limited the number of nodules and N-fixing capabilities of the native *Trifolium repens* by adversely affecting soil bacterial communities (Wardle et al. 1994). Other research has found that invasive plants decreased the abundances of mutualistic soil microbes, leading to changes in native plant abundance (Callaway et al. 2008). Since many soil animals rely on microbes as a food source (Scheu and Schaefer 1998), any declines in microbial communities resulting from plant invasion could impact primary and secondary consumers in the soil (Harkes et al. 2017; Abgrall et al. 2018). However, few studies have examined how decreases in soil microbial communities via plant invasion can affect soil food webs.

Garlic mustard (*Alliaria petiolata*), an invasive biennial herb that can negatively influence soil fungal communities, has been invading North America forests since the 1800s (Anderson et al. 1996). Garlic mustard invades a gradient of forest ecosystems, from mesic deciduous forests with low to full sunlight, to sandy-upland woodlands (Morris et al. 2012). Garlic mustard-infested stands can reach densities > 250 adults per m² (Nuzzo 1999), causing direct changes to soil communities via alterations of the physical environment. Furthermore, like other plants within the Brassicaceae (mustard) family, garlic mustard produces secondary compounds (glucosinolates) that are toxic to soil fungi, resulting in a competitive advantage over native plants that form mutualisms with mycorrhizal fungi (Roberts and Anderson 2001; McCary et al. 2019). Glucosinolates released from garlic mustard tissue reduce populations of both arbuscular mycorrhizae (Stinson et al. 2006) and ectomycorrhizal fungi (Wolfe et al. 2008). Given that soil fungi represent a critical resource for many soil micro-arthropods

(Newell 1984; Scheu and Simmerling 2004), the suppression of mycorrhizal fungi by garlic mustard will likely have cascading effects through the soil food web.

Previous research has shown that garlic mustard can affect arthropod primary and secondary consumers in forest ecosystems (deHart and Strand 2012; Alerding and Hunter 2013; Warrix et al. 2015), although these studies did not assess fungal abundance or composition. Furthermore, their findings are contradictory. For example, deHart and Strand (2012) examined isotopic ratios among soil arthropods in garlic mustard-invaded and uninvaded woodlands and found that predators in invaded areas were generally depleted of $\delta^{13}\text{C}$ and enriched with $\delta^{15}\text{N}$, suggesting that predators switched from fungivorous springtails to alternative prey resources. Their results indicate that predators shifted diets to compensate for reduced springtail densities in garlic mustard patches, though prey densities were not measured. In contrast, Alerding and Hunter (2013) found that in a forest recently invaded by garlic mustard, springtail abundance was 3 × higher compared to an adjacent uninvaded forest. Lastly, Warrix et al. (2015) examined garlic mustard effects on litter arthropods in a hardwood forest and found that invaded areas had lower arthropod richness than uninvaded patches, but overall arthropod abundance and Shannon diversity did not differ. Since these three studies were field surveys with limited replication, it is difficult to explain the contradictory results. To help resolve these inconsistent findings, and to better understand garlic mustard's effects on micro-arthropod fungivores and predators in the soil food web, we conducted a mensurative experiment across multiple sites combined with a complementary manipulative mesocosm experiment.

In the research reported here, we examined how garlic mustard affects soil food webs via changes to belowground fungal resources. For 2 years, we measured the responses of plants, soil fungi, and arthropod fungivores and predators to garlic mustard invasion. Two questions were addressed: (1) how does garlic mustard alter the soil fungal-based food web in woodland ecosystems? (2) Are garlic mustard-induced changes to higher trophic levels of the soil food web an indirect result of a reduction in soil fungi? To tackle the central questions of this research, we set up a mensurative experiment that measured how the presence/absence of garlic mustard correlated with the abundances of soil fungi and higher arthropod trophic groups. We then conducted a manipulative mesocosm experiment to tease apart the direct and indirect effects of garlic mustard on the soil food web by introducing garlic mustard to field plots. Since bottom-up control processes are strong in soil food webs of forests (Scheu and Schaefer 1998; Chen and Wise 1999), we predicted that garlic mustard invasion would decrease densities of soil fungi, fungivores, and predators, with effect size attenuating up the food chain [Prediction 1]. We further predicted that the

negative effects of garlic mustard on fungivores and predators would be indirect, i.e., due to garlic mustard's negative impact on fungi [Prediction 2].

Methods

Study 1: mensurative field study

Study sites Five separate oak-dominated (*Quercus alba* or *Q. rubra*) woodlands (each > 3 km apart) were sampled during the summers of 2014 and 2015. Soils ranged from silt loam to silty clay loam (NRCS 2016), and each site had been invaded by garlic mustard for at least 5 years before the study was initiated (R. London, S. Kobal, T. Simpson, pers. comm.). Within a designated area of each site (~ 1 ha), ten 1-m² plots were established: five plots in areas invaded by garlic mustard (hereafter referred to as “invaded”) and five plots in adjacent areas without evidence of garlic mustard invasion (hereafter referred to as the “control”). Invaded plots ($n = 5$ per site) were areas where garlic mustard covered the majority of the 1-m² plot and contained at least 50 flowering stems, though garlic mustard densities varied among the five sites (Supplementary Materials Appendix S1 Table S1). Control plots ($n = 5$ per site) contained herbaceous and shrub species (not all natives) but had no garlic mustard. To minimize major differences in soil characteristics, control plots were placed between 5 and 30 m away from the nearest invaded plot, with each control plot being at least 5 m from another plot. No management or restoration activities were conducted during the study. Refer to Appendix S1 for the experimental layout and full details on each site.

Vegetation survey Cover of garlic mustard (as second-year plants), bare ground, leaf litter, shrubs, trees, and herbaceous plants was each expressed using the Daubenmire cover scale (Daubenmire 1959) (1, < 1%; 2, 2–5%; 3, 6–25%; 4, 26–50%; 5, 51–75%; 6, 76–95%; 7, > 96%). Garlic mustard second-year plants were used as the metric for determining invasion severity because studies have suggested that reduction of soil fungi is most pronounced during the second-year phase (e.g., Rodgers et al. 2008 and Wolfe et al. 2008). Vegetation surveys were conducted in July of 2014 and 2015.

