Above- and below-ground community responses to a mutualism-disrupting allelopathic invasive plant

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ABSTRACT

To understand the dynamics of biological invasions, mechanisms of invaders' impacts must be assessed across scales of ecological organization, and under different biotic and abiotic conditions. Traits of invasive species that generate successful competitors such as high fecundity, efficient resource acquisition, or novel chemical weapons allow them to rapidly increase in abundance and negatively affect native communities. Mycorrhizal mutualism disruption is a common and well-supported mechanism of impact of invasive plants, where allelopathic chemicals indirectly impact native plant species via disruption of mycorrhizal soil resource uptake. However, not all native plant species depend on mycorrhizal fungi, creating opportunities for allelopathic plant invaders to have disparate impacts across plant communities. Therefore, direct impacts of allelopathic plant invasion on mycorrhizal fungal taxa and the subsequent indirect effects on native plant species are not expected to be universal. For my research, I use a model for allelopathic plant invasion, *Alliaria petiolata* (garlic mustard). My dissertation expands mechanistic understanding of impacts of allelopathic plant invasion, knowledge of the consequences of complex species interactions and allows for predictions of mutualism disruption across environments. I do this by 1) quantifying community change in mycorrhizal mutualists in plant roots exposed to garlic mustard in the field, 2) scaling up known physiological- and population-level effects of mutualism disruption to the plant community, and 3) understanding the impacts of garlic mustard and mutualism disruption across heterogenous environmental conditions and invader abundance. My results show that garlic mustard increased rare AM fungal taxa in plant roots, had a disproportionate negative effect on the mycorrhizal plant community relative to non-mycorrhizal plant community members, the negative effects of mutualism disruption on the native plant community are intensified at high garlic mustard abundance, and are evident across regional-scale despite heterogeneous environmental conditions. These results provide strong support for the mutualism disruption hypothesis as an important theory for understanding causes and consequences of allelopathic plant invasion and build links between the above- and below-ground community consequences of mutualism disruption. Mechanistic drivers of invader impact can scale across levels of

ecological organization and doing so enhances our predictive ability of the impacts of invasive species.

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List of plant species and mycorrhizal status in the Illinois Critical Trends Assessment Dataset (CTAP plant list.docx)

INTRODUCTION

Global change is increasing environmental stress for most organisms on Earth (Tylianakis et al. 2008). Major global threats include 1) habitat loss due to harvesting and encroachment by human development that increases fragmentation, decreases patch size, and alters gene flow (Brooks et al. 2002, Fahrig 2003, Aguilar et al. 2006), climate change that leads to shifts in phenology, physiology, and species ranges (Dillon et al. 2010, Bellard et al. 2012, Kharouba et al. 2018), and biological invasions (Bellard et al. 2016, Downey and Richardson 2016).

Biological invasions occur when a species is introduced to a novel environment and rapidly expands its range due to a removal of ecological barriers to population growth or spread (Valéry et al. 2008). Increasing global trade is expected to increase the number of introductions and thus the number of invasions globally (Levine and D'Antonio 2003), highlighting the growing threats of invasive species to native communities. Invasive species cause billions of dollars of economic damage in the United States alone (Pimentel et al. 2005). In addition, the ecological consequences can be detrimental for biodiversity within native ecosystems. Invasive species impact native communities largely in three ways. First, invasions can manipulate the abiotic environment in a way that is unfavorable to native species, such as increasing nutrient availability which alters ecosystem functioning (Ehrenfeld 2010) and mutualism dynamics (Johnson 2010, Grove et al. 2017), changing hydrological conditions such as water clarity (Strayer et al. 2019), or altering frequency of fire regimes (Brooks et al. 2004). Second, invasive species may directly diminish members of the native community through predation, herbivory, or pathogenicity (Orwig et al. 2002, Blehert et al. 2009, Loss et al. 2013). Finally, invasive species can directly (Brown et al. 2002, Vilà and Weiner 2004) or indirectly (White et al. 2006, Pringle et al. 2009) compete with native species for resources.

Since invasive species by definition join communities, they fundamentally alter species interactions (Mitchell et al. 2006, Tylianakis et al. 2008), frequently in a negative direction (Tylianakis et al. 2008, Kiers et al. 2010). New interactions between invasive and native members of the community are developed (Levine and D'Antonio 1999), but by interacting with native species the invader can negatively alter native-native interactions as well (Christian 2001, Brown et al. 2002, Cahill et al. 2008). The effects of

invasive species on mutualistic species interactions are particularly important to understand because beneficial partnerships may buffer each other from the impacts of an invasion (Fricke et al. 2017), but may also be the vector by which invasive species impact native species (White et al. 2006, Cipollini et al. 2012, Aslan et al. 2013, Rogers et al. 2017). For example, the novel weapons hypothesis postulates that invasive species can become problematic in the invaded range when they produce powerful chemical weapons that are evolutionarily novel to native species (Callaway and Ridenour 2004). These novel weapons can directly affect microbial communities (Callaway and Ridenour 2004, Cipollini et al. 2012), but assessments of these indirect effects are rare (White et al. 2006).

While it is apparent that invasive species can pose many threats to the ecology and evolution of natural communities, it remains challenging for scientists and land managers to predict the impact a specific non-native species may have on a specific community (Yokomizo et al. 2009, Hulme 2015). This is due to an incomplete understanding of the processes by which invasive species may alter the community, and how the success of the invader varies in response to the native community, time since invasion, and invader abundance. The impacts of invasions occur from lower levels of ecological organization, affecting species populations first and then, over time, possibly cascading up to communities and then to ecosystems (Vilà et al. 2011). For this reason, it is important to understand the mechanism(s) driving the impact of an invasion on individual organisms to provide explanations for processes that link ecological scales. For those invasive species that have well-understood mechanisms driving their invasion success at the level of individual species interactions, the next step is to assess the mechanistic process in a more complex and realistic community setting to build theory and better predict consequences of these interactions (Hausmann and Hawkes 2009, Lekberg et al. 2013, Simberloff et al. 2013). If a hypothesized mechanism predicts ecological outcomes across scales of ecological organization, there is a strong argument for a generalizable effect of that mechanism.

Alteration of species interactions by non-native plant species

Invasion of non-native plant species is one global change factor that can fundamentally alter interactions among native species both above and belowground

(Christian 2001, Traveset and Richardson 2014). The effects of invasions on species interactions are often overlooked, as they are assumed to only be locally important (Wiens 2011) in comparison to abiotic global change factors (e.g., climate, nitrogen [N] deposition), even though this is not always true (Wisz et al. 2013). By entering and establishing in a community, non-native invaders fundamentally alter species interactions and threaten the integrity of mutualistic relationships (Christian 2001, Traveset and Richardson 2014, Rodrigues et al. 2015). This process can occur when an invader creates novel interactions or when it directly or indirectly harms populations of any species that participates in a mutualistic partnership (Traveset and Richardson 2014). Invasive species can compete with native species for mutualism services (Brown et al. 2002), disrupt interactions (Rogers et al. 2017), or create environmental conditions that are unfavorable to mutualism persistence (Johnson 2010). The least obvious of these potential consequences occur when the invasive species creates environmental conditions that are unfavorable for the persistence of one partner, which indirectly impacts the interaction. This phenomenon is particularly common in plant invaders that alter the soil environment by changing soil nutrient properties or by releasing chemical compounds that are toxic to certain soil microbes (Cipollini et al. 2012, Grove et al. 2017). Since the introduction and spread of invasive species is one of the foremost threats to biodiversity (Bellard et al. 2016), an understanding of the relationship between biological invasion and species interactions is paramount to fully understanding threats to biodiversity (Aslan et al. 2013).

Beneficial interactions among species may rescue organisms from the detrimental consequences of global change (Bascompte et al. 2006, Fricke et al. 2017), but the strength and outcome of interactions themselves can be altered under changing environmental conditions (CaraDonna et al. 2017). Disruption of important mycorrhizal interactions is a common and potentially devastating consequence of anthropogenic global change. These mutualisms can be disrupted in multiple ways including (1) changing the context-dependency of plant-fungal interactions (Johnson 2010, Kiers et al. 2010), (2) shifting plant-fungal interactions and network structure (Kiers et al. 2010), or (3) shifting the composition of the plant or fungal community (Johnson et al. 2013). Arbuscular mycorrhizal (AM) fungi are one group of soil microbes that may be

vulnerable when plant invaders alter soil conditions (Hale and Kalisz 2012, Grove et al. 2017). Since an estimated 72% of land plants form relationships with AM fungi (Brundrett and Tedersoo 2018), a large number of plant species could be negatively impacted by this type of mutualism-disrupting plant invader. AM fungi are plant root symbionts that extend hyphae from persistent structures inside the plant root into the soil (Parniske 2008). These hyphae capture water and nutrients that are inaccessible to the plant root, and trade these soil resources for photosynthetically-derived carbon compounds from the plant (Parniske 2008). AM fungi can only briefly survive without a plant partner (Parniske 2008), and their plant partners suffer physiological declines when AM fungal function is disrupted (Hale et al. 2016).

One mechanism of invader soil conditioning is the release of antimicrobial allelochemicals from invasive plant tissues (Cantor et al. 2011, Del Fabbro and Prati 2015, Zhang et al. 2020) that kill fungal hyphae in the soil and cut off the transport of essential resources to the native plant (Vaughn and Berhow 1999, Hale et al. 2016). The loss of this critical nutritional mutualism has dramatic physiological consequences for native plants that rely on AM fungi (Hale et al. 2016, Bialic-Murphy et al. *in revision*). Numerous non-native plants are suspected of releasing antimicrobial chemicals into the soil (Kalisz et al. 2020, Zhang et al. 2020) that may cause resource stress in the large number of native plants that rely on those soil fungi. Exposure to the chemicals from one such invader, *Alliaria petiolata* (Brassicaceae, Bieb. Cavara & Grande, hereafter garlic mustard), disrupts the plant-AM fungal mutualism, which causes physiological stress, shifts carbon allocation, and lowers population growth rates in native plants (Hale et al. 2011, 2016, Brouwer et al. 2015, Bialic-Murphy et al. 2019).

Garlic mustard as a model organism

Garlic mustard is an invasive herbaceous plant that was introduced to Long Island, NY in 1868. Since its introduction, garlic mustard has spread across much of the United States and Southern Canada (Nuzzo 1999, Welk et al. 2002), where it has become a problematic forest invader. Although garlic mustard has many traits associated with weediness and invasibility, such as increasing availability of soil nitrogen and high seed production (Anderson et al. 1996, Rodgers et al. 2008), the most promising explanations of its success as an invasive species and its impact on native

communities lie in garlic mustard's allelopathic properties through the production of antimicrobial glucosinolates (Vaughn and Berhow 1999, Barto et al. 2010b). Glucosinolates produced by garlic mustard are novel in North America (Barto et al. 2010b) and are highly toxic to many North American AM fungi that are naïve to these compounds (Callaway et al. 2008), limiting fungal survival and spore germination even at very low concentrations (Cantor et al. 2011). An inhibited extraradicular hyphal network is hypothesized to reduce plant physiology by disrupting the exchange of water and resources from the soil to the plant root (Hale et al. 2016), while possibly incurring the carbon cost of maintaining parasitic internal fungal structures (Johnson 2010, Hale et al. 2016). In the short term, the effects of mutualism disruption manifest in reduced physiology of native plant species (Hale et al. 2011, 2016, Brouwer et al. 2015). Over time, this builds to shifts in vital rates and then demography (Stinson et al. 2006, Brouwer et al. 2015, Bialic-Murphy et al. 2019). It is unknown whether these negative demographic responses of native plants to garlic mustard invasion will cascade to altered abundance and diversity of plant communities. Importantly, the magnitude of glucosinolate concentration, and thus toxicity to mycorrhizal fungi, in plant tissues varies widely by invasion age, light availability, population density and environmental heterogeneity (Lewis et al. 2006, Lankau et al. 2009, Smith 2015, Evans et al. 2016). Because allelopathic glucosinolate production is difficult to predict, spatial patterns of garlic mustard invasion are challenging to disentangle.

