Invasive garlic mustard demonstrates stronger relationships with pathotrophic than mycorrhizal fungi

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Abstract

Garlic mustard (*Alliaria petiolata*) has long been known to degrade mycorrhizal mutualisms in soils it invades and may also promote the abundance of microbial pathogens harmful to native plants or alter saprotrophic communities to disrupt nutrient cycling. Phenology of other invasive species, like *Lepidium latifolium* and *Lonicera maackii*, plays a role in their interactions with soil microbial communities, and so we may expect garlic mustard phenology to influence its effects on native soil microbiomes as well. Here, we investigate differences in fungal, bacterial, and archaeal community structure, as well as the abundance of key functional groups, between garlic mustard present, absent, and removed treatments in central-Illinois forest soils across different stages of the garlic mustard life cycle. Across its phenology, garlic mustard present soils had different overall fungal community structure and greater abundance of pathotrophic fungi than soils where garlic mustard was absent or removed. However, abundance of ectomycorrhizal and saprotrophic fungi as well as bacterial and archaeal community structure were similar between treatments and did not interact with garlic mustard phenology. The most abundant overall fungal taxon was a plant pathogen, *Entorrhiza aschersoniana*, that was greatest in garlic mustard present soils, particularly while the plants were flowering. These results support the hypothesis that invasive plants form active relationships with microbial pathogens that could contribute to their overall success in invading ecosystems.

Keywords

Garlic mustard, forest soil, microbial-mediated plant invasion, fungal pathogen, mycorrhizal fungi, invasive plant phenology

Introduction

Novel interactions between exotic plants and native microbes can both facilitate the spread of invasive species and contribute to harmful legacies of invasion on native plant communities (Callaway et al. 2004; van der Putten 2010; Van der Putten et al. 2007). Some invasive plants can actively disrupt or degrade mutualistic interactions between soil microbes and native plants that depend on them (Pringle et al. 2009; Traveset and Richardson 2014), giving invaders a relative fitness advantage over native plants. Other invaders have been shown to accumulate local pathogens in soils (Eppinga et al. 2006; Mangla et al. 2008). Because invasive plants are often less susceptible to native pathogens than are native plants (Agrawal et al. 2005; Engelkes et al. 2008; Klironomos 2002; Mitchell and Power 2003), this "enemy release" can provide another relative fitness benefit to invasive plants over natives (Kardol et al. 2007; Keane and Crawley 2002). Invasive plant effects on soil microbial communities and their functions can vary with invasion age (Lankau 2011), plant phenology (Portier et al. 2019), and soil properties (Li et al. 2006). Therefore, it is important to investigate these plant-microbe interactions in different environmental contexts to avoid biases in the documented patterns (Hulme et al. 2013).

Garlic mustard (*Alliaria petiolata*, Cavara and Grande), a prominent invasive forest herb in North America (Rodgers et al. 2008), is often considered a classic example of invasion success driven by mutualism suppression (Callaway et al. 2008). Garlic mustard produces secondary metabolites that reduce inoculum potential, activity, and biomass of mycorrhizal fungi (Anderson et al. 1996; Roberts and Anderson 2001). Because garlic mustard is a non-mycorrhizal plant, this assault on the mycorrhizal mutualists of its competitors has been posited to explain garlic mustard’s high invasion success (Lankau et al. 2009; Stinson et al. 2006; Wolfe

et al. 2008). Concomitant with a decrease in the relative abundance of mycorrhizal fungi in garlic mustard invaded soils is an increase in the relative abundance of pathotrophic and saprotrophic fungi (Anthony et al. 2017; Anthony et al. 2019). Both of these groups could also contribute to garlic mustard's invasion success. Pathotrophic and saprotrophic fungi could fill niches made available by the suppression of mycorrhizal fungi (Anthony et al. 2017), with pathotroph accumulation potentially also benefitting garlic mustard as its growth rate is unaffected when inoculated with heterospecific pathogens (Klironomos 2002). Changes in saprotrophic bacterial and archaeal communities can alter soil nutrient cycling (Hawkes et al. 2007; Hawkes et al. 2005), potentially conferring advantage over native plants (Fraterrigo et al. 2011; Lee et al. 2018); however, garlic mustard effects on these communities are more poorly characterized than its effect on fungal communities (Cipollini and Cipollini 2016). Bacterial richness and diversity has been found to be consistent in soils under garlic mustard and other native forest herbs (Burke and Chan 2010). However, garlic mustard’s impact on bacterial richness and abundance is thought fluctuate with invasion age, increasing initially then eventually recovering in older garlic mustard populations (Lankau 2011). Although garlic mustard is well-known for its suppression of mycorrhizal fungi, it is not clear if its impacts on other functional groups within soil microbial communities also play a role in its invasion success.