Extraction of fungal hyphae Since arthropod fungivores graze on extraradical mycelia for food (Rusek 1998), measuring soil fungal lengths can provide an estimate of resource availability. Therefore, in July and August of 2014 and 2015, soil cores [5 cm in depth and diameter (98 cm³)] were collected from each plot to estimate soil fungal hyphae, which were extracted using an aqueous extraction/filtration method (Sylvia 1992). Arbuscular, ectomycorrhizal, and saprophytic fungi could not be morphologically differentiated using this technique. From each core, 5 g of air-dried soil

was added to a 100 mL of 5% sodium hexametaphosphate solution (39.5 g/L of DI water), shaken by hand, and sifted through sieve sizes of 500 and 45 μm . Water-suspended hyphae (10 mL) were then vacuum-filtered onto a 0.2- μm nitrocellulose filter (Merck Millipore Ltd., Tullagreen, Carrigtwohill, Ireland) and mounted on microscope slides using a polyvinyl-lactoglycerol (PVLG) solution. After drying for 48 h at 20 °C, hyphal lengths (m/g dry soil) were calculated using the grid-intersect method under 200 \times magnification (Newman 1966).

Molecular analyses of fungal community structure In August of 2015 (the end of the study), additional soil cores were collected to evaluate fungal community composition in each plot. DNA was extracted from soil cores using MoBio PowerSoil extraction kits (Carlsbad, CA, USA). We used fungal primers (FF390 and FR1) to target the 18S rRNA region of the genome (Vainio and Hantula 2000). PCR amplification was then performed as described by Green et al. (2015) and the resulting products were sequenced at the University of Illinois at Chicago DNA Services Facility on the Illumina MiSeq platform using standard v3 chemistry. The software package QIIME v1.8 was used to generate operational taxonomic units (OTU) and taxonomic summaries (Caporaso et al. 2010). OTU clusters were created de novo using the UCLUST algorithm with a 97% similarity threshold (Edgar 2010). Fungal OTUs were annotated against the Silva 119 database (Quast et al. 2013); summaries of absolute abundances of taxa were calculated for all phyla, classes, orders, families, genera, and species (Caporaso et al. 2010). While analysis of the 18S rRNA region is informative for describing differences in fungal composition, it does not precisely discriminate between ectomycorrhizal and saprophytic fungal guilds. Thus, we used family level identification because it provided the most fungal taxonomic information without a high level of uncertainty. Refer to Appendix S2 for the full details on the methodology used to characterize soil fungal composition.

Arthropod sampling Arthropods were sampled with 98-cm³ soil cores the same days that soil for hyphal extractions was collected (July and August of 2014 and 2015). Only the soil horizons beneath the litter layer were sampled because leaf litter was sparse across the sites.

Densities of soil fungivores (Collembola and Oribatida) and predators (predatory mites [Mesostigmata and Prostigmata]) were assessed by extracting them from each core using Berlese–Tullgren funnels. Springtails (Entognatha, Collembola) and oribatid mites (Acari, Oribatida) were identified as arthropod fungivores because research has extensively demonstrated that these taxa eat soil fungal hyphae (Lenoir et al. 2007; Crotty and Adl 2019). These arthropod fungivores are important prey for mesostigmatid (Acari, Mesostigmata) and prostigmatid (Acari, Prostigmata) mites (Coleman et al. 1999; Lenoir et al. 2007; Shao et al. 2015);

however, these predators are also known to be generalists, preying on nematodes and insect larva as well (Moore et al. 2003; Bardgett and Wardle 2010). Following 72-h extractions, all target taxa were preserved in 70% ethanol, identified to the lowest taxonomic level (from order to family depending on the organism), and counted. In this study, we collected a total of 3550 arthropods, in which oribatids were the most abundant taxon (56% of the collection). The second most abundant group, mesostigmatid mites, represented 15% of the sampled arthropods. Refer to Appendix S3 Table S1 for full details on the arthropod collection.

Statistical analyses Fungal hyphal lengths, and fungivore and predator densities were analyzed using linear mixed-effects models (LMMs; Zuur et al. 2009) with three fixed factors: (1) Treatment [control vs. invaded] (2) Month [July and August], and (3) Year [2014 and 2015]. We also included a Treatment \times Month and a Treatment \times Year interaction to evaluate if garlic mustard effects were consistent during the experiment. Since we were mostly interested in treatment impacts across years, we did not include a Year \times Month interaction in our models. The random effects included plot nested in site to account for the repeated sampling of plots, and a site effect to account for variability across the five study sites. To mitigate the influence of extreme values and not violate normality assumptions, all data were 4th-root transformed. LMMs were performed using the “nlme” package in R (R Development Core Team 2018). See Appendix S3 for alternative model fits, such as the use of generalized mixed-effects models (Table S2); all analyses gave broadly similar results.

Permutational analysis of variance (PERMANOVA: 9999 permutations; Type III SS) and principal coordinate analysis (PCO) were used to evaluate differences in fungal composition between invaded and control plots using arthropods as vector overlays (Pearson’s correlation; Anderson et al. 2008). Fungal OTU’s data were standardized to relative abundances, 4th-root transformed, and then a distance matrix was calculated using the Bray–Curtis dissimilarity measure. PERMANOVAs and PCOs were conducted using PRIMER-E/PERMANOVA + software (Anderson et al. 2008).

Structural equation modeling (SEM) was used to distinguish between direct and indirect pathways of the impact of garlic mustard in the soil food web (Grace 2006). We employed piecewise SEM (Lefcheck 2016), which allows the joining of multiple linear mixed-effects models into one global SEM. As such, piecewise SEM can include random effects and variance structures that are characteristic of linear mixed-effects models. We initially built a hypothesized a priori structural model containing variables for garlic mustard (i.e., GM stem density per m^2), plant community (i.e., herbaceous plant cover), fungivores (sums of densities of springtails and fungivorous mites per m^2) and predators

(sums of predaceous mite densities per m^2) using LMMs (Fig. 1). Several competing models were also constructed with the same basic structure but with altered pathways that were biologically realistic. To evaluate the overall fit of the SEMs, Shipley’s test of d-separation (Shipley 2013) was performed; P values derived from Fisher’s C test statistic that were >0.05 indicated adequate model fits (Shipley 2013). Since several SEMs (including our a priori model) had acceptable model fits and were within 2 AIC units of each other (see Appendix S3), we only report on the SEM associated with our initial hypothesized structural model. We estimated indirect effects by multiplying the direct path coefficients between variables of interest. Before conducting SEMs, hyphal lengths, and fungivore and predator densities were 4th-root transformed to meet parametric requirements of homoscedasticity of errors; no violations occurred following data transformations. Piecewise SEM was performed using the “piecewiseSEM” package in R (Lefcheck 2016, R Development Core Team 2018).