Garlic mustard directly affects mycorrhizal fungi with toxic secondary metabolites that are released into the soil (Roberts and Anderson 2001). Exposure to these chemicals at biologically realistic concentrations inhibits AM fungal hyphal growth and spore germination (Cantor et al. 2011). In field conditions, garlic mustard presence decreases soil hyphal abundance (Rodgers et al. 2008, Cantor et al. 2011, Koch et al. 2011, Hale et al. 2016), and higher soil AM fungal diversity (Anthony et al. 2019, Bialic-Murphy et al. *in revision*). These effects are thought to contribute to the impact of garlic mustard on plant physiological responses to garlic mustard invasion (Hale et al. 2016), but it is unknown how the mutualist community within plant roots mediates the effects of garlic mustard on extraradicular hyphae.

Although it has been established that garlic mustard negatively indirectly affects native mycorrhizal plants through inhibition of their fungal symbionts, it has been questioned how widespread and generalizable the impact of mutualism disruption is on native plant species (Cipollini and Cipollini 2016). The most detailed study of the effects of mutualism disruption have been shown in the perennial mycorrhizal herb *Maianthemum racemosum*, which shows declines in physiology, reproduction, and root AM fungal diversity (richness) in the presence of garlic mustard and glucosinolate exudates (Burke 2008, Hale et al. 2011, 2016, Brouwer et al. 2015). However, plant species exist on a spectrum of mycorrhizal dependency (i.e. biomass response to mycorrhizal colonization) from obligately mycorrhizal to non-mycorrhizal (Tawaraya 2003). *Maianthemum racemosum* produces no fine root hairs, and is highly colonized by AM fungi (Brundrett and Kendrick 1988), and thus presumably has a high dependence on AM fungi. Thus, we expect that mutualism disruption would have high consequences for a species with traits similar to *Maianthemum*. Under the assumption that mutualism disruption only negatively effects plants that have high mycorrhizal dependency, we would not expect all members of the plant community to respond in the same way to garlic mustard invasion and mutualism disruption.

Within the native plant species that associate with AM fungi and have been experimentally tested, responses to garlic mustard differ. The AM fungal community within *Maianthemum* roots significantly shifted after exposure to garlic mustard, but there were no associated declines in richness of AM fungal taxa colonizing mycorrhizal herbs, *Trillium grandiflorum* or *Arisaema triphyllum* (Burke 2008). However, co-occurrence with garlic mustard negatively impacts population growth rates of *Trillium grandiflorum* (Bialic-Murphy et al. 2019). Seedling growth and arbuscular mycorrhizal colonization of three native trees were highly decreased by garlic mustard conditioned soil and exposure to garlic mustard tissue extracts (Stinson et al. 2006). Other plant species may not be as sensitive to garlic mustard. For example, negative effects of garlic mustard on *Impatiens pallida* growth were seen only before colonization by AM fungi, but not after (Barto et al. 2010a). This suggests that *Impatiens* pre-symbiosis growth stage is sensitive to direct effects of garlic mustard, but that this species may be buffered from any direct effects of garlic mustard by fungal partners (Barto et al. 2010a).

However, this study is limited in that the authors exposed *Impatiens* plants to two chemical extracts from garlic mustard instead of the full chemical profile of the plant. They also did not assess a mechanism to explain this response of *Impatiens* plants to exposure to garlic mustard extracts. Since the impacts of mutualism disruption can be mediated through water stress (Hale et al. 2016) or nutrient stress, an alteration of the plant's physiological mechanisms likely explain their results. Other work has shown that a native mycorrhizal herb, *Pilea pumila,* has evolved a tolerance to soil conditions created by garlic mustard (Lankau 2012). Further, growth of the non-mycorrhizal plant *Arabidopsis thaliana* (Brassicaceae) was not impacted by garlic mustard extracts (Cipollini et al. 2008), although this plant species also produces glucosinolates. While prior studies of the individual effects of garlic mustard on individual plant species have yielded major insights regarding the mechanism of the invader's impacts, such studies cannot be extrapolated to draw conclusions regarding garlic mustard's effects at the plant community level.

An above- and below-ground community approach is especially important because garlic mustard is expected to affect members of both the plant and the fungal communities in different ways. Because garlic mustard's impact on native plants is mediated by disruption of beneficial interactions with AM fungi (Hale et al. 2016), individual members of plant communities that vary in mycorrhizal dependency will likely not respond to garlic mustard exposure in the same manner. Plant species that are highly dependent on their mycorrhizal associations may exhibit strong negative reactions when exposed to garlic mustard's allelochemistry, whereas those that form weak or no mycorrhizal associations may initially respond very little, or not at all. Moreover, it is possible that AM fungal taxa may vary in their tolerance to the presence of garlic mustard. For example, AM fungal taxa that tend to produce less extraradicular biomass, such as those within the Glomeraceae (Chagnon et al. 2013), may be guarded from some of the stresses of exposure to garlic mustard's allelochemicals, and may be favored in the presence of garlic mustard. If there are differential responses of AM fungal taxa to the invasion of garlic mustard, this may lead to altered interactions among plants and AM fungal symbionts in addition or instead of changes in above or belowground species richness. In this way, garlic mustard may be filtering the

understory communities it invades by changing belowground soil conditions via allelochemistry to alter the fungal community, and in turn, selectively impacting plant species that rely heavily on their fungal partners. There is evidence that garlic mustard is a poor direct competitor in the invaded range (Meekins and McCarthy 1999, Bossdorf et al. 2004), but there may be processes mitigating the impact of garlic mustard on the plant community, such as direct competition or abiotic conditions. A community approach will establish the generality of the effects of mutualism disruption in contrast to other potential processes.

Although it is clear that there are negative effects of garlic mustard on individual members of both the plant and fungal communities, it is unclear how both of these communities are responding to garlic mustard. We expect decreased diversity in both AM fungal and plant communities exposed to garlic mustard. However, since garlic mustard is predicted to impact the plant community only through disruption of mycorrhizal mutualisms, we expect that only the mycorrhizal component of the plant community will decrease in diversity. We also expect that this pattern will hold across heterogeneous environmental conditions and become stronger as garlic mustard increases in abundance.

A shift in the AM fungal community within plant roots would show that garlic mustard is altering how the root fungal community interacts with the plant community and depending on the structural components driving that shift, could suggest either resilience or vulnerability of the interactions. Since mutualism disruption by garlic mustard has been clear in a plant species that has presumably high dependence on mycorrhizal fungi (*Maianthemum*) contrasted by a lack of response by a non-mycorrhizal plant (*Arabidopsis*), testing for the effects of mutualism disruption across a plant community that varies in mycorrhizal status is an important step in understanding native plant species vulnerability to garlic mustard via mutualism disruption. Third, addressing the scale at which mutualism disruption is detectable will provide insights into whether this mechanism shapes plant communities invaded by garlic mustard at the regional level. The forest understory hosts a large component of the diversity of Eastern deciduous forests (Gilliam 2006). In addition, these forests house species that are diverse in their mycorrhizal associations (Brundrett and Kendrick 1990, Soudzilovskaia

et al. 2020). For these reasons, forest understories are vulnerable to mutualism disruption, and are a key study system for assessing the impacts of allelopathic plant invasion.

My dissertation explores the impacts of mutualism disruption by garlic mustard on ecological communities to determine the generality and scope of mutualism disruption by invasive plants. Simultaneous evaluation of the mutualism disruption hypothesis and realized, community-level impacts of this plant invader will associate the mechanistic process with the community-level consequences to link key attributes of invasion biology. I evaluated the above- and below-ground community consequences of mutualism disruption by addressing the following questions.

Chapter 1: In what ways does garlic mustard invasion change the community of AM fungi residing in native plant roots relative to plants in invader removal plots?

Chapter 2: To what extent can changes in plant community composition and diversity in garlic mustard invaded communities be explained by shifts in abundance of mycorrhizal and non-mycorrhizal plant species?

Chapter 3: How does the abundance of garlic mustard relate to its impact on plant abundance and diversity across a heterogeneous environment?

Answers to these questions will provide critical insights into the plant and fungal community level effects of garlic mustard by linking a mechanistic process (mutualism disruption) with a biological invasion. Mechanisms of invasion are seldom well-understood. But when they are, context-dependence in community level impacts can cloud the broad conclusions sought out by researchers. Even with a highly studied invasive plant, such as garlic mustard, conclusions about its impacts on individual members of the native plant community can differ. However, use of mechanistic hypotheses can help explain different responses of native species to garlic mustard. Tying together the above- and below-ground community consequences of garlic mustard invasion will advance our understanding of allelopathy as a mechanism for

plant invasion (Zhang et al. 2020). My research describes the impact of garlic mustard invasion on belowground root fungal communities as well as the large-scale responses of plant communities across the invaded range. Part of these species-specific responses may be explained by the plant species' ability to attract and maintain different AM fungal taxa in the face of mutualism disruption by garlic mustard. I will assess this possibility by quantifying garlic mustard-mediated changes in the AM fungal communities within plant roots. Since the response to garlic mustard is expected to first impact interactions between plants and AM fungi, but has also been detected as declines in population growth rates (Bialic-Murphy et al. 2019), the next step is to assess whether garlic mustard causes a predictable impact on native plant communities. A predictive framework is only useful if it holds up across heterogeneous conditions. In the final chapter, I described changes in the plant community at varying invasion intensity and environmental conditions. Bridging the gaps between belowground connections, and large-scale community change will provide a powerful assessment of the scale and direction of the effects of garlic mustard invasion as well as the mutualism disruption hypothesis as a whole. Most studies on allelopathic invasions occur in controlled lab settings (Zhang et al. 2020), which although needed for careful examination of allelopathic properties, does not explore the whole picture. Novel weapons such as allelopathy may seem to show inconsistent impacts, but we know that it is the recipient of those allelopathic effects (i.e. the native plant) that explains more of the variance in impacts rather than the identity of the allelopathic plant (Zhang et al. 2020). My work provides a first assessment of how traits of the native species (here mycorrhizal status) dictate the response of the native community to allelopathic plant invasion. For this reason, it is important to understand the underlying mechanism that predicts why portions of the native community may be more vulnerable to those novel weapons.

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CHAPTER 1 REMOVAL OF ALLELOPATHIC PLANT INVADER DECREASES RARE ARBUSCULAR MYCORRHIZAL FUNGI IN NATIVE PLANT ROOTS

Abstract

Invasive species fundamentally alter species interactions when they enter and establish in a community. Understanding the process by which this occurs, and the impact of these altered species interactions is foundational to predictions of future success of invasive species. Disruption of mycorrhizal fungal mutualisms is a well-established mechanism by which allelopathic invasive plants indirectly gain a fitness advantage over native plant species that rely on mycorrhizal mutualists. Allelopathic plant invasion can reduce soil hyphal abundance of arbuscular mycorrhizal (AM) fungal hyphae, which causes physiological stress in native plants, alters vital rates, decreases population growth rates, and changes plant community composition. However, the role of the AM fungal community in this mechanistic process remain unclear. I sampled the root AM fungal community in native herbaceous plant species from a 12-year experiment in a deciduous forest in Pennsylvania in which plots experienced ambient levels of the allelopathic invasive plant, *Alliaria petiolata* (garlic mustard) or were weeded to remove the invader. Richness and Shannon diversity, a measure of evenness, of AM fungi in plant roots was higher in all plant species in the garlic mustard ambient treatment. However, garlic mustard treatments did not change the AM fungal community composition or inverse Simpson diversity, which are both more sensitive to changes in abundance of common taxa. By inhibiting AM fungal growth in the soil and promoting increases in less common AM fungal species, garlic mustard's allelochemistry may be creating an environment that forces plants to be less selective with AM fungal partners. Since previous work at in these plots found that plants exposed to garlic mustard show signs of physiological stress and decreased population growth rates, these rare AM fungi may be playing a different role than the more highly abundant fungi that are found in roots in both garlic mustard treatments, either by moderating or exacerbating the effects of garlic mustard on native plant species.