Our understanding of garlic mustard impacts on soil microbial communities derives largely from studies on persistent invasions with high plant densities, mostly located in the north-eastern United States (Anthony et al. 2017; Anthony et al. 2019; Burke et al. 2019; Stinson et al. 2006; Wolfe et al. 2008) but also in the midwestern United States (Lankau 2011; Lankau et al. 2014). After initial invasion, garlic mustard population densities increase rapidly then become relatively stable (Anderson et al. 1996; Nuzzo 1999). Although garlic mustard population

densities do not appear to decrease over time, its influence on microbial communities changes with invasion age, as garlic mustard produces less allelochemicals over time (Lankau et al. 2009) thereby weakening its influence on soil microbes (Lankau et al. 2010). Soil bacterial and fungal communities also evolve as invasions age, leading to a recovery in the diversity of these communities in older garlic mustard stands (Lankau et al. 2011). Furthermore, landscape factors like disturbance regime, soil types, and surrounding plant communities also influence how quickly garlic mustard invasions spread and their population distribution patterns in invaded forests (Haines et al. 2018; Urbanowicz et al. 2019). Because garlic mustard may interact with soil microbiomes differently depending on ecoregions and invasion patterns (Urbanowicz et al. 2019), it is important to broaden our understanding of these interactions beyond the historically studied settings.

Garlic mustard studies often focus on plant-microbe interactions during the active life stage of garlic mustard earlier in the growing season (e.g., Anthony et al. 2017; Burke et al. 2019). However, invasive plant effects on soil microbial communities (Arthur et al. 2012) and microbial processes (Portier et al. 2019) can vary with plant phenology. As garlic mustard exhibits an early phenology relative to native competitors (Engelhardt and Anderson 2011), its effects on soil microbial communities may depend on its own life stages or broader temporal changes associated with surrounding plants and ecosystem conditions (Bennett et al. 2013; Saikkonen 2007). Effects like mutualist suppression that confer a competitive advantage may be more important for garlic mustard during its early phenology, but garlic mustard could also produce allelochemical and soil ecological legacy effects that continue to indirectly influence soil microbial communities in the non-growing season. The long-term persistence of indirect garlic mustard effects is supported by observations that soil microbial communities can take

several years to return to pre-invasion condition after garlic mustard eradication (Anthony et al. 2019; Lankau et al. 2014). To our knowledge, no studies have explicitly investigated how garlic mustard impacts on soil microbial communities change over the course of its life cycle.

Here, we investigated how garlic mustard affects the relative abundance of soil microbial functional groups (ectomycorrhizal fungi, saprotrophic fungi, pathotrophic fungi, bacteria, and archaea) across garlic mustard phenology in relatively recently invaded hardwood forests in the midwestern United States. We hypothesized that the degree of garlic mustard effects on soil microbial community composition varies with its phenological stage, reflecting changes in the production of allelochemicals that directly affect soil microbes. We compared soil microbial community composition among three garlic mustard treatments (present, absent, and experimentally removed) established in multiple forests across central Illinois where most garlic mustard populations were estimated to be 20-30 years old at the time of the study. We expected to observe a decrease in ectomycorrhizal fungi relative abundance, an increase in pathotrophic and saprotrophic fungi relative abundance, and a change in bacterial and archaeal community composition associated with the presence of garlic mustard throughout the growing season and persisting after senescence, but with the magnitude of garlic mustard effects declining at flowering and even more so after senescence.

Methods

Study Sites

In 2017, we measured garlic mustard impacts on soil microbial communities across five forests in central Illinois: Brownfield Woods, Collins Pond Woods, Hart Woods, Richter Woods,

and Trelease Woods. These sites are located within a 30 km north-south range and a 40 km east-west range. Geographical coordinates, climate, and soil variables are reported in Table S1. All sites are managed by the Illinois Natural History Survey (INHS; https://www.inhs.illinois.edu/research/natural-areas-uiuc). According to documentation from INHS managers, all of these sites began experiencing invasion between 1991 and 1995. Site managers attempted to control garlic mustard populations through hand-pulling, herbicide application, and "weed whacking", and as a result, garlic mustard densities at these sites were generally maintained below the high densities often associated with a decline in garlic mustard allelochemical production (Lankau 2011; Lankau et al. 2009).

In 2018, eight forests in central Illinois were sampled to expand the range of our inference to a greater number of geographical sites: Allerton Park, Breens Woods, Bunny Forest, Funk Woods, Richter Woods, Trelease Woods, Warbler Ridge, and the Vermillion River Observatory. Two of the sites, Richter Woods and Trelease Woods, were sampled in both years; garlic mustard populations in the other three 2017 sites were intensively managed via herbicide application in early 2018 so those sites were excluded. Site descriptions can be found in Table S1. These sites are located within a 130 km north-south range and a 140 km east-west range. As the 2018 study sites are managed by multiple different agencies, records for invasion ages and management were not available for some of the sites depending on the agency. Plant communities from forests sampled in 2017 and 2018 are relatively homogenous across these sites, consisting of a “prairie grove” species mix with *Quercus, Fraxinus, Carya,* & *Acer* species dominating the canopies. The understory these forests are made up of a diverse mix of tree seedlings, *Asimina triloba,* and other shrubs including *Sanicula, Laportea, Ageratina, Ribes,* and *Trillium* species