Study 2: manipulative mesocosm experiment

To uncover potential mechanisms causing patterns revealed in Study 1, a 2-year manipulative experiment was performed in a pin oak (*Quercus palustris*) woodland at the Morton Arboretum, IL (41°48’50.94N, 88°4’16.17W). We designed the experiment to determine if (1) densities of hyphae and arthropods were lower in the garlic mustard plots than uninvaded plots of Study 1 because of conditions not caused by garlic mustard; (2) to establish whether garlic mustard reduced soil fungi in the sites of Study 1, or if garlic mustard instead tended to invade soils with lower hyphal densities; and (3) to separate direct effects of garlic mustard from indirect effects (i.e., via effects on fungi) on soil arthropods.

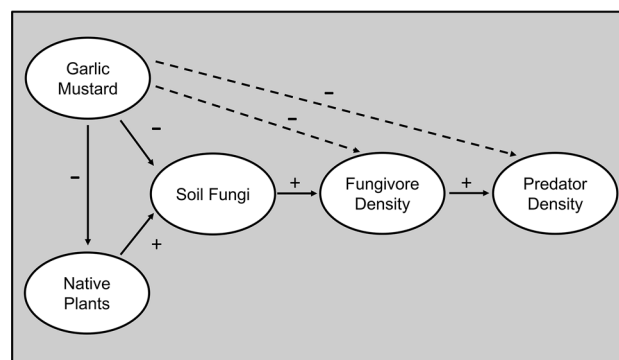


Fig. 1 Hypothesized structural model showing how garlic mustard may affect soil arthropod abundance both directly and indirectly. Solid lines represent direct effects, and dashed lines illustrate indirect effects. Plus (+) or minus (-) symbols represent the hypothesized effect of one variable on another

Each plot (“mesocosm”) was created by first removing soil to a depth of 0.25 m from a 0.5 m × 0.5-m area. Weed Barrier® Pro Landscape Fabric (Home Depot, Atlanta, GA, USA) was then placed in the bottom of each plot to separate underlying soil from the soil that would be added (see below) while still allowing for drainage. Aluminum sheeting was installed 22 cm into the soil around each plot with ~2.5 cm of metal exposed above the surface of the added soil. Plots were arranged in a 4 × 8 grid, with plots separated by 2.5 m within a row and rows 5-m apart. We added soil from a garlic mustard patch to half of the plots ($n = 16$), to the others we added soil from an area where no garlic mustard had grown. Plots were then planted with seedlings of either *Alliaria petiolata* (garlic mustard) or a native mycorrhizal biennial, *Lactuca floridana* (blue lettuce). Throughout the experiment, half of the plots were treated with a fungicide. Thus, the experiment had a fully crossed 2 × 2 × 2 factorial design, with the three treatments (soil, plant, and fungicide) applied at random to four replicates of each treatment combination.

Soil treatment Garlic mustard-invaded soil was collected from Derwen Mawr Forest Preserve (Study 1), and uninvaded soil was collected from the same site where there was no record of garlic mustard invasion. The soils were hand-sifted to eliminate roots and large debris and then mixed gently by hand to minimize disturbance of the soil fauna. Soils were then added to each plot (~250 L per plot).

Plant treatment Seedlings of either *L. floridana* (grown at the University of Illinois at Chicago greenhouse) or garlic mustard (transported from Nippersink Canoe Base; see Study 1 above) were planted at a density of 10 individuals per 0.25 m². We used *L. floridana* as the native plant comparison because it is native to the Chicago region, has a 2-year life cycle like garlic mustard, and occupies habitats that garlic mustard invades. Seedlings were replaced if they died within the first month after planting; all plots maintained 10 plants per plot throughout the experiment.

Fungicide treatment To manipulate the relative abundance of fungi, fungicide 3336 WP (Cleary Chemical Corporation, Dayton, NJ, USA) was applied to half of the experimental plots. The fungal suppression treatment was applied every 3 weeks at a concentration of 1 g/L during the growing season (Wilson and Williamson 2008). In 2014, each plot received 0.5 L per application, which was doubled to 1 L in 2015 to limit fungal populations further. Previous research has failed to find any negative effects of Cleary’s fungicide 3336 WP on organisms other than fungi, including no toxic effects on nearby plants (Wilson and Williamson 2008). To serve as a control, treatments without fungicide application received 0.5 L of deionized water in 2014 and 1 L in 2015.

Sampling of soil biota Soil cores (3 cm in diameter and depth [21.2 cm³]) were taken from each plot while seedlings were being planted and before fungicide was added to survey

the soil biota before the experiment commenced. Four more sampling events then followed: July 2014, August 2014, May 2015, and July 2015. To measure fungal lengths, soil cores were collected to extract soil hyphae using an aqueous extraction/filtration method (Sylvia 1992). See Study 1 for details of the collection of fungal lengths.

Soil cores (without leaf litter) collected on the same day as fungal lengths were placed in Berlese-Tullgren funnels to extract fungivores [springtails (Collembola) and oribatid mites (Acari, Oribatida)] and predators [(predaceous mites (Acari, Mesostigmata and Prostigmata)]. All arthropods were counted and identified to the lowest possible taxonomic level (from order to family depending on the organism). In this study, we collected 1339 arthropods. Oribatids comprised the highest proportion (39%); the Collembolan family Isotomidae represented the second most abundant group (21%). Mesostigmatid mites comprised 5% of the entire collection. For full details on the arthropod collection, refer to Appendix S4 Table S1.

Statistical analysis Since garlic mustard has a biennial life cycle with effects on fungal hyphae most pronounced during the second year (Rodgers et al. 2008; Wolfe et al. 2008), we performed analyses of variance (ANOVAs) on densities of hyphae, fungivores, and predators for the final time point of the 2-year experiment. Fungivore and predator densities were square-root transformed to ensure appropriate model fits.