Introduction

Although plant invasions are among the leading drivers of biodiversity loss, the mechanisms facilitating impacts of invaders are often unclear (Levine et al. 2003, Ricciardi et al. 2013, Simberloff et al. 2013). Many hypotheses have been proposed to explain the success of invasive species (e.g. high invader fecundity, rapid evolution and adaptation to new habitat, novel resource use, ecosystem engineering, novel weapons; Crooks 2002, Callaway and Ridenour 2004, Funk and Vitousek 2007, Whitney and Gabler 2008, Jelbert et al. 2015), but there remain few conclusive studies and therefore generalizations about the relevance of these hypotheses (Ricciardi et al. 2013). Since generalizations that lead to broad understanding of invasion impact are a primary goal of invasion ecology, thorough assessments of these hypotheses are vital for forward progress and ultimately management of invaded ecosystems.

Understanding the role of the native community is critical in uncovering patterns of invasion success. The native community can exhibit biotic resistance to invasion, which limits the abundance of the invader (Levine et al. 2004). It has been shown that at small spatial scales, communities with higher species richness are more resistant to invasion (Levine and D'Antonio 1999, Fridley et al. 2007) and are less likely to experience strong impacts of invasion (Levine et al. 2004). Microbes in particular are important mediators of the effects of plant invasions on native communities (Hawkes et al. 2005, Cipollini et al. 2012, Rodrigues et al. 2015, Dawson and Schrama 2016), particularly because of the important roles of microbes in plant productivity and nutrient cycling in the soil (Klironomos et al. 2000, Van Der Heijden et al. 2008).

Many invasive species gain an advantage over native species by disrupting native mutualisms (Hale and Kalisz 2012, Traveset and Richardson 2014, Grove et al. 2017). By joining a native community and increasing in abundance, invasive plants necessarily alter important interactions between native species (Russo et al. 2014). Belowground, invasive plants disrupt native plant-microbial mutualisms by changing aboveground plant communities (Kivlin and Hawkes 2011), altering the soil biogeochemical conditions (Johnson 2010, Grove et al. 2017), and releasing novel allelopathic compounds that are toxic to naïve microbial mutualists (Roberts and Anderson 2001, Callaway et al. 2008, Barto et al. 2010, Cantor et al. 2011). Uncovering

the response of the microbial mutualist community to allelopathic plant invasion is an important step towards understanding how mutualisms facilitate or buffer the effects of plant invasion.

Plant invaders commonly produce secondary metabolites that impede the growth or establishment of other nearby plant species (i.e. allelopathy) (Kalisz et al. 2020, Zhang et al. 2020) either by direct phytotoxicity or indirectly through disruption of mutualisms (Hale and Kalisz 2012). Mycorrhizal mutualism disruption by allelopathic compounds is thought to decrease soil hyphal abundance (Cantor et al. 2011) and disrupt the uptake and exchange of soil resources for photosynthetically-derived carbon compounds, which negatively affects plant physiology and vital rates (Stinson et al. 2006, Meinhardt and Gehring 2012, Brouwer et al. 2015, Hale et al. 2016), population growth rates (Bialic-Murphy et al. 2019), and plant communities (Stinson et al. 2007, Roche et al. 2020). Although allelopathy is common across plant families (Kalisz et al. 2020), and is highly associated with plant invasion, there are few systems in which these effects have been uncovered (Hale and Kalisz 2012, Grove et al. 2017, Kalisz et al. 2020, Zhang et al. 2020), so the threat of allelopathy in plant invasion is likely underestimated. Arbuscular mycorrhizal (AM) fungi are a group of critical plant root obligate mutualists, that form associations with an estimated 72% of flowering plants (Brundrett and Tedersoo 2019, Soudzilovskaia et al. 2020). AM fungi provide the plant partner with increased acquisition of water and soil resources in exchange for carbon compounds from the plant (Parniske 2008). A disruption of this important mutualism, for example by allelopathic plant invasion, can have extensive consequences for the plant partner (Stinson et al. 2006, Callaway et al. 2008, Meinhardt and Gehring 2012, Hale et al. 2016).

If the impacts of allelopathic plant invasion on the native plant community are mediated by AM fungal mutualisms, it is imperative that we understand how the AM fungal community either mitigates or exacerbates the detrimental effects of invasion for native plants. Invasion by allelopathic plants can result in multiple outcomes for AM fungal communities of native plant roots depending on how resilient or resistant the AM fungi are to anti-microbial compounds. Persistent invasion and subsequent alteration of soil fungal communities (Anthony et al. 2019) could lead to the depletion of AM fungi in

roots of long-lived perennial plant species, conversely, successful removal of plant invasion could allow eventual AM fungal recolonization (Lankau et al. 2014). A change in root AM fungal community composition would suggest that plants exposed to an allelopathic invasion may be able to select fungal partners that are more tolerant of the novel invaded soil conditions, and thus more cooperative mutualists (Kiers et al. 2011). However, these new partners may not be functionally redundant with the pre-invasion fungal community (Klironomos 2000). Finally, AM fungal biomass as a whole may be reduced (Cantor et al. 2011), with a higher chance of local extinction of AM taxa in roots exposed to invaded soil conditions.

Using a long-term (12 year) invader removal/invader ambient experiment, I investigated the effects of *Alliaria petiolata* (Brassicaceae, Bieb. Cavara & Grande, hereafter garlic mustard) on the AM fungal community within roots of five native plant species. Specifically, I aimed to understand how garlic mustard impacted 1) AM fungal biomass within plant roots, as well as the 2) diversity and composition of AM fungi in plant roots. I expected biomass of AM fungi in plant roots would be reduced in garlic mustard ambient treatments because allelopathic chemicals released by garlic mustard over 12 years has inhibited fungal growth and spore germination in soil, thus diminishing the mutualism's function (Cantor et al. 2011). I also expected that lower biomass of AM fungi in plant roots would subsequently lead to a reduction in AM fungal diversity and a shift in community composition within plant roots. Here I explore the extent to which the AM fungal community within native roots is altered by garlic mustard invasion to gain deeper insights into the consequences of mutualism disruption by allelopathic plant invasion.

Methods

Study species and Experimental Design

Garlic mustard is an invasive biennial plant that was first recorded in the United States in 1863 (Nuzzo 1999) and has since invaded forest understories across much of the east coast of the United States and southern Canada. Like many invasive plants (Kalisz et al. 2020, Zhang et al. 2020), garlic mustard alters soil conditions via

allelopathy (Roberts and Anderson 2001). Part of the success of garlic mustard stems from its novel secondary metabolites (Barto et al. 2010), which are released into the soil, kill extraradicualar fungal hyphae and reduce fungal spore germination (Cantor et al. 2011). This exposure to garlic mustard's allelochemicals in the field consequently decreases abundance of soil AM fungal hyphae (Rodgers et al. 2008, Cantor et al. 2011, Koch et al. 2011, Hale et al. 2016) and increases AM fungal richness in bulk soils (Anthony et al. 2019, Bialic-Murphy et al. *in revision*) but not soil resource availability between garlic mustard weeded and ambient treatments (Bialic-Murphy et al. *in revision*). It is currently unknown how the AM fungal community within plant roots responds to garlic mustard invasion.

In 2003, we established five paired 14 m x 14 m study plots (10 plots total) in the Trillium Trail Nature Reserve (50.520237, -79.900932), Fox Chapel, Pennsylvania. One pair from each plot was randomly assigned as deer access and the other deer exclusion. The deer access plots remained unaltered, while the deer exclusion plots were surrounded by a 2.5 m tall wire fence to prevent deer access but allow smaller animals to pass freely. I included the data from both deer treatments in the analyses and in the error structure for all statistical analyses but did not specifically evaluate the effect of deer. One half of each deer treatment plot was assigned as garlic mustard "ambient" and remained unaltered and at ambient levels of garlic mustard invasion while the other half was assigned as garlic mustard "weeded". All garlic mustard plants were removed from the weeded treatment plots annually and disposed of off site. Focal plant species for this study were selected based on their occurrences in the plots. Only native species with a minimum of three individuals in both treatments within a plot and occurring in at least three plots were included in this study. Of the 75 herbaceous species recorded to be present at Trillium Trail over the last 12 years, and the 21 herbaceous plant species present at the time of sampling, five species met the criteria for inclusion in this study. These species are *Arisaema triphyllum*, *Impatiens spp.*, *Maianthemum racemosum*, *Podophyllum peltatum*, and *Trillium spp.*, all of which are common herbaceous understory species. All are perennial except for *Impatiens spp.*, which is an annual species.

Sample collection and processing

In June of 2018, I harvested three individual plants of each of the five focal species from each treatment in deer access plots, and for *Maianthemum* and *Trillium* in deer exclusion plots. In June of 2019, I collected additional samples for *Maianthemum*, *Trillium*, and *Impatiens* from deer exclusion plots. For the subset of plant species that were sampled in both 2018 and 2019, I never detected a difference in the AM fungal community between samples from the same garlic mustard treatments, so year was not included in subsequent analyses. Roots from sampled plants were surface sterilized and pooled within 24 hours. I froze 25-50 mg of plant tissue at -80 °C in preparation for DNA extraction. I pooled three individuals per sample so that any patterns will be driven by plant species, rather than individual effects.

We extracted DNA from 25-50 mg of plant root tissue with a Qiagen DNeasy Plant Mini Kit (Valencia, CA), and used a nested PCR approach to identify AM fungal sequence variants. We amplified the 18S (small ribosomal subunit) region using first general eukaryotic primers NL1-NL4 (O'Donnell 1993). Each PCR reaction contained 21.5 µL of Platinum PCR Supermix (Invitrogen, Carlsbad, CA, USA), 1.25 µL of each primer (10 µM), 0.5 µL of BSA (20 mg/mL), and 2 µL (~20 ng) of DNA. The thermocycler conditions for the first PCR with NS1 and NS4 primers were as follows: 94 °C for 3 minutes, followed by 30 cycles of 94 °C for 30 seconds, 58 °C for 60 seconds, and 72 °C for 60 seconds and 72 °C for 10 minutes. I used 2 µL of the first PCR product as the DNA template for the second PCR step, and AM fungal specific primers NS31-AM1 (Lekberg et al. 2012). All other reaction volumes remained identical to the first PCR step. Thermocycler conditions for the second PCR step were as follows: 94°C for 5 minutes, followed by 40 cycles of 94°C for 45 seconds, 63.1°C for 60 seconds, and 72°C for 90 seconds and finally 72°C for 10 minutes. This PCR step was run in triplicate, combined, and quantified on a qubit fluorometer. Samples were purified with magnetic beads (Agencourt) and quantitated to equal concentration before unique barcodes were ligated onto each sample. Samples were sequenced with Illumina MiSeq (2 x 275bp PE run) at the University of Tennessee, Knoxville Genomics Core. I cleaned sequences and grouped them into exact sequence variants using default settings in the DADA2 pipeline (Callahan et al. 2016).

Root colonization by AM fungi

I tested for differences in intraradicular hyphal biomass between garlic mustard treatments by scoring host plant roots for percent colonization by mycorrhizal fungi. To prepare roots for microscopy, I cleared roots in 10% KOH for 5 days, acidified them in 1% HCl for 1 minute, stained them in 0.01% acid fucshin in 14:1:1 lactic acid:glycerol:deionized H_2O for 1 day, and destained them in 14:1:1 lactic acid:glycerol:deionized H_2O for at least 1 day (Grace and Stribley 1991). I scored for hyphal abundance at 200X following a gridline intersection method for ~ 100 root intersections per individual (McGonigle et al. 1990) on a LAXCO LMC-3000 upright compound microscope. Percent root colonization was arcsin square root transformed to meet assumptions of normality. For each plant host species, I used a linear model to explain differences in percent root colonization by garlic mustard treatments.