Study Design

In mid-March of each year of this study, we established 10-m^2 experimental blocks in each forest. Each block contained 1-m^2 plots for each of the following three treatments: garlic mustard present (present), garlic mustard absent (absent), and garlic mustard removed via hand-pulling (removed). We established the garlic mustard removed treatment as a control to account for meter-scale differences in soils within each block that might promote the growth of garlic mustard and to understand if live garlic mustard plants are required to see their effects. In observational studies such as these it is often difficult to differentiate the effects of invasive plants on soil conditions from preexisting edaphic characteristics that may have promoted garlic mustard colonization. We accounted for this challenge by comparing soils with active garlic mustard plant present and those that were previously invaded but live plants were removed. This allowed us to better attribute the presence of live garlic mustard plants to changes in microbial communities, rather than simply draw association between invaded and uninvaded soils. In 2017, we established 4 replicate blocks in each of the five forests and collected soil samples in May and August, corresponding with the rosette and senescent life stages of garlic mustard, respectively. In 2018, only a single treatment block was established in each forest because site was considered the experimental unit for replication. This allowed us to increase the number of sites (from 5 to 8) and sampling dates per site (from 2 to 3) compared to 2017. In 2018, we also added a soil collection in June, during garlic mustard flowering, to account for potential changes in garlic mustard allelochemical production with changes in reproductive status (Cipollini and Cipollini 2016) or light availability (Smith 2015).

Soil samples were collected from 0-10 cm depth from the soil surface using a 2 cm diameter hand corer. There was very little, if any, development of organic horizons in any of our plots, so all samples consisted primarily of mineral soils. Four soil cores were collected from each 1 m^2 treatment plot, composited, and homogenized by hand in the field. On the same day as collection, the soil samples were transported to the University of Illinois at Urbana-Champaign and frozen at -20□C for later analysis.

DNA extraction, sequencing, identification, and assignments

We used high-throughput DNA sequencing to determine the impact of garlic mustard on microbial community structure as well as the abundance of key functional groups and individual taxa. DNA was extracted from 500 mg of freeze-dried soil using the FastDNA SPIN Kit for Soils (MP Biomedicals, Santa Ana, USA). The extracts were purified using cetyl trimethyl ammonium bromide (CTAB). DNA extracts were submitted to the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign for Fluidigm amplification (Fluidigm, San Francisco, USA) and Illumina sequencing (Illumina, San Diego, USA). We assessed fungal communities via the *ITS2* gene region using ITS3 and ITS4 (Ihrmark et al. 2012) primers to amplify DNA and sequence amplicons via MiSeq bulk 2 x 250 bp V2. As we were interested in the broad fungal community effect of garlic mustard invasion, we chose to use a universal ITS2 barcode region (Schoch et al. 2012). However, this barcode is known to discriminate against early-diverging fungal lineages like arbuscular mycorrhizal fungi (Stockinger et al. 2010), so this group was not included in our analysis. We assessed bacterial communities via the bacterial and archaeal *16S* rRNA genes. The 2017 samples were amplified using V4_515 forward and V4_806 reverse primers, and amplicons were sequences via MiSeq bulk 2 x 250 bp V2. The 2018

samples were amplified using Arch519 forward and BAC785 reverse primers, and amplicons were sequenced via NovaSeq 2 x 250 bp. Primer sequences and references can be found in Table S2. We changed the *16S* primers from 2017 to a more permissive primer in 2018 to capture a wider range of the bacterial and archaeal community in our analysis. Because different primer sets could lead to differences in amplification efficiency between different bacterial and archaeal taxa, we do not directly compare 2017 and 2018 *16S* datasets in our analysis.

A standard bioinformatics workflow was used to process sequences. Briefly, FLASH (Magoč and Salzberg 2011) was used for merging paired-end reads before quality filtering via the FLASHX-Toolkit (Gordon and Hannon 2010) to remove sequences with quality scores of 30 or less for 10% of bases or more. USEARCH (Edgar 2010) was used to cluster sequences with 97% similarity into operational taxonomic units (OTUs) as well as remove chimeric sequences and singletons. QIIME (Caporaso et al. 2010) was used to convert OTU clusters into an OTU table, assign taxonomy using the UNITE reference database (version 8, 2019 release), and then to rarify all samples to a uniform read depth of 7172 (ITS-2017), 2776 (ITS-2018), 13263 (16S-2017), and 153507 (16S-2018). The final fungal OTU tables were analyzed via the FUN-Guild (Nguyen et al. 2016) database to assign OTUs with probable or highly probable matches to ectomycorrhizal (ECM), pathotroph, or saprotroph functional groups.

Statistical Analysis

All statistical analysis was performed in the R statistical environment (Team 2013). Overall patterns of beta diversity and community structure were analyzed with the VEGAN package (Oksanen et al. 2010). To determine differences community composition based on invasion status (treatment), garlic mustard phenological stage (sampling date), and the

interaction between treatment and phenological stage, we performed a permutational analysis of variance (PERMANOVA) with the *adonis* function on Hellinger-transformed rarified OTU count numbers. We added site (forest) as a term in the *adonis* model, rather than stratifying, to account for the variance contributed by site to microbial communities. This allowed us to keep the model consistent between sampling years, as there was no replication within sites in our 2018 sampling year. We also assessed differences in community composition and heterogeneity using nonmetric multidimensional scaling (NMDS) ordinations on Bray-Curtis dissimilarity of rarified OTU count numbers. Differences in the relative abundance of functional groups (i.e., pathotroph, saprotroph, and ECM) and individual OTUs were determined using a linear-mixed model with the LMER package (De Boeck et al. 2011). In this model, we used invasion status, phenological stage, and their interaction as fixed effects and site as a random effect. Relative abundances were log-transformed to meet assumptions of normality and homogeneity of variance. Post-hoc pairwise differences between treatments and between time points were determined using a Tukey test. Statistical significance was assessed as $P < 0.05$.