Results

Study 1: mensurative field study

Garlic mustard’s impact on fungal community structure and arthropod taxa Garlic mustard invasion affected fungal community composition on all sites (Fig. 2), although the treatment effect differed across sites [$P(\text{Treatment} \times \text{Site Pseudo-}F_{4, 39}) < 0.001$; Appendix S3 Table S3]. Fungal communities in control and invaded plots in West Campus woods were separated along Axis 1 of the PCO (Fig. 2a). Oribatid mites, the most abundant fungivore, were less abundant in the invaded plots (i.e., their vector was positively associated with the control plots; Fig. 2a). Similarly, fungal communities at Nippersink Canoe Base clearly differed between invaded and uninvaded (control) plots in ordination space, and oribatids also were more abundant in the uninvaded plots (Fig. 2b). Unlike the pattern for West Campus woods, the predatory mesostigmatid mites were also more numerous in control plots.

Garlic mustard also affected the fungal communities for the other three sites, but there was slightly more overlap between invaded and control plots, and relationships with arthropod taxa were not as consistent (Fig. 2c–e). For

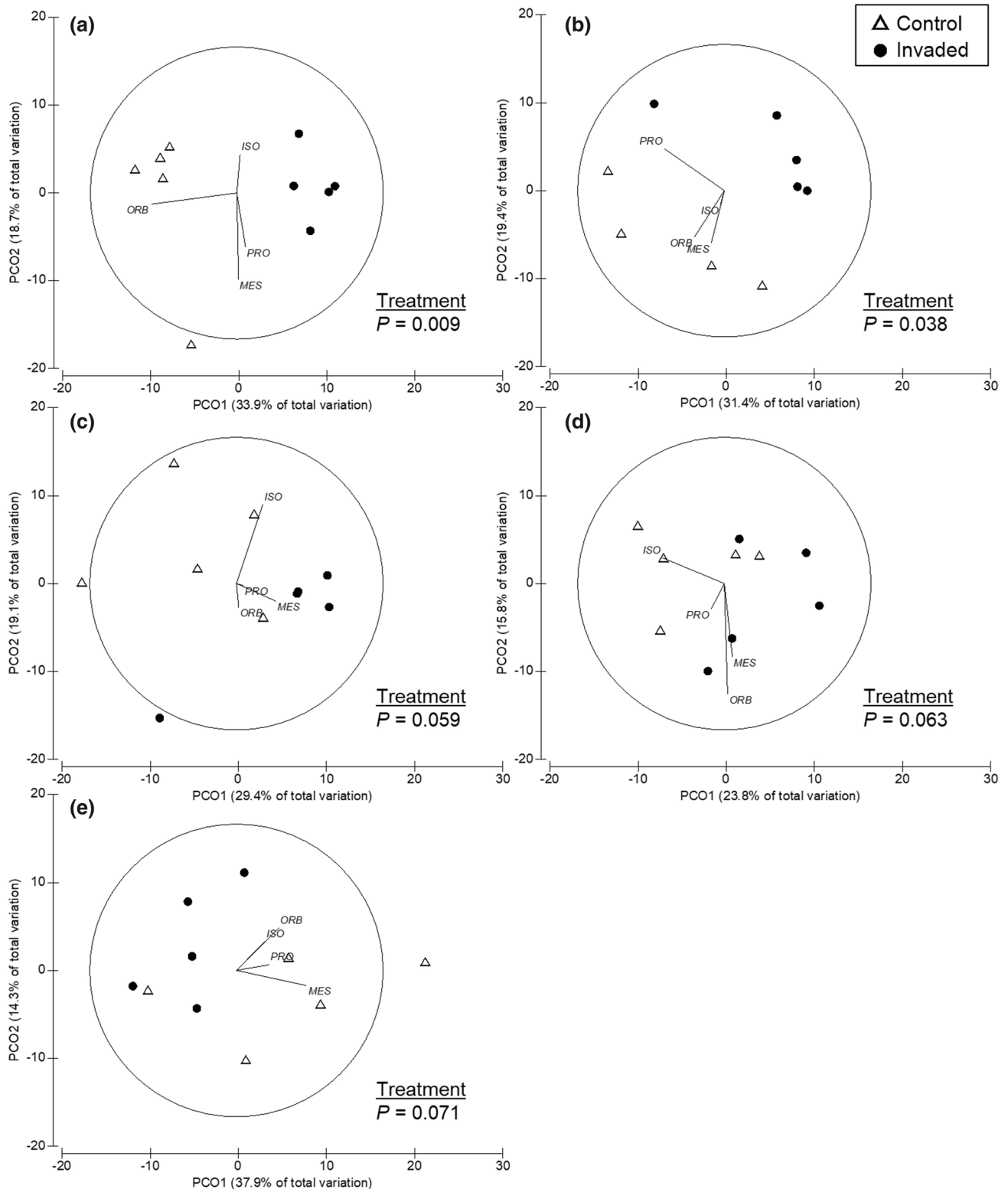


Fig. 2 Principal component analysis (PCO) of fungal community composition according to garlic mustard treatment for **a** West Campus, **b** Nippersink Canoe Base, **c** Pioneer Road, **d** Derwen Mawr, and **e** Greene Valley in Study 1. Patterns are presented separately by site because the effect of garlic mustard varied across sites [$P(\text{Treatment} \times \text{Site } Pseudo-F_{4,39}) < 0.001$; Appendix S3 Table S3]. Each symbol indicates one of the ten plots for each site. The length

of each vector represents the strength of the correlation of that taxon with the two axes (circle indicates a correlation coefficient of 1). The two most abundant fungivores (*ISO* Isotomidae, *ORB* Oribatida) and predators (*MES* Mesostigmata and *PRO* Prostigmata) are given. *P* values are for the treatment $Pseudo-F_{1,8 \text{ or } 9}$ from PERMANOVA (error *df* differ because fungal DNA could not be analyzed for some samples)

Pioneer Road woods, oribatids showed no correlation with the garlic mustard treatment, and mesostigmatid predators appeared to be more abundant in invaded, not control, plots (Fig. 2c). Oribatids, isotomid Collembola, and mesostigmatid predators exhibited variable patterns of correlation with control and invaded plots for Derwen Mawr and Greene Valley (Fig. 2d, e). All three groups were weakly positively correlated with three or four of the control plots for Greene Valley (Fig. 2e), whereas they showed no clear pattern with uninvaded and invaded plots at Derwen Mawr (Fig. 2d).