AM fungal diversity

To understand changes in the root AM fungal community between garlic mustard treatments, I assessed AM fungal diversity within plant roots. I calculated species richness, Shannon diversity and Inverse Simpson diversity. Species richness does not account for changes in abundance, which makes this metric sensitive to changes in rare species. Conversely, Inverse Simpson diversity is more sensitive to changes in common species because it accounts for taxon abundance (Morris et al. 2014). For each metric, I used a general linear mixed effects model with an interaction between garlic mustard treatment and plant host species as a fixed effect, and garlic mustard treatment nested within deer treatment nested within plot as a random effect. I used a negative binomial error structure to account for overdispersion in species richness and inverse Simpson diversity.

AM fungal community composition

I used distance-based redundancy analysis to compare dissimilarity in AM fungal composition within plant roots between ambient and garlic mustard weeded treatments. I expected that AM fungal communities would be significantly different between garlic mustard weeded and ambient treatments. The model was structured with an interaction

between garlic mustard treatment and plant host species to explain jaccard dissimilarities between AM fungal communities in the garlic mustard ambient and garlic mustard weeded treatments.

Statistical analyses

All statistical analyses were conducted using R version 3.6.2 (R Core Team 2020). I calculated diversity metrics, conducted distance-based redundancy analysis, and made ordinations using the vegan package version 2.5-6 (Oksanen et al. 2019). Linear models were estimated using the stats package in base R. Linear mixed effects models were estimated using glmmTMB version 0.2.3 (Brooks et al. 2017) and the fit of those models were tested using DHARMa version 0.2.6 (Hartig 2016).

Results

Root colonization of AM fungi

The effect of garlic mustard on percent root colonization varied by species. Garlic mustard weeding increased percent root colonization by 18.2% for *Arisaema* and 9.2% for *Maianthemum* (Figure 1.1a, 1c; *Arisaema*: $F = 5.89$, $p = 0.07$; *Maianthemum*: $F = 6.49$, $p = 0.04$). However, colonization of *Impatiens* roots responded in the opposite direction, and there was 3.4% less root length colonized when garlic mustard had been weeded (Figure 1.1b; $F = 8.94$, $p = 0.02$). There was no effect of garlic mustard treatment on percent root colonization of *Podophyllum* or *Trillium* (Figure 1.1d, 1.1e; *Podophyllum*: $F = 0.25$, $p = 0.6$; *Trillium*: $F = 2.06$, $p = 0.19$).

AM fungal diversity

I identified a total of 877 ASVs, from five genus's (Acaulospora, Archaeospora, Diversispora, Glomus, and Kuklospora), the vast majority being from the genus Glomus (See supplement). Richness of the root AM fungal community was 7.8% lower (Figure 1.2a) and Shannon diversity was 15.5% lower (Figure 1.2b) in garlic mustard weeded treatments in comparison with garlic mustard ambient treatments. There was no significant interaction between garlic mustard treatment and host species on species richness or Shannon diversity. Plant host species did not have a significant effect on

richness or Shannon diversity (Figures 1.2a, 1.2b). There was no effect of garlic mustard treatment or plant host species on inverse Simpson diversity (Figure 1.2c).

Intraradicular AM fungal community composition

There was no significant interaction between garlic mustard treatment and plant host species. Composition of AM fungal communities were indistinguishable between garlic mustard ambient and weeded treatments (Figure 1.3). However, plant species did host different AM fungal communities within their roots (Figure 1.4).

Discussion

To fully anticipate the threat of invasive species, it is imperative that the mechanistic drivers of invader impact are carefully evaluated. It is becoming more clear that mutualism disruption is the basis for many of the negative effects garlic mustard has on plants in communities it invades (e.g. Roberts and Anderson 2001, Stinson et al. 2006, 2007, Callaway et al. 2008, Hale et al. 2011, 2016, Bialic-Murphy et al. 2019, Roche et al. 2020). In this study, I evaluated the effects of garlic mustard's allelochemicals on the AM fungal community within native plant roots to understand the microbial underpinnings of mutualism disruption. After 12 years of garlic mustard treatments, I found rare AM fungal taxa in plant roots in garlic mustard ambient plots that are not present in plant roots when garlic mustard has been weeded, as indicated by higher AM fungal richness in roots in the garlic mustard weeded treatments. Further, there was no change in AM fungal community composition between garlic mustard treatments. Biomass of AM fungi in plant roots did not consistently change across plant species or garlic mustard treatments, indicating that host plant species may have different strategies for interacting with AM fungi under the two invasion conditions created by the treatments. My results shed first light on the belowground microbial community responsible for mediating the impacts of mutualism disruption by garlic mustard. I found that the abundant (i.e. most common) AM fungal taxa are maintained in both garlic mustard treatments, but that instead of the expected loss of AM fungal taxa in garlic mustard ambient treatments, there are additions of rare AM fungi.

My results on root AM fungal communities are consistent with previous work on the effect of garlic mustard on soil AM fungal communities also showing increased diversity of fungal taxa in the presence of garlic mustard (Lankau et al. 2014, Anthony et al. 2019, Bialic-Murphy et al. *in revision*). I showed that richness and Shannon diversity of the root-associated AM fungal community was higher in garlic mustard ambient treatments than weeded treatments while there was no change in inverse Simpson diversity or community composition between the garlic mustard treatments. This indicates that host plants house the same communities of common, abundant AM fungi, but that there are additional rare AM fungi in plant roots in the garlic mustard ambient treatments. These results are consistent with other studies that have found additional fungal taxa present in invaded plots in comparison with uninvaded plots (Anthony et al. 2017). There are multiple potential consequences of these additional AM fungi. Since it is likely that not all AM fungi are equally susceptible to garlic mustard's allelochemistry (Callaway et al. 2008, Chagnon et al. 2013, Anthony et al. 2017), resistant AM fungal taxa could be ones that can persist in the harsh soil environment created by garlic mustard, and could be providing some benefit that the plant would not be receiving from the shared common fungi; mediating the negative effects of garlic mustard.

Interestingly, despite this increase in AM fungal species richness, mycorrhizal plant species exposed to garlic mustard at our site have decreased vital rates and population growth rates in comparison with plants in weeded treatments (Brouwer et al. 2015, Bialic-Murphy et al. 2019). Further, plant species diversity also declines in the garlic mustard ambient treatments, relative to the weeded treatments (Roche et al. 2020). Therefore, under the altered conditions created by garlic mustard invasion, native plants may be tolerating colonization by less beneficial mutualists (Kiers and Denison 2008), and as a result, experience decreases in the delivery of the expected soil resources. Interestingly, I found that plant host mycorrhizal colonization responded in different ways to the garlic mustard treatments, in contrast to previous work in which there was not a significant effect of garlic mustard on percent mycorrhizal colonization after four years of garlic mustard ambient/weeding treatments (Burke 2008). However, the direction of changes in percent mycorrhizal colonization in this study did not correspond consistently to the direction of changes in plant species abundance at our

site in response to garlic mustard treatments (Roche et al. 2020). This indicates that these plant species could be using different resource allocation strategies, with some allowing AM fungal biomass to persist in their roots, while others may stop providing resources to uncooperative fungi (Kiers et al. 2010). Alternatively, some AM fungi are tolerant to garlic mustard's allelochemicals (Callaway et al. 2008). It is possible the rare AM fungal taxa detected in my experiment are tolerant to garlic mustard's allelochemicals and thus increase in abundance in garlic mustard ambient plots. These additional AM fungal taxa in the roots may then be providing some mutualism services to the native plants and moderating the effects of mutualism disruption when the function of AM fungi more sensitive to garlic mustard's allelochemicals is decreased.

The garlic mustard removal experiment shows responses of the intraradicular AM fungal community to garlic mustard weeding. However a weeding experiment, even one that was conducted for 12 years, may not be synonymous with uninvaded conditions (Barney et al. 2015, Anthony et al. 2019). Garlic mustard is a biennial, so its abundance within a plot can change across years. Thus, my findings could also be affected by variable garlic mustard abundance and its local allelochemical concentrations within and among plots. Garlic mustard's impact on plant diversity and abundance is greater when it reaches high densities (Roche Chapter 3). Further, concentrations of the allelochemicals released by garlic mustard are influenced by garlic mustard density and light availability (Roberts and Anderson 2001, Lankau et al. 2009, Smith 2015). Since I did not measure allelochemical concentrations in the soil over time, my study reflects an overall long-term effect of garlic mustard presence over 12 years. However, previous work at this field site has shown that allyl-isothiocyanate (AITC), an antimicrobial compound released by garlic mustard, is detectable in garlic mustard ambient treatments at concentrations that are known to inhibit AM fungal spore germination; AITC was never detected in garlic mustard uninvaded areas (Cantor et al. 2011). Therefore, despite inevitable unmeasured variation in exposure to allelochemicals released by garlic mustard, I am confident that the garlic mustard treatments were effective.

My results shed light on the belowground community responses within plant roots to garlic mustard invasion. I investigated beyond the physiological responses of native

plants to biomass of AM fungi in soils or roots, to understand how the AM fungal community within plant roots change in response to garlic mustard ambient and weeded treatments. These results provide a deeper insight into how invasive species change interactions between plants and AM fungi and uncover the role of the AM fungal community in plant response to disturbance. Although I focus on the effects of garlic mustard, microbes frequently mediate the effects of allelopathic invasion (reviewed in Cipollini et al. 2012) and plant invasions in general (Dawson and Schrama 2016). In addition, invasive plants across the tree of life express allelopathic traits (Kalisz et al. 2020). Consequently, I expect my findings of increased rare AM fungi in ambient garlic mustard treatments to have broad implications for understanding the microbial drivers of plant invasion. I provide further support that plant invasions generally increase richness of AM fungi (Lekberg et al. 2013, Anthony et al. 2019), and that this pattern in soils is matched in plant roots (Kivlin and Hawkes 2011). Further investigation will determine whether these shifts in the root AM fungal community mediate or exacerbate the effects of allelopathic plant invasion.