Results

Soil microbial community composition

In both 2017 and 2018, overall fungal (and to a lesser extent bacterial and archaeal) community composition differed based on garlic mustard treatment and phenology, with no interactions between those effects (Table 1). Garlic mustard treatment accounted for 1.8% (p=0.04; Table 1; Figure 1A) and 3% (p=0.002; Table 1; Figure 1B) of the variation in the fungal overall community composition in 2017 and 2018, respectively. Garlic mustard treatment accounted for a smaller portion of bacterial and archaeal community variation relative to fungi,

with 1.1% (p=0.78; Figure 2A) and 2.8% (p=0.05; Table 1; Figure 2B) in 2017 and 2018, respectively. Phenology was associated with a greater portion of community variation than garlic mustard treatment: 3.3% (p=0.001; Table 1; Figure 1C) and 4.6% (p=0.001; Table 1; Figure 1D) for fungal communities in 2017 and 2018, respectively, and 6.8% (p=0.001; Table 1; Figure 2C) and 4.2% (p=0.001; Table 1; Figure 2D) for bacterial and archaeal communities in 2017 and 2018, respectively. There was no significant interaction between garlic mustard treatment and phenology on fungal or bacterial and archaeal community composition in either year (Table 1).

Functional group relative abundance

Garlic mustard invasion did not have a consistent effect on soil microbes across all functional groups (Figure 3). Garlic mustard did not significantly affect the relative abundance of ECM or saprotrophs in soil fungal communities (Table 2, Figure 3AB). ECM relative abundance increased as the growing season progressed in both 2017 ($F_{1,110}$=29.88, p<0.001) and 2018 ($F_{2,56}$=11.85, p<0.001; Table 2; Figure 3CD), with no interaction between phenology and garlic mustard treatment (Table 2). In contrast, the relative abundance of saprotrophs was not significantly affected by sampling date in either year (Table 2, Figure 3). The relative abundance of pathotrophic fungi within the fungal community was significantly increased by garlic mustard presence compared to the absent or removed treatments in both 2017 ($F_{2,110}$=6.12, p<0.001) and 2018 ($F_{2,56}$=7.02, p=0.002; Table 2; Figure 3AB). In 2017, plant phenology did not significantly affect pathotroph relative abundance (Figure 3C), but in 2018, pathotroph relative abundance was greatest while plants were flowering and decreased significantly after senescence ($F_{2,56}$=2.73, p=0.03; Table 2; Figure 3D). The average relative abundances of the four most

abundant OTUs in each functional group (including the 4 most abundant OTUs overall) are listed in Table S3.

OTU abundance

The most abundant fungal OTU was *Entorrhiza aschersoniana*, a pathogen which accounted for an average of 3.4% of fungal communities across all treatments and sampling dates in 2017 (with a maximum of 50.1%) and an average of 6.7% (with a maximum of 72.2%) across all samples in 2018. This OTU was found in 85% of garlic mustard present samples (averaging 10% of the fungal community in this treatment) and was the most abundant taxon in 30% of these samples in 2017. In 2018, this OTU was found in 80% of garlic mustard present samples (averaging 8% of the fungal community in this treatment) and was the most abundant taxon in 41% of these samples. In contrast, this taxon was found in only 13% of garlic mustard absent samples (averaging 0.0003% of the fungal community in this treatment) in 2017 and 16% of garlic mustard absent samples (averaging 0.008% of the fungal community in this treatment) in 2018. *E. aschersoniana* relative abundance was consistently greater in garlic mustard present plots than in absent or removed plots across sampling dates (2017: $F_{2,110}$=21.07, p<0.001; 2018: $F_{2,56}$=16.528, p<0.001; Table 2; Figure 4). In garlic mustard present and removed soils, *E. aschersoniana* relative abundance decreased after garlic mustard present plots senesced (2017: $F_{2,110}$=4.10, p=0.01; 2018: $F_{4,56}$=6.08, p<0.001; Table 2; Figure 4). In 2018, *E. aschersoniana* abundance was greatest in garlic mustard present treatments when the plants were flowering (Figure 4). Garlic mustard did not significantly affect any of the other most abundant OTUs based on the same statistical test used for *E. aschersoniana*.

Discussion

Garlic mustard's suppression of mycorrhizal fungi have long been known (Roberts and Anderson 2001; Vaughn and Berhow 1999) while evidence for its interactions with other microbial functional groups in more recent (Anthony et al. 2017). Here, we found to garlic mustard to promote pathotrophic fungi, especially during its flowering stage, without significantly affecting other microbial groups like ECM, saprotrophic fungi, or bacteria and archaea. Though plant phenological stage is important for microbial community interactions with other invasive species (Arthur et al. 2012; Portier et al. 2019), garlic mustard's phenology did not significantly influence ECM and saprotrophic abundance or community structure. However, phenological stage did appear to play a role in the abundance of a prominent soil pathogen, *E. aschersoniana,* which was greatest when garlic mustard plants were active. The evidence we found of garlic mustard promoting pathotrophic fungi, namely a specific pathotrophic taxon whose abundance peaked at the plant's flowering stage, supports the broader hypothesis that microbial pathogens are important for understanding plant invasions.