Impact of garlic mustard on the three trophic levels
 No trophic level exhibited an interaction between Treatment and time ($P > 0.2$ for Treatment \times Month or Treatment \times Year), suggesting garlic mustard effects were consistent throughout the study (Table 1). Fungal hyphae were $2.8 \times$ higher in uninvaded plots (LMM, $F_{1,92} = 11.53$, $P = 0.001$, Fig. 3a). Arthropod fungivores were $1.4 \times$ more abundant in uninvaded plots than the invaded plots (LMM, $F_{1,92} = 4.54$, $P = 0.04$, Fig. 3b). In contrast, predator densities did not differ between invaded and uninvaded plots (LMM, $F_{1,92} = 0.02$, $P = 0.87$, Fig. 3c).

Direct and indirect effects of garlic mustard on the soil food web
 The use of path-related networks demonstrated that garlic mustard influenced the soil food web. Our a priori SEM was a good fit for the collected data (Fisher's $C = 10.28$, $P = 0.42$, Fig. 4). The SEM indicates garlic mustard had a strong negative direct effect on herbaceous plant

Table 1 Results from the linear mixed-effects models testing for the main and interactive effects of Treatment, Month, and Year on (a) soil fungal length, (b) fungivore density, and (c) predator density in Study 1

Factor	Residual <i>df</i>	<i>F</i>	<i>P</i>
<i>A. Hyphal lengths</i>			
Treatment	92	11.53	0.001
Month	97	2.85	0.095
Year	92	10.74	0.002
Treatment \times Month	97	1.83	0.179
Treatment \times Year	92	1.63	0.204
<i>B. Fungivores</i>			
Treatment	92	4.54	0.036
Month	97	0.19	0.662
Year	92	6.44	0.013
Treatment \times Month	97	0.97	0.326
Treatment \times Year	92	0.12	0.730
<i>C. Predators</i>			
Treatment	92	0.02	0.872
Month	97	2.42	0.123
Year	92	3.75	0.056
Treatment \times Month	97	0.90	0.345
Treatment \times Year	92	0.00	0.976

Numerator *df* = 1 for all treatments

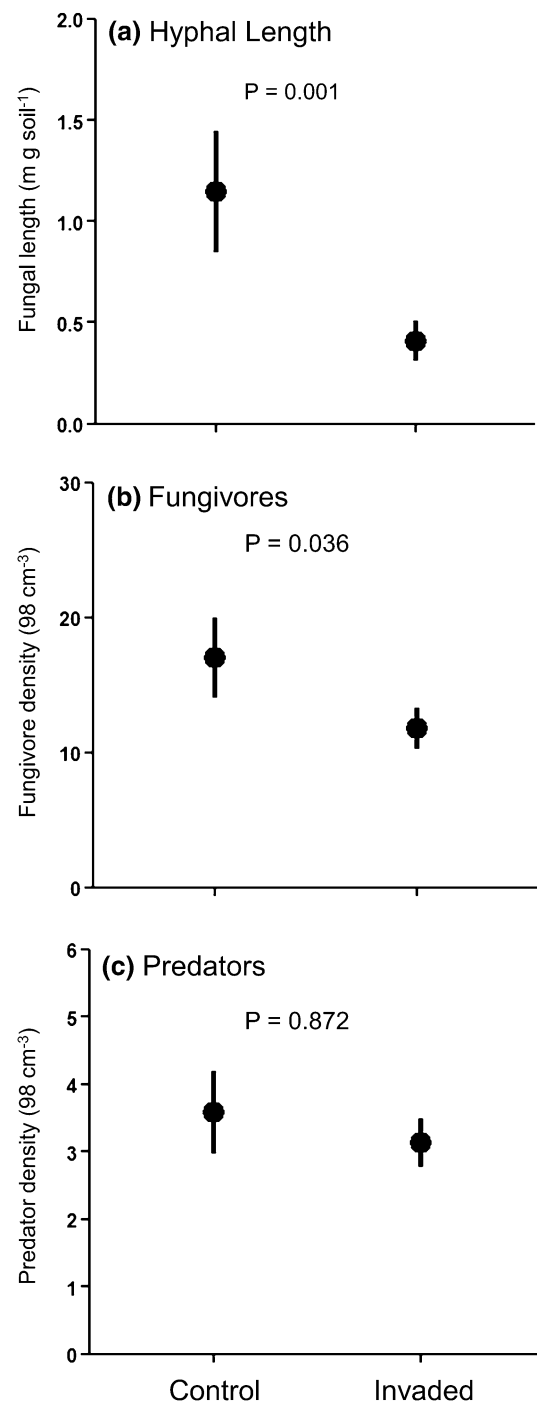


Fig. 3 The influence of garlic mustard invasion on **a** soil hyphal length, **b** fungivore, and **c** predator density. Garlic mustard-invaded plots had lower amounts of soil fungal hyphal and arthropod fungivores compared to the uninvaded plots, whereas predators were unaffected. *P* values were calculated from linear mixed-effects models (see Table 1); points represent mean \pm SE

cover (-0.45 [standardized coefficient], $P < 0.001$) and a weaker direct negative effect on soil hyphal length (-0.22 , $P = 0.04$). Soil fungal hyphae had a weak positive effect on

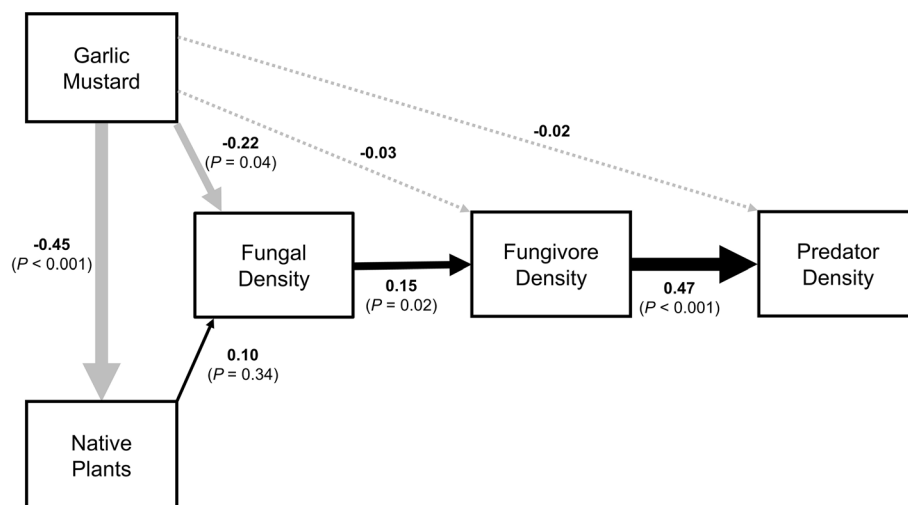


Fig. 4 Structural equation model illustrating the direct and indirect effects of garlic mustard on the soil food web. Bold numbers adjacent to arrows are the standardized coefficients. The P value gives the strength of evidence for each direct causal path for the coefficient. The width of the arrows is an approximate indication of the strength