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Appendix

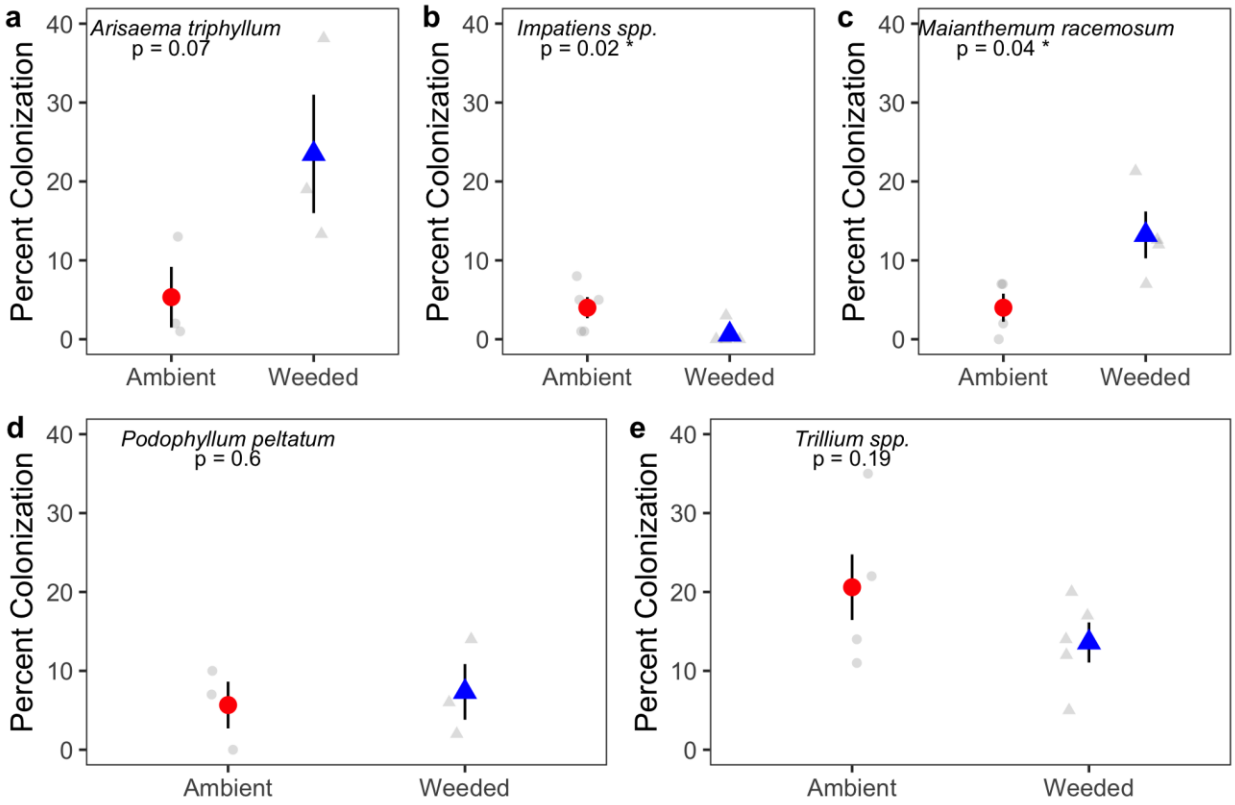


Figure 1.1 Effect of garlic mustard treatment on root length colonized by AM fungi for a) *Arisaema triphyllum*, b) *Impatiens spp*. c) *Maianthemum racemosum*, d) *Podophyllum peltatum*, and e) *Trillium spp*. Points are mean percent root colonization for garlic mustard ambient (red circles) and garlic mustard weeded (blue triangles) treatments. Gray points are raw data for ambient (circles) and weeded (triangles) garlic mustard treatments. Bars are +/- 1 SE.

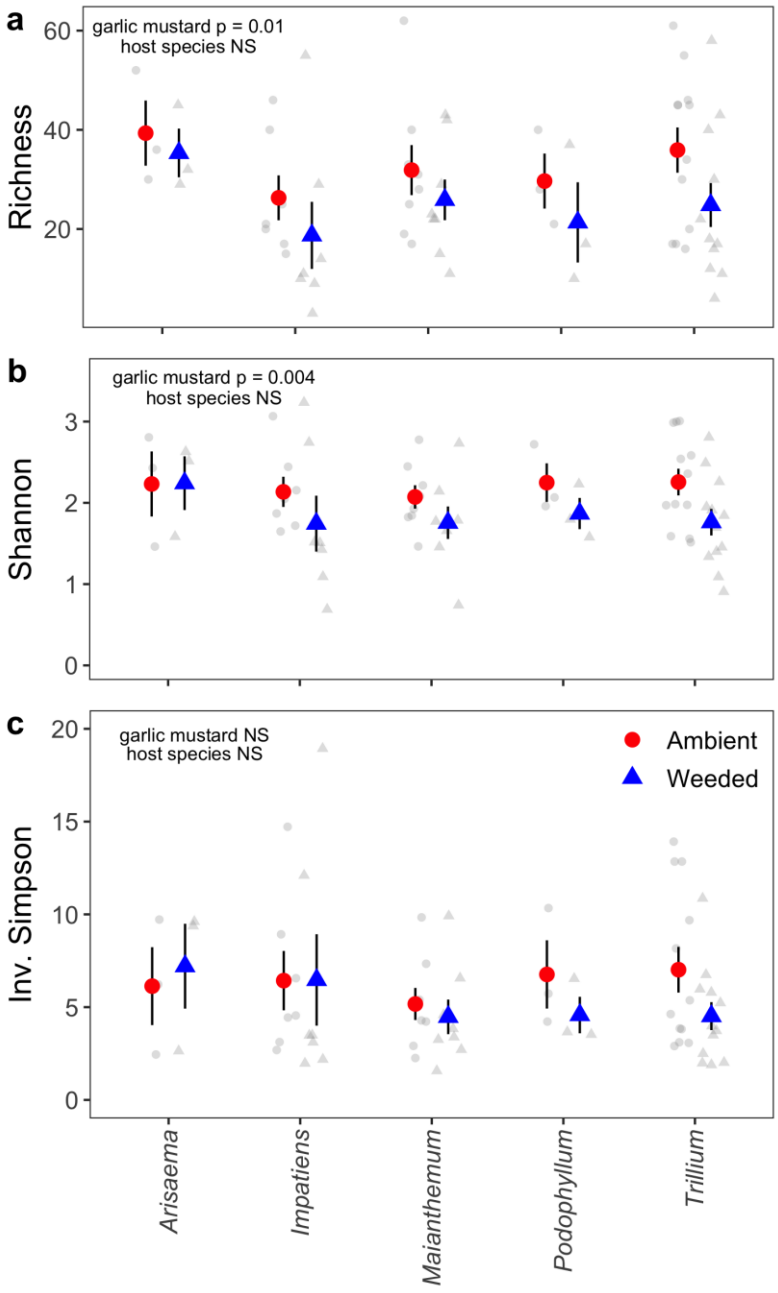


Figure 1.2 Effect of garlic mustard treatment and plant host species on AM fungal a) richness, b) Shannon diversity and c) inverse Simpson diversity. Points are means for diversity metrics in garlic mustard ambient (red circles) and garlic mustard weeded (blue triangles) treatments. Gray points are raw data for garlic mustard ambient (circles) and weeded (triangles) treatments. Bars are +/- 1 SE.

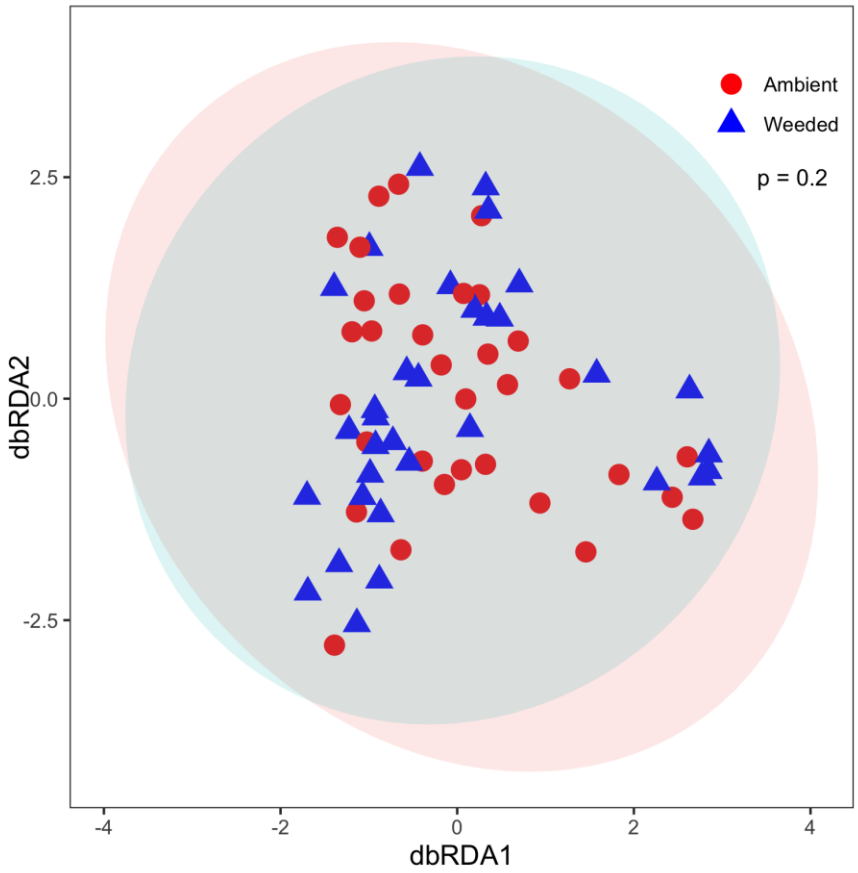


Figure 1.2 Ordination plot of Bray-Curtis dissimilarities of root AM fungal communities between garlic mustard ambient (red circles) and weeded (blue triangles) treatments. Ellipses represent one standard deviation.

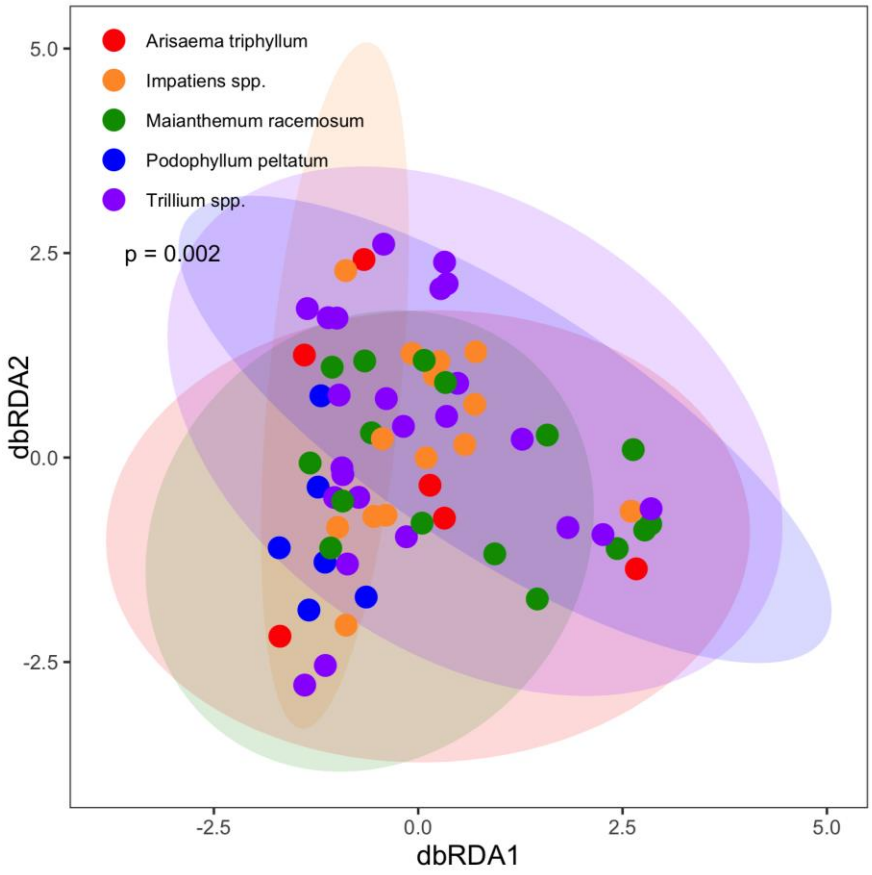


Figure 1.3 Ordination plot of Bray-Curtis dissimilarities of root AM fungal communities between *Arisaema triphyllum* (red), *Impatiens spp*. (orange), *Maianthemum racemosum* (green), *Podophyllum peltatum* (blue), and *Trillium spp*. (purple). Ellipses represent one standard deviation.