Although invasive plants can acquire a competitive advantage over native species via passive immunity to native pathogens, referred to as "enemy release", we found soils beneath garlic mustard to actively accumulate the pathotrophic fungi *E. aschersoniana.* The recruitment of *E. aschersoniana* resulted from direct influence of live garlic mustard, as the relative abundance of E. *aschersoniana* declined over time in the garlic mustard removed treatment and after garlic mustard senescence in the present treatment. Pathogen accumulation by invasive plants is often associated with generalist pathogens (Eppinga et al. 2006; Mangla et al. 2008). However, this pathogen recruited by garlic mustard specifically targets the roots of other

common forest understory plants (sedges and rushes; Bauer et al. 2015) that might compete with garlic mustard. *E. aschersoniana* may not infect garlic mustard due to significant differences in root architecture between garlic mustard and this taxon's preferred hosts (Fineran 1980). We did not observe any root galls characteristic of *E. aschersoniana* infection on garlic mustard plants we removed when establishing our treatments. Furthermore, we could find no evidence in the literature of this fungus, or any other members of its phylum, infecting Brassicaceae roots. Without performing reciprocal transplant studies to determine whether the fungi cultivated by garlic mustard would negatively affect native plants (or garlic mustard itself), we do not know if this relationship confers garlic mustard a competitive advantage. *E. aschersoniana* has not been documented in garlic mustard invasions in the northeast U.S. where mutualism suppression has been demonstrated to facilitate garlic mustard invasion. Given that garlic mustard recruitment of *E. aschersoniana* occurred across all 11 independent forest sites in our study, where we did not observe evidence of mutualism suppression, the potential for this pathogen to play a role in garlic mustard invasion is worthy of exploration.

Garlic mustard's inhibition of mycorrhizal fungi in its invasive range is one of its most widely observed traits (Anderson et al. 2010; Anthony et al. 2017; Callaway et al. 2008; Pringle et al. 2009; Rodgers et al. 2008; Stinson et al. 2006; Wolfe et al. 2008), though we did not find support for mycorrhizal inhibition in this study. Evidence for garlic mustard mycorrhizal suppression often comes from potting studies using extracts from garlic mustard tissues (Callaway et al. 2008; Stinson et al. 2006). Though mycorrhizal suppression has been demonstrated under field conditions (Anthony et al. 2017), it is difficult to quantify ecologically relevant allelochemical concentrations from garlic mustard plants *in situ*, particularly as these concentrations fluctuate with population age (Lankau et al. 2009). Further, many of the soils in

our study had higher clay contents than the characteristically sandy soil of more northern and eastern North American forests invaded by garlic mustard (Table S1). Allelochemicals produced by garlic mustard plants could have bound to these clay particles (Blair et al. 2005), potentially ameliorating their effects on mycorrhizal fungi. As garlic mustard has been found to have diminished effects on intact, rather than establishing, mycorrhizal networks (Barto et al. 2010), this could help explain why we observed shifts in fungal community structure and pathotrophic relative abundance without reductions in ECM abundance. The increases in pathotroph relative abundance in garlic mustard invaded soils without evidence for mycorrhizal inhibition observed here suggest a relationship between garlic mustard and pathotrophic fungi independent of mycorrhizal allelopathy. Pathotroph accumulation can occur in the absence of mutualism suppression for garlic mustard, but its role in promoting garlic mustard invasion will require further investigation.

Our results demonstrate that plant-microbe interactions thought to facilitate garlic mustard invasion, like mutualism suppression or enemy release, may vary with invasion age and geography. Many studies investigating microbial community impacts of garlic mustard invasion are conducted on long-term, high-density populations (Anthony et al. 2017; Anthony et al. 2019; Burke et al. 2019; Stinson et al. 2006; Wolfe et al. 2008), demonstrating the eventual effects of garlic mustard invasion. However, based on these studies it is difficult to predict microbial community responses to more recent garlic mustard invasions (Corbin et al. 2017). Microbial community suppression by garlic mustard peaks when invasive populations are around 40 years old (Lankau 2011), which may represent a potential ecological filter on microbial communities in garlic mustard invaded soils. Most garlic mustard populations in our study were younger than 40 years with low garlic mustard plant density characteristic of this age range, thus, the soils

surrounding these populations have not likely experienced garlic mustard's peak microbial suppression capacity. Filtering of microbial communities in garlic mustard invaded soils caused by peaks in allelopathy based on invasion age could explain differences in our results, particularly mycorrhizal suppression, from studies conducted on older, higher density garlic mustard populations. However, without direct comparisons we cannot definitively conclude whether these differences are due to invasion age or other regional characterizes. Our results indicate that garlic mustard invasions can occur without strong evidence for mycorrhizal inhibition, challenging the paradigm that mutualism suppression is a critical driver of garlic mustard invasiveness. It is possible that other microbially-mediated mechanisms, like pathogen accumulation, play a larger role in garlic mustard invasion in some environmental contexts.