of evidence. Grey lines indicate a negative relationship, black lines a positive relationship. Note that P values cannot be calculated for the indirect effects, which are the products of the coefficients in a path. Indirect effects are indicated only if there is reasonably strong evidence for each direct effect in the pathway

fungivore density (0.15 , $P = 0.02$, Fig. 4), while fungivore density had a strong positive impact on predator density (0.47 , $P < 0.001$, Fig. 4). Garlic mustard had very weak negative indirect effects on the second and third trophic levels (-0.03 and -0.02 for fungivores and predators, respectively). Herbaceous plant cover had no effects on soil fungal hyphae (Fig. 4). This SEM model explained 21% of the variance (i.e., marginal coefficient of determination) for herbaceous plant cover, 9% for soil hyphae, 2% for fungivores, and 21% for predators. Furthermore, in an alternative SEM with a direct link between garlic mustard and arthropod fungivores (Appendix S3 Fig. S1), we found that garlic mustard had no direct impact on fungivore densities ($P = 0.36$). See Appendix S3 for full details on alternative SEM fits.

Study 2: manipulative mesocosm experiment

Hyphal length Garlic mustard clearly suppressed fungal growth. In fungicide-free plots, the mean length of fungal hyphae at the end of the experiment in plots with *L. floridiana* was ca. $5 \times$ greater than in fungicide-free plots planted with garlic mustard (Fig. 5). It is striking that fungi were rare in all plots except those with *L. floridiana* (Fig. 5), a pattern confirmed by the Plant \times Fungicide interaction in the ANOVA ($P = 0.03$, Table 2a). The type of soil (from either invaded or uninvaded plots) had no impact on fungal growth [$P(\text{Soil} \times \text{Fungicide}) = 0.88$, $P(\text{Plant} \times \text{Soil}) = 0.46$, $P(\text{Soil}) = 0.39$; Table 2a].

Fungivores The pattern of treatment effects on fungivores at the end of the experiment was more complex, as indicated by the 3-way (Plant \times Soil \times Fungicide) interaction in

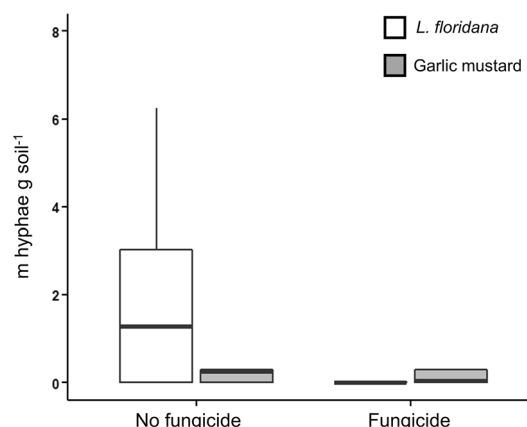


Fig. 5 At the end of Study 2, fungal hyphae were most abundant [measured as hyphal length (g soil^{-1})] in the fungicide-free plots planted with the native species *L. floridiana* [$P(\text{Plant} \times \text{Fungicide}) = 0.034$, Table 2a]. The top and bottom of the boxes indicate the first and third quartiles, with the centerline denoting the median. The whiskers show 1.5 times the interquartile range

the ANOVA ($P = 0.03$, Table 2b). Fungivore densities were highly variable among treatments in plots without fungicide (Fig. 6a); and surprisingly high and similar in magnitude in all Plant and Soil treatments that had been sprayed with fungicide (Fig. 6b). The 3-way interaction in the ANOVA results came from the Plant \times Soil interaction only in the fungicide-free plots (Fig. 6a, b; Table 2b). In uninvaded soils without fungicides, fungivores were almost $5 \times$ more abundant in plots with the native plant, *L. floridiana* (Fig. 6a), in agreement with the fungicidal effects of garlic mustard

Table 2 Analysis of variance (ANOVA) results testing for the main and interactive effects of Plant, Soil, and Fungicide on (a) hyphal length, (b) fungivore density and (c) predator density at the end of Study 2

Factor	F value	P
<i>A. Hyphal lengths</i>		
Plant	1.32	0.261
Soil	0.75	0.394
Fungicide	5.79	0.024
Plant × Soil	0.56	0.461
Plant × Fungicide	5.03	0.034
Soil × Fungicide	0.02	0.881
Plant × Soil × Fungicide	0.65	0.427
<i>B. Fungivores</i>		
Plant	0.58	0.453
Soil	0.17	0.685
Fungicide	1.79	0.155
Plant × Soil	4.54	0.028
Plant × Fungicide	0.19	0.665
Soil × Fungicide	0.03	0.874
Plant × Soil × Fungicide	5.28	0.031
<i>C. Predators</i>		
Plant	0.14	0.704
Soil	0.09	0.768
Fungicide	0.34	0.566
Plant × Soil	0.03	0.855
Plant × Fungicide	1.13	0.298
Soil × Fungicide	0.05	0.831
Plant × Soil × Fungicide	1.14	0.296

(Fig. 5). Surprisingly, this pattern was reversed in garlic mustard-invaded soils, in which fungivores were more abundant in plots planted with garlic mustard compared with those containing *L. floridiana* (Fig. 6a).

Predators At the end of Study 2, predator densities had shown no response to any of the three treatments (Fig. 6c, d; Table 2c).

Discussion

Our findings show that invasive garlic mustard alters soil fungal composition and reduces the overall density of fungal hyphae, which indirectly depresses densities of major soil fungivores (oribatid mites and springtails). Surprisingly, we found no indirect effects of garlic mustard on predators that consume fungivores, although the SEM analysis reveals a clear positive impact of fungivore numbers on predator densities. Thus, by depressing abundances of soil fungi, invasive garlic mustard indirectly lowers numbers of primary consumers of the soil food web, but this indirect effect has no discernible impact on the next higher trophic level.