CHAPTER 2 NEGATIVE EFFECTS OF AN ALLELOPATHIC INVADER ON AM FUNGAL PLANT SPECIES DRIVE COMMUNITY-LEVEL RESPONSES

This chapter orgininally published by Morgan D. Roche, Ian S. Pearse, Lalasia Bialic-Murphy, Helen R. Sofaer, Stephanie N. Kivlin and Susan Kalisz

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Abstract

The mechanisms causing invasive species impact are rarely empirically tested, limiting our ability to understand and predict subsequent changes in invaded plant communities. Invader disruption of native mutualistic interactions is a mechanism expected to have negative effects on native plant species. Specifically, disruption of native plant-fungal mutualisms may provide non-mycorrhizal plant invaders an advantage over mycorrhizal native plants. Invasive Alliaria petiolata (garlic mustard) produces secondary chemicals toxic to soil microorganisms including mycorrhizal fungi, and is known to induce physiological stress and reduce population growth rates of native forest understory plant species. Here, we report on a 10-year manipulative field experiment in replicated forest plots testing if the effects of removal of garlic mustard on the plant community support the mutualism disruption hypothesis within the entire understory herbaceous community. We compare community responses for two functional groups: the mycorrhizal vs. the non-mycorrhizal plant communities. Our results show that garlic mustard weeding alters the community composition, decreases community evenness, and increases the abundance of understory herbs that associate with mycorrhizal fungi. Conversely, garlic mustard has no significant effects on the non-mycorrhizal plant community. Consistent with the mutualism disruption hypothesis, our results demonstrate that allelochemical producing invaders modify the plant community by disproportionately impacting mycorrhizal plant species. We also demonstrate the importance of incorporating causal mechanisms of biological invasion to elucidate patterns and predict community-level responses.

Introduction

Biological invasions threaten biodiversity, with broad impacts on the economy, environment, health, and culture. Global meta-analyses demonstrate that invasive species can have a range of negative impacts that include lower abundance and fitness of native species and altered microbial activity and nutrient availability in invaded sites (Vilà et al. 2011, Pyšek et al. 2012). Despite these observed negative consequences, we are still largely unable to predict the potential outcomes of an introduced species prior to its establishment and spread (Ricciardi et al. 2013, Sofaer et al. 2018). Since the impacts of invasion depend on the interaction of traits of individual invasive species (Gurevitch and Padilla 2004) and traits of the invaded community (Hejda et al. 2009), it is unlikely that a single mechanism will fully explain the impacts of invasion. However, understanding causal mechanisms by which certain types of invasive species exert impacts on species in the resident community can lead to more accurate predictions of where and when negative effects will occur in the invaded range (Levine et al. 2003).

Many studies have analyzed spatiotemporal patterns of community change to assess the impacts of an invasive species, but knowledge of the mechanism of impact is indispensable for generalizing individual or species-level effects to those expected at the community level. Studies of community-level patterns show that invasions can lead to biotic homogenization across locations (Sax et al. 2002) but rarely reduce local species richness within one location (Powell et al. 2011). In addition, invasive species alter community evenness by disproportionately displacing dominant native species (Powell et al. 2013, Pearse et al. 2019). Yet such patterns do not generally predict which species within the resident community will be most vulnerable to the invader, and thereby reliably link invader impacts at the individual, population, and community levels. In contrast, by understanding mechanisms of impact, we may be able to predict responses across levels of biological organization. For example, by understanding how herbivorous insects identify their host plants, we can anticipate the species-specific impacts of invasive plants or insects on other trophic levels in the native food web (Pearse and Altermatt 2013, Desurmont and Pearse 2014). Moreover, evolutionary divergence time between native range host tree and invaded range host trees is a strong predictor of high impacts of introduced insects on forests (Mech et al. 2019). For

trophic interactions (such as herbivory, above), the mechanism of impact is a function of diet, and can be relatively straightforward and consistent across space and time.

The indirect or competitive interactions that are typical of plant invaders are particularly difficult to detect and predict (Suding et al. 2008), and understanding mechanisms of invasion involving these types of interactions typically requires manipulative studies. Many processes can underlie invader impact (Ricciardi et al. 2013), but the mutualism disruption hypothesis (Hale and Kalisz 2012) is particularly well suited to evaluating invader impact from individual species to the community because we can make predictions about which native species within a community will be affected based on their known mutualism dependence. Under this hypothesis, an invasive species can gain a competitive advantage over mutualism-dependent native species by reducing or eliminating the benefits of these mutualisms (Hale and Kalisz 2012, Traveset and Richardson 2014). The effects of mutualism disruption have been observed in a variety of invaded systems. Compelling examples include the disruption of ant seed dispersal in the presence of the Argentine ant (Linepithema humile) that resulted in a shift of the resident plant community based on seed and elaiosome size (Christian 2001, Rowles and Dowd 2009), the predation of seed dispersing birds to near extinction by the brown tree snake (Boiga irregularis) that severely reduced native tree recruitment on Guam (Rogers et al. 2017), and the usurpation of native pollination services by the showy flowered purple loosestrife (Lythrum salicaria) that led to a steep decline in pollinator visitation to native flowers (Brown et al. 2002). Mutualism disruption is widespread, and can negatively affect dispersal, reproductive, or nutritive mutualisms.

The global threat of mutualism disruption is particularly substantial for plant-mycorrhizal mutualisms because of their ubiquity (Soudzilovskaia et al. 2020) and their importance for plant growth, function, and community dynamics (Van Der Heijden et al. 2008). Invasive plants can impact plant-mycorrhizal mutualisms in various ways (Grove et al. 2017): by directly competing with native plants, which weakens the native plant's contribution to their mycorrhizal partnership (Grove et al. 2017), by changing soil properties to alter the balance of resource exchange between plant and fungal partners (Allison et al. 2006, Johnson 2010), or by directly inhibiting the mutualism via invader-produced toxic secondary chemistry (allelochemicals) (Hale and Kalisz 2012). The

specific mechanism of mycorrhizal mutualism disruption may vary among invasive species but toxic allelochemistry is common. Using published lists of invasive plant families (Pyšek et al. 2012) and plant families with known allelopathic properties (Hale and Kalisz 2012), we estimate that 41% of invasive plant families are allelopathic (unpublished data). Of these allelopathic plant invaders, Alliaria petiolata (Brassicaceae, Bieb. Cavara & Grande, hereafter garlic mustard) is emerging as a model for understanding allelopathic mycorrhizal mutualism disruption (Cipollini and Cipollini 2016).

Experimental evidence in both controlled and field settings has established that garlic mustard reduces native plant performance and population growth through the disruption of their mycorrhizal associations (e.g., Stinson et al. 2006, Callaway et al. 2008, Wolfe et al. 2008, Barto et al. 2011, Hale et al. 2011, 2016, Brouwer et al. 2015, Bialic-Murphy et al. 2019). Furthermore, garlic mustard is a poor direct competitor (Meekins and McCarthy 1999, Bossdorf et al. 2004), and has no known direct phytotoxic effects (Brouwer et al. 2015, Hale et al. 2016). In addition, there is evidence that an increase in garlic mustard abundance is associated with lower native plant diversity (Stinson et al. 2007), and that community level responses to garlic mustard vary by species and by abiotic conditions (Haines et al. 2018). However, it is unknown whether the physiological consequences of mutualism disruption for individual plant species, mediated by declines in function of plant-mycorrhizal fungal partnerships, are linked to the shifts in plant community diversity and composition following garlic mustard invasion (Stinson et al. 2007, Callaway et al. 2008, Cipollini and Cipollini 2016, McCary et al. 2019).

Here, we exploit the power of this well-established mechanism of invasion impact to test for a link between mutualism disruption of mycorrhizal plant individuals and community-level response of the forest understory to garlic mustard invasion. A large portion of the diversity in most Eastern deciduous forests exists in the understory, and these species are also diverse in their mycorrhizal associations (Gilliam 2007, Soudzilovskaia et al. 2020), making the forest understory community a highly relevant study system. We present analyses of data collected from a 10-year garlic mustard weeding experiment in replicated plots to assess whether garlic mustard differentially

impacts native mycorrhizal versus non-mycorrhizal herbaceous understory plant species. We ask two questions: 1) Does the release and recovery from garlic mustard invasion (i.e. garlic mustard weeded treatment) differentially effect the community composition and diversity of mycorrhizal and non-mycorrhizal herbaceous understory plant communities? 2) Are changes in community composition and diversity explained by shifts in abundance of mycorrhizal and non-mycorrhizal plant species? Based on the mutualism disruption hypothesis, we expect a greater change in the community composition, diversity, and abundance of mycorrhizal plants (i.e., the functional groups most sensitive to garlic mustard's allelochemicals) than non-mycorrhizal plants, culminating in a distinct signature of mutualism disruption at the community level.

Methods

Study system

Trillium Trail Nature Reserve in Fox Chapel, PA (50.520237, -79.900932) is a beech-maple forest with a diverse understory plant community (Hale et al. 2011). The majority (~73%) of understory herbs at Trillium Trail partner with arbuscular mycorrhizal (AM) fungi (Hale et al. 2011), and provide photosynthetically derived carbon compounds in exchange for water and nutrients acquired by the fungi (Augé 2001, Van Der Heijden et al. 2008). Many of these mycorrhizal plant species have coarse roots with no root hairs (Brundrett and Kendrick 1988), which suggests that they rely heavily on their fungal partners for soil resources. For these reasons, we expect that the effects of mycorrhizal mutualism disruption by allelopathic plant invaders could be dramatic at Trillium Trail, making it an ideal location to test for evidence of mutualism disruption at the community level.

Garlic mustard, a biennial herb, is invasive to temperate forests of North America and colonizes forest understories (Anderson et al. 1996, Nuzzo 1999). Garlic mustard's allelochemicals are toxic to AM fungi (Cantor et al. 2011). Exposure to these allelochemicals decreases growth and abundance of AM fungal hyphae in soil and roots (Rodgers et al. 2008, Cantor et al. 2011, Hale et al. 2011, 2016, Poon and Maherali 2015). As a result, native plants that rely on AM fungi for nutrient and water acquisition

are negatively affected. Garlic mustard was first documented at Trillium Trail in the early 1990's (Knight et al. 2009). Maianthemum racemosum, a common species at Trillium Trail with coarse roots and high root AM fungal colonization rates, exhibited declines in carbon fixation and shifts in carbon storage, and altered vital rates following disruption of the mycorrhizal mutualism by garlic mustard (Brouwer et al. 2015, Hale et al. 2016). For another common mycorrhizal species at our site, Trillium erectum, garlic mustard invasion altered plant vital rates and reduced the population growth rate (Bialic-Murphy et al. 2019). It is clear from these prior studies that garlic mustard disrupts the plant root-AM fungal mutualism, the consequences of mutualism disruption are physiological in nature, and the physiological effects of mutualism disruption are strong enough to reduce population growth rates.

In 2006, we began an experimental treatment in five 14 m x 14 m replicate plots that were surrounded by 2.5 m tall wire fencing (erected in 2002) to prevent deer access. Each plot was divided into 36, 2 m x 2 m subplots. After sampling in 2006, the 18 subplots on the left half of each plot were weeded of all garlic mustard plants and the right half of each plot was not manipulated and remained at ambient levels of garlic mustard invasion. All garlic mustard individuals, including roots, were hand pulled from the weeded side of each plot, bagged and disposed of off-site. In every subsequent year, weeding occurred in the spring when garlic mustard germinates and all weeded material was removed from the site. To preclude garlic mustard seed dispersal into the weeded half of each plot, we installed temporary barriers annually just prior to garlic mustard seed dispersal that we removed when seed dispersal was complete. Persistent weeding was an effective method for removing the invader. Garlic mustard relative abundance in ambient plots averaged 15.2% and ranged from 0.5% to 71.6%. After weeding in 2006, garlic mustard abundance averaged 0.08% in the weeded plots due to infrequent late emerging seedlings (Figure 2.1). These seedlings were subsequently removed.

In late June to early July in even years beginning in 2006, all herbaceous species within each subplot were identified to the species- or genus-level. Since some species within a genus are not distinguishable from each other when they are not flowering (e.g. Impatiens, Trillium), these species were grouped by genus when estimating abundance.

To estimate species abundance, two observers were trained using standardized percent cover area templates. Within a 2 m x 2 m subplot, total leaf area occupied by each species was independently estimated by each observer, and we averaged estimated cover of the two observers. Total abundance across all species for any subplot could be >100% due to leaf overlap. We calculated the mean abundance of each species across the 18 subplots for the garlic mustard weeded and garlic mustard ambient treatments within each plot in each sampling year.