Conclusions

Garlic mustard is often cited as a key example of the mutualism degradation hypothesis for plant invasion (Pringle et al. 2009; van der Putten 2010). However, we were unable to detect this phenomenon for garlic mustard invasions in Illinois forests with relatively young invasions. Instead, we demonstrated that garlic mustard actively promoted the relative abundance of fungal pathotrophs, particularly *E. aschersoniana*, which could confer a competitive advantage over native plants. While we did not observe garlic mustard phenological stage to influence its effect on the abundance and structure of microbial functional groups, *E. aschersoniana* relative abundance was affected by phenology. This observation together with a rapid decline in *E. aschersoniana* relative abundance after garlic mustard removal suggests that live garlic mustard directly recruits this pathogen. Overall, our study shows that in some contexts, either associated

with invasion age or regional environmental differences, garlic mustard interacts more strongly with pathotrophic fungi than mycorrhizal fungi.

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Table 1. Results from PERMANOVA tests for differences in overall community composition for fungal (*ITS*) and bacterial (*16S*) communities based on garlic mustard treatments, phenology, and this interaction of treatment and phenology for 2017 and 2018 datasets.

Barcode Gene	**Main Fixed Effect**	**2017**			**2018**		
		F-value	R^2	*p*-value	*F*-value	R^2	*p*-value
ITS	Treatment	1.224	0.018	**0.042**	1.656	0.030	**0.002**
	Phenology	4.509	0.033	**0.001**	2.529	0.046	**0.001**
	Treatment*Phenology	0.893	0.012	0.977	0.938	0.034	0.719
	Site	4.668	0.137	**0.001**	4.943	0.361	**0.001**
16S	Treatment	0.781	0.011	0.781	1.175	0.028	**0.050**
	Phenology	9.889	0.068	**0.001**	2.093	0.025	**0.001**
	Treatment*Phenology	0.541	0.008	0.999	0.908	0.021	0.811
	Site	5.847	0.161	**0.001**	0.244	0.244	**0.001**

Table 2. ANOVA results for main effects on the relative abundance of fungal guilds (ECM, pathotroph, or saprotroph) and the fungal pathogen, *E. aschersoniana*. Site was included as a random effect in all models.

Fungal Functional Group or Taxon	**Main fixed effect**	***2017***			***2018***		
		df	*F*-value	*p*-value	*df*	*F*-value	*p*-value
ECM	Treatment	2	1.397	0.252	2	0.514	0.601
	Phenology	1	29.882	**<0.001**	2	11.855	**<0.001**
	Treatment*Phenology	2	0.953	0.388	4	0.908	0.466
PATHOTROPH	Treatment	2	6.129	**<0.001**	2	7.023	**0.002**
	Phenology	1	0.735	0.391	2	3.943	**0.026**
	Treatment*Phenology	2	0.108	0.897	4	2.737	**0.039**
SAPROTROPH	Treatment	2	0.168	0.845	2	1.234	0.300
	Phenology	1	0.002	0.965	2	0.846	0.435
	Treatment*Phenology	2	0.009	0.991	4	0.415	0.797
E. ASCHERSONIANA	Treatment	2	21.079	**<0.001**	2	16.528	**<0.001**
	Phenology	1	6.958	**0.009**	2	7.478	**0.001**
	Treatment*Phenology	2	4.108	**0.019**	4	6.088	**<0.001**

Figure 1. Fungal community composition expressed as nonmetric multidimensional scaling axes of Bray-Curtis dissimilarities of rarified count numbers for OTUs within each sample (circle), convex hulls (lines) depict the range of community composition across garlic mustard treatment (AB) and phenology (CD) in 2017 (AC; stress = 0.25) and 2018 (BD; stress = 0.23). Left panels include data across all sampling dates together for each treatment and right panels include data from all treatments together for a given sampling date.