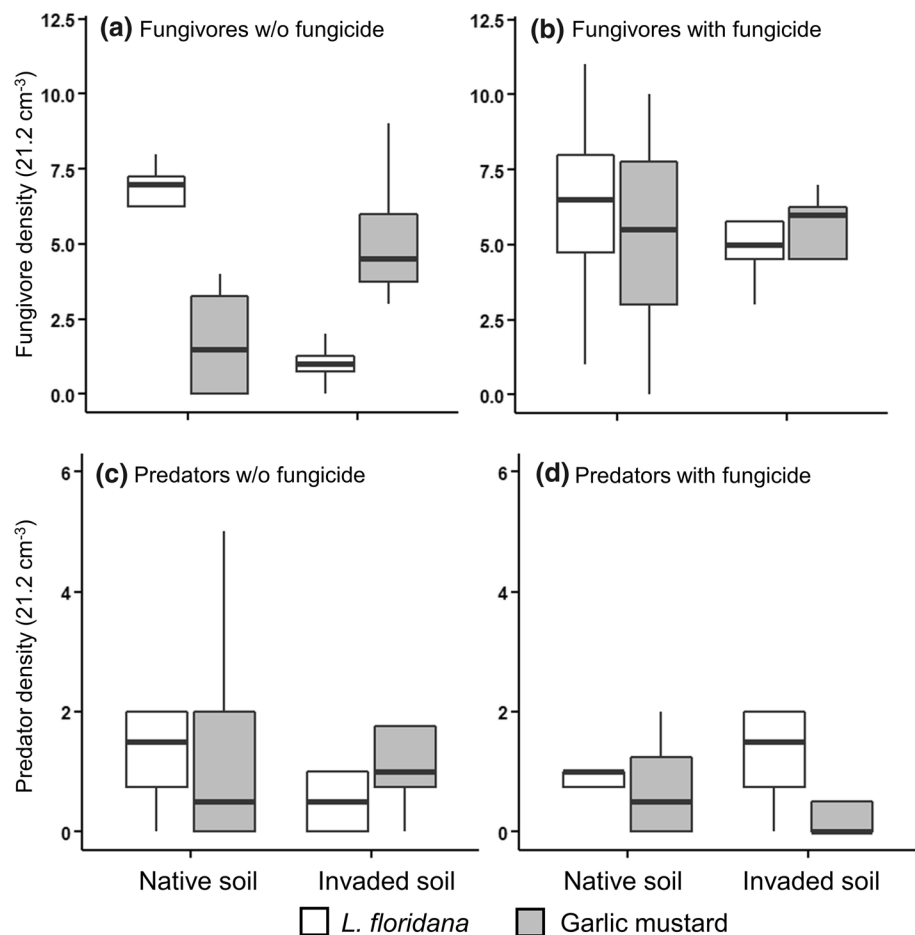
Garlic mustard alters soil food via changes to fungal resources

Our first prediction [Prediction 1] stated that garlic mustard invasion would have a negative effect on soil fungi, fungivores, and predators, with the effect attenuating up the food chain. Data from the mensurative study support this prediction but with the amendment that there is a greater weakening of the bottom-up control processes than we predicted, i.e., garlic mustard had negative effects on soil fungi and arthropod fungivores but not predators. Our mesocosm experiment demonstrated the same pattern but only in non-invaded soils. These findings support recent meta-analyses showing that invasive plants can alter soil food webs (Abgrall et al. 2019; Zhang et al. 2019), with primary consumers generally being more sensitive to invasion than secondary consumers.

We also predicted that garlic mustard would indirectly affect higher trophic levels via changes to soil fungi [Prediction 2]. The data from our experiments also support this prediction. First, our SEM analysis indicated a negative indirect effect of garlic mustard on fungivores, though the coefficient was not strong (fungivores = −3%). Second, the multivariate analyses of fungal community composition in garlic mustard-invaded vs. uninvaded plots show that changes in fungal resources were associated with differences in fungivore densities. In general, densities of fungivores (oribatids or isotomids) were positively correlated with uninvaded plots, except for Greene Valley Forest Preserve—the site with the fewest garlic mustard stems per m² (see Appendix S1 Table S1). Since fungal communities consistently differed between invaded and uninvaded plots across sites, the correlations with fungivores indicate that changes in fungal composition influenced their responses to garlic mustard. To our knowledge, this is the first study to show this relationship between arthropod fungivores and fungal composition as a response to plant invasion.

Overall, the manipulative mesocosm experiment provided further support that the effects of garlic mustard on soil food webs were indirect, but the results were complex. We expected plots with either garlic mustard and/or fungicide to harbor similar fungivore densities if the negative impact of garlic mustard on fungivores had been via reduction in fungal hyphae. Plots with garlic mustard and/or fungicide did have the lowest fungal abundances at the end of the experiment; however, plant identity, soil history, and fungicide application all had important effects on fungivore densities. We found that the fungicide-free treatment with *L. floridiana* and native soils had many more fungivores than garlic mustard in the same treatment combination. These differences in fungivore densities between *L. floridiana* and garlic mustard in native soils disappear with fungicide application, an expected result if fungi were important for

Fig. 6 Responses of fungivores (a, b) and predators (c, d) to treatments at the end of Study 2. Only fungivores in plots without fungicide responded to the treatments, but the effect of plant type upon fungivore densities was opposite in the two soil types [invaded (garlic mustard) or native (*L. floridana*)]. The top and bottom of the boxes indicate the first and third quartiles, with the center-line denoting the median. The whiskers show 1.5 times the interquartile range



fungivores. However, this predicted pattern was only evident when comparing *L. floridana* and garlic mustard in native soils; all other results were not as straightforward.

We provide several possible explanations for the complex fungivore patterns stemming from a subset of treatment combinations from the manipulative experiment. Although the fungicide plots harbored similar fungivore densities across treatment combinations (which we anticipated), the densities were higher than predicted. This non-intuitive finding from the fungicide plots might be due to the unintended consequences of fungicide application. Studies show that fungicides reduce extraradical hyphae, but some chemical formulations can stimulate fungal spore production (Jabaji-Hare and Kendrick 1987; Von Alten et al. 1993). Since soil fungivores feed on all life stages of fungi (Behan and Hill 1978), including the spores (Nakamori and Suzuki 2005), it is possible that the stimulatory effects of fungicides on spore production were enough to support fungivore populations despite the presence of garlic mustard, its invaded soil, or reduced abundances of extraradical hyphae. Thus, it appears that the fungicide application created opposing effects on fungal components, leading to similar availability of overall fungal resources for arthropod fungivores.