Data Analyses

We assigned a mycorrhizal status (mycorrhizal or non-mycorrhizal) to each recorded plant species based on available published and unpublished data (Table 2.S1 and citations therein). Species that form associations with AM fungi were assigned to the "mycorrhizal" functional group and species that do not form mycorrhizal associations were assigned to the "non-mycorrhizal" functional group. None of the understory herbaceous species in our study formed mycorrhizal associations with non-AM fungi (i.e. ectomycorrhizal, ericoid). We excluded species from our analyses with unknown mycorrhizal status. The relative abundance of species with an unknown mycorrhizal status averaged 1.6% of the total abundance. Since we manipulated garlic mustard abundance by weeding half of each plot, garlic mustard was also excluded from all analyses. In total, we were able to evaluate garlic mustard's effects on 28 plant taxa (19 mycorrhizal, 9 non-mycorrhizal) across our experimental plots.

We characterized shifts in mycorrhizal and non-mycorrhizal components of the plant community by quantifying changes in community dissimilarity in response to garlic mustard weeding and year. We further described patterns of community change by analyzing the effects of garlic mustard weeding and year on species richness, evenness, and diversity of mycorrhizal and non-mycorrhizal plant species. We also tested for a difference in the abundance of mycorrhizal and non-mycorrhizal plants between the garlic mustard ambient and weeded treatments. Finally, we asked whether individual species showed consistent responses that could be predicted by their functional group (i.e., mycorrhizal status).

Community composition

We tested for the effects of garlic mustard on community composition for the mycorrhizal and the non-mycorrhizal plant communities separately because we expected only mycorrhizal plant species to directly respond to garlic mustard removal. We predicted that weeding would affect mycorrhizal plant communities, with increasing dissimilarity between garlic mustard ambient and weeded treatments over time, whereas we did not expect differences between treatments for non-mycorrhizal plant communities. PERMANOVA is based on the ratio of differences in dissimilarity between versus within groups (Anderson 2014), which we used to compare dissimilarity in composition between ambient and garlic mustard weeded treatments and to test for different trends over time within those treatments. For each plant functional group, we used a PERMANOVA with an interaction between garlic mustard treatment and year of sampling to explain Bray-Curtis dissimilarities between plant communities in the garlic mustard ambient and garlic mustard weeded treatments. The permutations were constrained within garlic mustard treatment nested within plot nested within year.

Diversity

To determine whether garlic mustard weeding changed diversity in the plots over time, we calculated species richness, Pielou's evenness, Shannon's diversity, and Inverse Simpson diversity of the mycorrhizal plant community and of the non-mycorrhizal plant community every year for each treatment in each plot. Of the two diversity indices (Shannon and Inverse Simpson), Shannon's diversity is more sensitive to changes in rare species, whereas Inverse Simpson diversity is more sensitive to changes in highly abundant species (Morris et al. 2014). We included all of these metrics because compound measurements such as Shannon and Inverse Simpson diversity contain slightly different information about which piece of diversity is changing (rare or abundant species). In addition, Pielou's evenness tends to correlate highly with Shannon's diversity, so it is useful to interpret both of these indices alongside Inverse Simpson diversity, because it is independent from evenness and Shannon's diversity (Morris et al. 2014). For each of these four diversity metrics, we fit a linear model with garlic mustard treatment, year, and their interaction as fixed effects. We included plot as a random effect to account for repeated measurements of each plot across years.

Abundance

We tested for changes in abundance within each functional group (i.e., mycorrhizal status) between garlic mustard ambient and weeded treatments using a generalized linear mixed effects model including the garlic mustard treatment, year, and their interaction as fixed effects. We fit separate models for each functional group, with the summed percent cover of all mycorrhizal species or of all non-mycorrhizal species, as the response variable, respectively. To control for non-independence due to repeated sampling of plots over time, we included plot as a random effect. Summed percent cover for each functional group never exceeded 100% so we modeled proportional cover using a beta distribution.

Individual species response

We tested whether mycorrhizal status affected species-specific responses to garlic mustard treatments. To do this, we used the Relative Interaction Index (RII) (Armas et al. 2004), which calculates the biomass response to a biological interaction, here, the relative biomass difference with and without garlic mustard.

$$RII = \frac{Ba - Bw}{Ba + Bw}$$

B_w in our study is the abundance of the focal species in the garlic mustard weeded treatment, and B_a is abundance in the ambient treatment within the same plot. Therefore, any negative RII values (i.e., $B_w > B_a$) indicate a negative effect of garlic mustard on abundance, while positive RII values indicate a positive effect. To assess differences in RII we fit a general linear mixed effects model including the garlic mustard treatment, year, and their interaction as fixed effects and plant species and plot as random effects. We predicted that mycorrhizal plant species would have more negative RII values, as well as a more negative temporal trend in RII than non-mycorrhizal plant species. RII is only a meaningful metric when abundance is greater than zero in both the presence and absence of the biological interaction, so we only calculated RII when the focal species was present in both treatments for a plot and year (16 mycorrhizal species and 6 non-mycorrhizal species).

Native plant response to garlic mustard invasion may be dependent on evolutionary history (e.g., have a phylogenetic signal). We tested for a phylogenetic

signal (K; Blomberg et al. 2003) in all metrics of native plant response separately for each year and treatment on an ultrametric super-tree phylogeny created following methods in Worchel et al. (2013). After correcting for multiple comparisons with a Bonferroni correction, there was no consistent effect of phylogenetic history of native plant responses to invasion and therefore we did not account for phylogenetic history in our statistical analyses.

All statistical analyses were conducted in R version 3.6.2 (R Core Team 201). We tested for phylogenetic signal using the Picante package version 1.8 (Kembel et al. 2010). Linear models were estimated with the glmmTMB package version 0.2.2.0 (Brooks et al. 2017). We assessed the fit of these models using the DHARMa package version 0.2.0 (Hartig 2016). Treatment effects of the linear models were tested using a Wald chi-squared test. We analyzed linear model interaction terms using Type III sum of squares and, when interactions were not significant, we analyzed main effects using Type II sum of squares. We tested for significant pairwise comparisons between garlic mustard treatments within year at $p = 0.05$ using the emmeans package version 1.3.5.1 (Lenth 2019). We used the vegan package version 2.5-3 (Oksanen et al. 2019) to calculate diversity metrics and to test for changes in community composition using PERMANOVA.

Results

All analyses were performed with data from years 2006 – 2016. There were no significant differences in composition, diversity or abundance of the mycorrhizal or non-mycorrhizal plant communities prior to establishment of the garlic mustard ambient and weeded treatments in 2006. These results suggest that the garlic mustard weeded treatment drove any subsequent changes in the mycorrhizal and non-mycorrhizal plant communities. Since we manipulated the presence of garlic mustard as part of the experimental design, data on garlic mustard abundance were excluded from all analyses. We report the test statistics for the main effects between the garlic mustard treatment and year in Table 3.S2 for the PERMANOVA, and in Table 3.S3 for the linear models. All significant differences are for $p \leq 0.05$.

Community composition

Composition of the mycorrhizal plant community (Figure 2.2a) differed between the garlic mustard ambient and garlic mustard weeded treatments. Conversely, the non-mycorrhizal plant community did not differ significantly between treatments (Figure 2.2b). Community composition of both functional groups (mycorrhizal and non-mycorrhizal) also changed over time (Figure 2.S1), However, we found no significant interaction between community composition and year in either functional group.

Diversity

Garlic mustard weeding changed the diversity of mycorrhizal but not non-mycorrhizal functional group. There was no effect of garlic mustard treatment or year on species richness of mycorrhizal plants (Figure 2.3a). Richness of non-mycorrhizal plants did not respond to garlic mustard treatment but changed over time (Figure 2.3b). Evenness of the mycorrhizal functional group overall was 15.2% lower in the garlic mustard weeded treatments but did not change over time (Figure 2.3a). In contrast, evenness of the non-mycorrhizal functional group did not change between garlic mustard treatments but did change over time (Figure 2.3b). Shannon and Inverse Simpson diversity of the mycorrhizal group decreased overall by 14% and 23% respectively in garlic mustard weeded treatments (Figure 2.3a), but garlic mustard treatment had no effect on Shannon or Inverse Simpson diversity of non-mycorrhizal group (Figure 2.3b). There was no temporal trend in Shannon or Inverse Simpson diversity for the mycorrhizal functional group, but there was a significant effect of year on Shannon and Inverse Simpson diversity for the non-mycorrhizal functional group.

Abundance

Weeding garlic mustard from the plots resulted in an overall 19.7% increase in abundance of mycorrhizal plant species relative to those in the ambient treatment (Figure 2.4a). In contrast, there was no change in abundance of non-mycorrhizal species between garlic mustard treatments (Figure 2.4b). The overall abundance of both mycorrhizal and non-mycorrhizal species changed over time, but these changes were independent of garlic mustard weeding. There was no significant interaction

between garlic mustard treatment and year for abundance of mycorrhizal or non-mycorrhizal species.

Individual species response

Within functional group (mycorrhizal status), there was variation in strength and direction of each plant species RII response (Figure 2.5). Ten mycorrhizal plant species increased in abundance in garlic mustard weeded versus ambient treatments resulting in a negative RII, whereas four mycorrhizal species had a positive RII. One non-mycorrhizal plant species had a higher abundance in garlic mustard weeded versus ambient plots whereas two non-mycorrhizal species had a positive RII. There was no effect of year or mycorrhizal status on the effect of garlic mustard (RII).

Discussion

Numerous studies demonstrate that invasive plants have negative impacts on native species (Vilà et al. 2011, Pyšek et al. 2012). However, to anticipate how resident plant communities shift and which native species are most likely to be impacted by an invader, we must explore the mechanisms by which invasive plants displace natives (Levine et al. 2003, Sofaer et al. 2018). Using detailed data from a 10-year manipulative field experiment, we found that the removal of garlic mustard (i.e. weeded treatment) altered the composition, decreased the diversity, and increased the abundance of the mycorrhizal plant community in comparison to ambient levels of garlic mustard invasion. In contrast, there were no effects of the garlic mustard treatments on the non-mycorrhizal plant community, which is not susceptible to garlic mustard's disruption of mycorrhizal mutualisms. Our study illustrates that the consequences of garlic mustard invasion for the plant community may be anticipated by incorporating an understanding of mutualism disruption into future invasion predictions, because garlic mustard weeding favored mycorrhizal, but not non-mycorrhizal plant species. Our results are consistent with previous work that found variation in response to garlic mustard by mycorrhizal dependency of forest tree seedlings (Stinson et al. 2006). Together, our findings and previous work demonstrate that garlic mustard invasion results in a functional shift in

plant community membership that can be reversed over time with removal of the invader.

The shifts in the composition, diversity, and abundance of the mycorrhizal – but not the non-mycorrhizal – functional group in response to garlic mustard weeding are indicative of mutualism disruption. Other mechanisms of impact, such as direct competition or direct phytotoxicity would not disproportionately affect species that form mycorrhizal associations. Garlic mustard is a poor direct competitor in its invasive range (Meekins and McCarthy 1999, Bossdorf et al. 2004), and disrupts mycorrhizal mutualisms to indirectly compete against other plants (Brouwer et al. 2015, Hale et al. 2016). Removal of a competitive dominant plant species from a community is expected to result in an increase in abundance of other community members (MacDougall and Turkington 2005), but unlike mutualism disruption, exploitative competition would not explicitly predict different responses for mycorrhizal and non-mycorrhizal plants to the removal of a competitor. Since mycorrhizal and non-mycorrhizal plants differ in their methods of resource acquisition (via roots alone or via fungal partners), they may respond differently to limiting resources under exploitative competition. However, soil resources do not differ between garlic mustard ambient and weeded treatments at our site (Bialic-Murphy et al. in prep) and are, therefore, unlikely to cause the trends we observed here. Observed patterns of the plant community response to garlic mustard in our field site are consistent with those predicted by the mechanistic process of mutualism disruption.