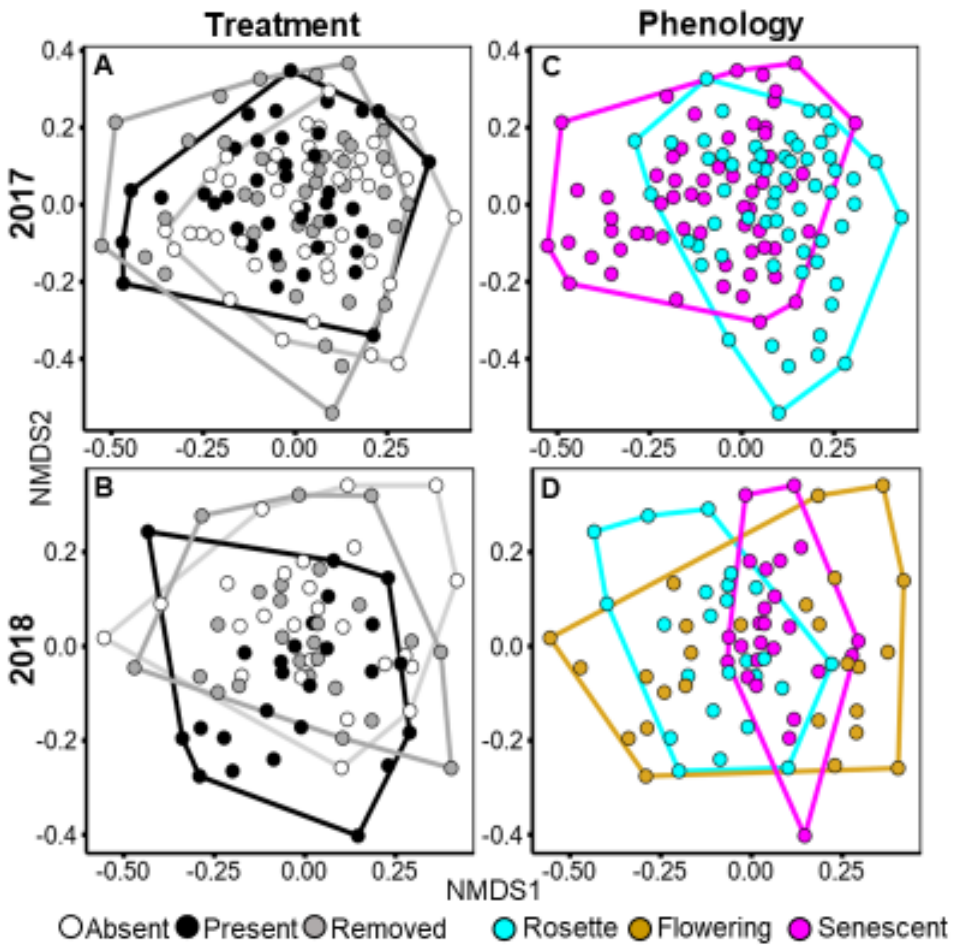

Figure 2. Bacterial and archaeal community composition expressed as nonmetric multidimensional scaling axes of Bray-Curtis dissimilarities of rarified count numbers for OTUs within each sample (circle), convex hulls (lines) depict the range of community composition across garlic mustard treatment (AB) and phenology (CD) in 2017 (AC; stress = 0.12) and 2018 (BD; stress = 0.17). Left panels include data across all sampling dates together for each treatment and right panels include data from all treatments together for a given sampling date.

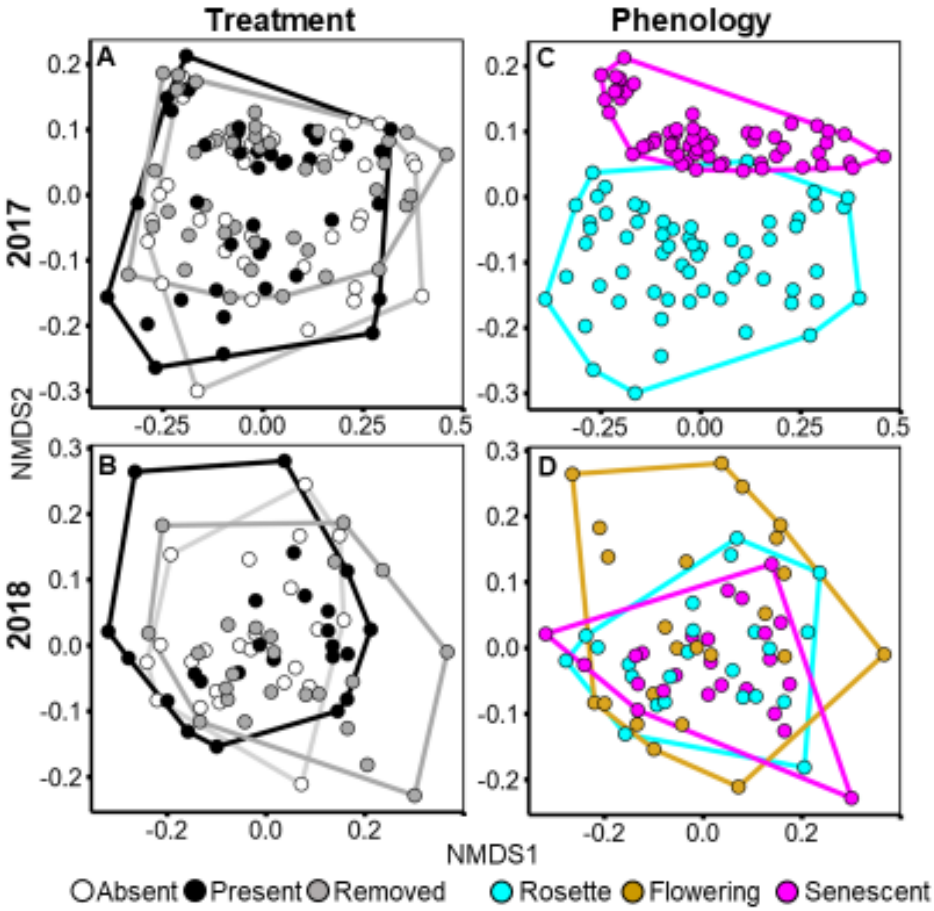

Figure 3. Relative abundance (%; mean ± SE) of ectomycorrhizal (ECM), pathotroph, and saprotroph functional groups based on garlic mustard treatment (AB) and phenology (CD) in 2017 (AC) and 2018 (BD). Different letters indicate statistically significant differences ($p < 0.05$) within each functional group in a given sampling year.