We found few fungivores in the *L. floridana* plots with fungicide-free invaded soil. This unexpected result might reflect the absence of high-quality fungi that can establish in soils that have experienced long-term invasion by garlic mustard. The recovery of soil fungal communities can take more than 6 years following the removal of intense and longstanding infestations of garlic mustard (Lankau et al. 2014). Consequently, fungal communities that could have fostered high fungivore numbers may have been unable to establish in *L. floridana* plots with garlic mustard-invaded soils, resulting in lower densities of fungivores. On the other hand, it appeared that garlic mustard in fungicide-free invaded soils had higher fungivore densities. Given the small sample size and high variation of fungivore densities in this treatment, we recommend evaluating this result with caution, particularly since our statistical modeling found no evidence for a difference between these plots and the other treatment combinations. The totality of these results suggests that fungal abundance is vital for predicting fungivore densities in garlic mustard-invaded and uninvaded areas, but other unmeasured environmental factors are also relevant in influencing fungivore populations.

Soil predator responses to garlic mustard

Densities of arthropod predators were highly correlated with fungivore densities in our SEM analysis, but overall densities of predators did not differ between treatments in either the mensurative or manipulative experiment, even though fungivores did differ. This pattern likely reflects the fact that soil predators are opportunistic generalist hunters, which will increase feeding on fungivores when these prey are very abundant (with resulting increases in population density) but will switch to the root- or bacteria-based food web when fungivores are less abundant. Although $2.8\times$ more fungi in uninvaded than invaded plots elevated fungivore densities by 40%, this increase in fungivores was insufficient to encourage soil predators to feed more heavily from the fungal channel compared to other energy pathways. When the fungal signal is much stronger, and resulting fungivore densities are much higher [as in manipulative experiments that increased fungivores much more than the contrast between garlic mustard-invaded and uninvaded plots (e.g. Chen and Wise 1999 and Lawrence and Wise 2017)], there is a detectable predator response to increased fungivore densities. Thus, differences in the origin and strength of the fungal signal could explain why we found unchanged predator densities across treatments but discovered a strong correlation between fungivores and predators in the SEM analysis. After controlling for variation across plots and study variables in the SEM, plots with higher densities of fungivores generally exhibited higher numbers of predators, but this relationship presumably breaks down when fungivores are rare. These results suggest that soil arthropod predators are linked to the fungal channel (i.e. direct effect of fungivores in SEM); however, the higher densities of fungivores in uninvaded versus invaded plots were not enough to shift predator feeding away from the bacterial and root-based channels. Thus, the clear signal from garlic mustard-to-fungi-to-fungivores disappeared at the predator level.

Comparison to other studies examining garlic mustard effects on soil arthropods

Since we found negative effects of garlic mustard on fungivores, our results support the findings of deHart and Strand (2012), which showed that arthropod predators shifted their diet to compensate for reduced springtail densities in garlic mustard patches. Interestingly, their study also provides a mechanism to explain why we did not detect a predator response: predators switched their diets to offset the reduction of fungivores via garlic mustard invasion. In contrast, Alerding and Hunter (2013) found that fungivores (springtails) were nearly $3\times$ higher in garlic mustard-invaded than an uninvaded forest, whereas Warrix et al. (2015) revealed no difference in litter arthropod abundance or diversity in

garlic mustard-invaded and uninvaded plots. A possible explanation for the conflicting findings is that both studies sampled arthropod communities that mostly reside in the leaf litter. Litter abundance, diversity, and structure are known to be important factors for litter arthropod abundance (Bultman and Uetz 1984); thus, variation in leaf litter abundance across the studies could have contributed to the differences in results. In our study sites, leaf litter was sparse, so we only sampled the lower soil horizons, which is where garlic mustard's allelopathy will most directly impact mycorrhizal fungi.

Future research directions

In longstanding populations of garlic mustard, we found negative effects of invasion on fungi and fungivores but not predators. Though we uncovered similar findings in our manipulative experiment with mesocosms, the overall pattern in the experiment was more complex and did not always reflect the pattern in the mensurative experiment. One explanation for the inconsistency is that our manipulations imposed a strong perturbation to the soil community, and our sampling occurred when the system had yet to reach an equilibrium. Effects of garlic mustard may take several generations to accumulate before producing the consistent and predictable impacts on soil food webs that we detected in our mensurative experiment. Future research should expand the timescale of garlic mustard manipulations to uncover the number of generations needed before the indirect effects of garlic mustard propagate to higher trophic levels. Using a finer taxonomic resolution of soil arthropods could also help elucidate in more detail how the soil community changes in response to garlic mustard invasion.

The strength and nature of the indirect effects of garlic mustard on soil communities likely correlate with direct impacts via invasion severity, i.e., the number of garlic mustard stems per m^2 . Higher densities of garlic mustard can directly modify the soil environment but may also lead to more pronounced allelopathic effects on the soil community. We found the most distinct differences in fungal composition between invaded and uninvaded plots in sites where garlic mustard densities were high (> 100 garlic plants per m^2). However, our manipulative mesocosm experiment simulated densities of only ~ 40 garlic mustard plants per m^2 , well below the mean densities measured in the mensurative experiment. Future studies should evaluate how the direct and indirect impacts of garlic mustard on soil food webs change across a gradient of invasion severity, as our research suggests there might be a threshold at which garlic mustard effects are most pronounced. Furthermore, future research should study the potential impacts of garlic mustard on saprophytic fungi, which is also an important resource for arthropod fungivores such as springtails (Klironomos et al.

1992). Our use of the 18S rRNA region of the fungal genome precluded the ability to distinguish mycorrhizal and saprophytic fungi. Since this fungal guild represents an important resource for soil fungivores, quantifying the responses of saprotrophic fungi will provide a more complete picture of how garlic mustard can impact soil food webs.

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Author contribution statement MM and DW conceived and designed the experiments. MM performed the experiments. MM analyzed the data. MM and DH wrote the manuscript; both the authors contributed substantially to manuscript revisions and gave final approval.

Data availability The data supporting the results of this manuscript are available on GitHub: https://github.com/mmccar26/McCary_Wise_2019_Garlic_Mustard.

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