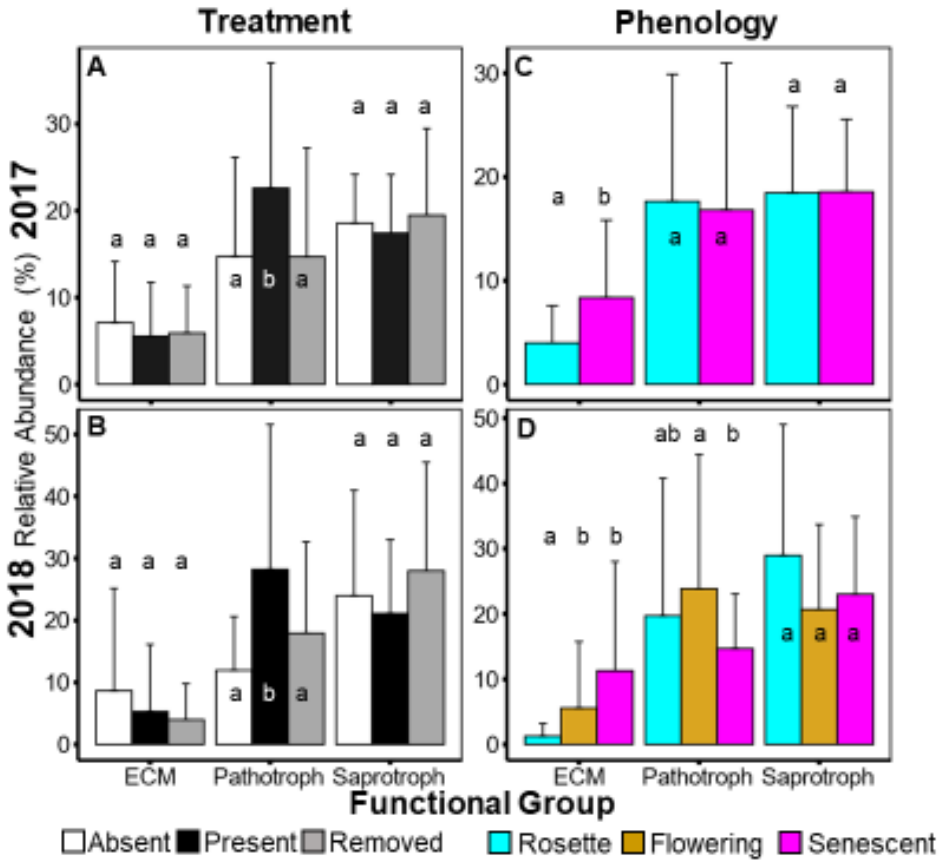
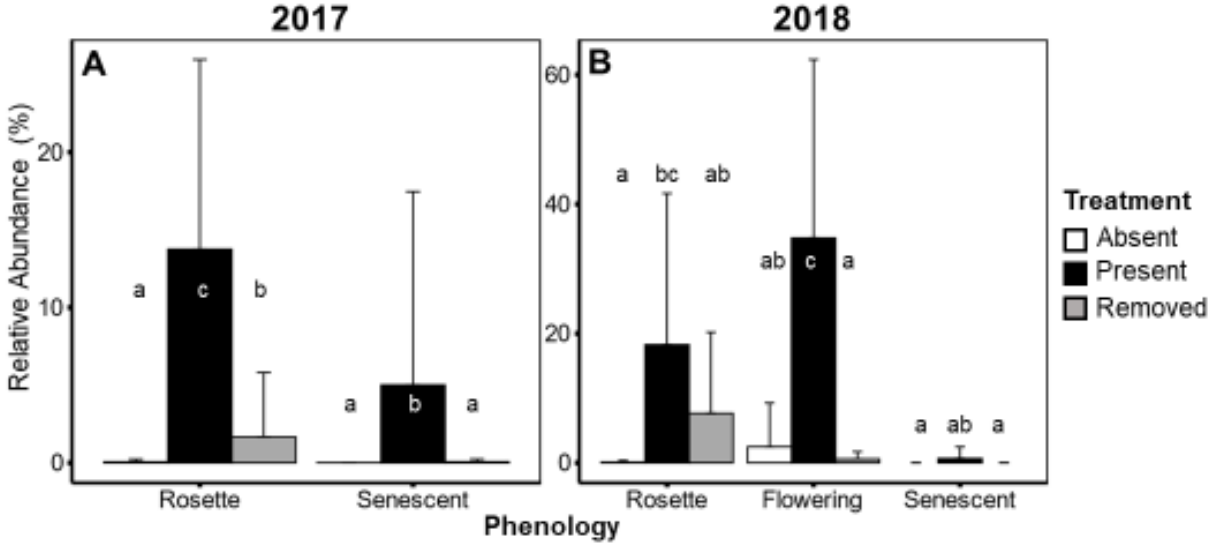
Figure 4. Relative abundance (%; mean ± SE) of the pathogenic fungi, *Entorrhiza aschersoniana*, based on garlic mustard treatment in 2017 (A) and 2018 (B). Different letters indicate statistically significant differences ($p < 0.05$) within a given sample year.