Previous work has quantified responses of root fungal communities to garlic mustard and provides context for interpreting our results. At our study site, root fungal communities within some species' roots changed in response to our garlic mustard treatments (Burke 2008), particularly in the presence of deer (Burke et al. 2019). Ongoing work shows that species in the ambient garlic mustard treatments have reduced hyphal colonization in their roots, and higher evenness in the soil AM fungal community (Bialic-Murphy et al. in prep). In other locations, studies have documented no recovery of mycorrhizal fungi after three years (Anthony et al. 2019) or only partial recovery after six years (Lankau et al. 2014). Since we did not observe a response in the aboveground community until 4-6 years of garlic mustard weeding, it is possible that

the aboveground response is mirroring the recovery of the belowground mycorrhizal fungal community, or that the mycorrhizal fungal community has not yet responded to garlic mustard removal. In addition, it is possible that changes in the abiotic soil conditions that are associated with garlic mustard invasion attribute to the observed response of mycorrhizal plant species (Rodgers et al. 2008, Anthony et al. 2019). However, no differences in abiotic soil conditions between ambient and weeded garlic mustard treatments after 14 years were found by Bialic-Murphy et al. (in prep). Thus, findings from our experiment provide a link between mechanistic evidence of mutualism disruption from careful fine-scale experiments (e.g. Stinson et al. 2006, Hale et al. 2011, 2016, Brouwer et al. 2015) to observed shifts in mycorrhizal plant communities.

Our findings support the utility of our coarse classification into mycorrhizal and non-mycorrhizal functional groups for anticipating responses to invasions but also highlight limitations in the degree of predictability within ecological communities. In particular, within the mycorrhizal group there was considerable variation in response to garlic mustard (RII) (Figure 2.5), such that a simple binary assignment of mycorrhizal status would be insufficient to reliably predict changes in species-level abundance. Plant species' dependency on mycorrhizal partners (i.e. biomass response to mycorrhizal colonization) generally falls on a spectrum from highly positive plant biomass responses to mycorrhizal colonization to somewhat negative (Tawaraya 2003), and variation in dependence of mycorrhizal plants on their fungal partners is known to influence the response of a particular species to mutualism disruption (Stinson et al. 2006, McCary et al. 2019). We do not know where our naturally occurring plant species fall along the spectrum of dependency on their mycorrhizal partners, and it is possible that differences among trajectories of the experimental plots in response to garlic mustard weeding (Figure 2.2a) arise from variation in the dependency of the mycorrhizal species naturally occurring in those plots. Exploring the factors that may have driven the different trajectories of the unique plant communities in each plot is an important next step in understanding the intricacies of community responses to garlic mustard invasion.

Our results revealed that the mycorrhizal plant community had decreased evenness and diversity in the garlic mustard weeded treatments. The decreases in

Shannon and Inverse Simpson diversity of the mycorrhizal plant community are likely driven by the lower evenness in the garlic mustard weeded treatments, since there was no change in species richness for this group. Evenness of the mycorrhizal plant community decreased in the garlic mustard weeded plots because of the dramatic increase in the abundance of a common annual species, Impatiens spp. after 2006 (Figure 2.S2). This created a less balanced community with low evenness where one species (Impatiens spp.) has a very high abundance whereas the rest of the species remain at a low abundance. Since Impatiens spp. have an annual life history and often exhibit ruderal growth patterns in comparison to many of the long-lived perennials at our site, we expect that the long-lived perennial species will rebound as well with more time.

Over the course of this study, we found that the main effect of time on the abundance of both plant communities was significant, but the interactions between the garlic mustard treatment and year were not. The non-mycorrhizal plant abundance decreased over time in both garlic mustard treatments. Therefore, this decline in non-mycorrhizal plant abundance cannot be attributable only to the increase in mycorrhizal plant abundance in the garlic mustard weeded treatment. We see similar declines in non-mycorrhizal plant abundance in other plots at our site that are not protected from deer herbivory by fences (unpublished data), indicating that exclusion of deer is also not driving the declines in non-mycorrhizal plant abundance. These results suggest that there are other sources of environmental variability (e.g., annual fluctuations in precipitation patterns) that likely influenced the abundance of both plant community types over time. Despite this, there is a strong signature of the effects of garlic mustard weeding on mycorrhizal plant abundance.

Garlic mustard consistently has negative effects on native mycorrhizal plant species (e.g. Stinson et al. 2006, Cipollini et al. 2008, Wolfe et al. 2008). These negative effects on mycorrhizal plant species are consistent with garlic mustard's disruption of native mycorrhizal mutualisms which leads to water stress and carbon stress (Brouwer et al. 2015, Hale et al. 2016) and decreases in vital rates (Brouwer et al. 2015) and population growth rates (Bialic-Murphy et al. 2019). In addition, garlic mustard alters plant communities (Stinson et al. 2007). Our results build on these findings to show that the outcomes symptomatic of mutualism disruption in individual

plants follow a similar pattern for plant communities in the field. Our 10-year long-term field experiment shows that even at the modest garlic mustard abundances at Trillium Trail, mutualism disruption alters the community composition, changes diversity and reduces the abundance of only the mycorrhizal plants within the entire plant community. We stress that the length of our experiment provides strong support for the long-term effects of garlic mustard on the mycorrhizal plant community. Most studies that evaluate the impacts of invasive species last only 1 year (Stricker et al. 2015) even though the effects of invaders are known to lag after the invasion establishes and have legacy effects after they are removed. Land managers attempting to restore native understories through garlic mustard removal may expect to see an increase in abundance of mycorrhizal plant species, but likely no changes in species richness. However, responses may differ by species' sensitivity to garlic mustard (see Figure 2.5 and Figure 2.S3), and may require multiple years (in our experimental study 4-6 years) of annual garlic mustard removal to occur.

Many invasive plant species are thought to impact native species by allelopathy and/or mutualism disruption (e.g., Alliaria petiolata, Tamarisk spp., various Fallopia species; Stinson et al. 2006, Wolfe et al. 2008, Bainard et al. 2009, Murrell et al. 2011, Hale and Kalisz 2012, Meinhardt and Gehring 2012), so our findings likely apply to the impacts of invasive plants beyond garlic mustard. In addition, since approximately 80% of understory herbs globally (and two thirds of the study species in our experimental plots; Table 2.S1) form mycorrhizal associations (Soudzilovskaia et al. 2020), there is potential for garlic mustard to drastically shift understory plant communities across its invasive range. We demonstrate that mycorrhizal mutualism disruption by an invasive plant leads to a shift in plant communities but found that responses were variable among species within functional groups. This suggests that the ability to predict invader impacts across space and time may require refinement of the focal functional trait, in this case a shift from a binary to a continuous view of mycorrhizal dependency. Our study highlights the utility of exploring the mechanisms by which invasive species cause impact because we find a shift in functional groups of the resident plant community that would only be anticipated by knowing that mechanism.

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Appendix

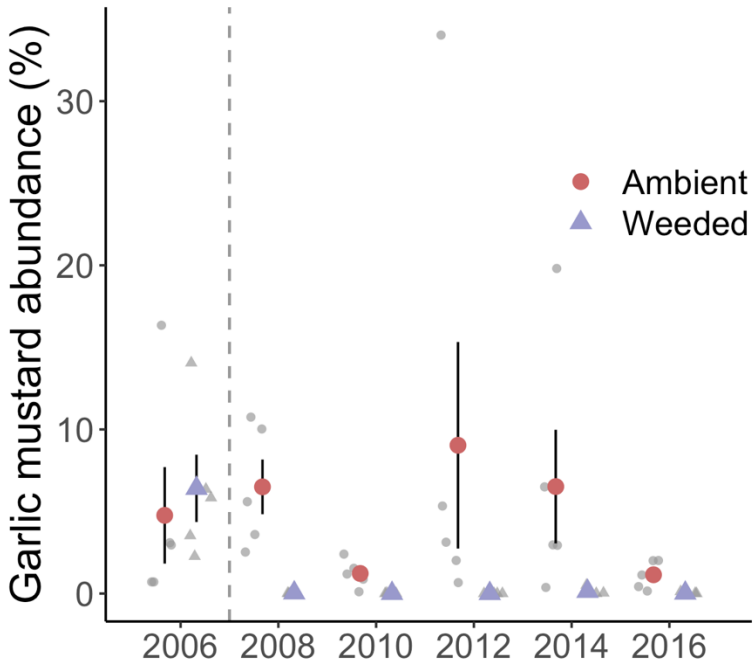


Figure 2.1 Graphical representation of garlic mustard treatment efficacy. Mean garlic mustard abundance +/- 1 SE in ambient (red circles) and weeded (purple triangles) treatments (2006-2016). Jittered data points for each plot in gray. The first year of garlic mustard weeding occurred after data collection in 2006. Vertical gray dashed line separates pre-treatment (2006) from post-treatment abundance values.

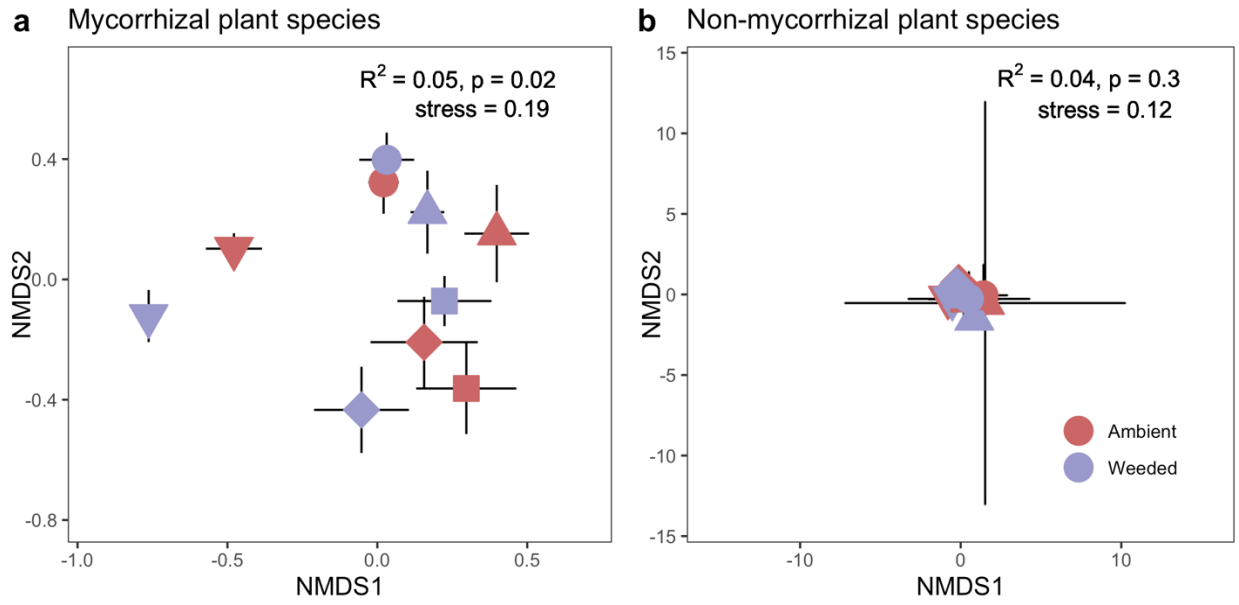


Figure 2.2 Ordination plots of Bray-Curtis dissimilarities illustrating differences in composition between garlic mustard ambient (red) and garlic mustard weeded (purple) treatments for a) mycorrhizal and b) non-mycorrhizal plant communities. Shapes represent different plots, plot 1 = down pointing triangle, plot 2 = square, plot 3 = diamond, plot 4 = up pointing triangle, plot 5 = circle. Symbols are plot means with 95% confidence intervals for data collected every other year over 11 years (2006-2016). Garlic mustard data was excluded from PERMANOVA and ordinations. Mycorrhizal plant communities differ significantly between treatments while non-mycorrhizal plant communities did not.

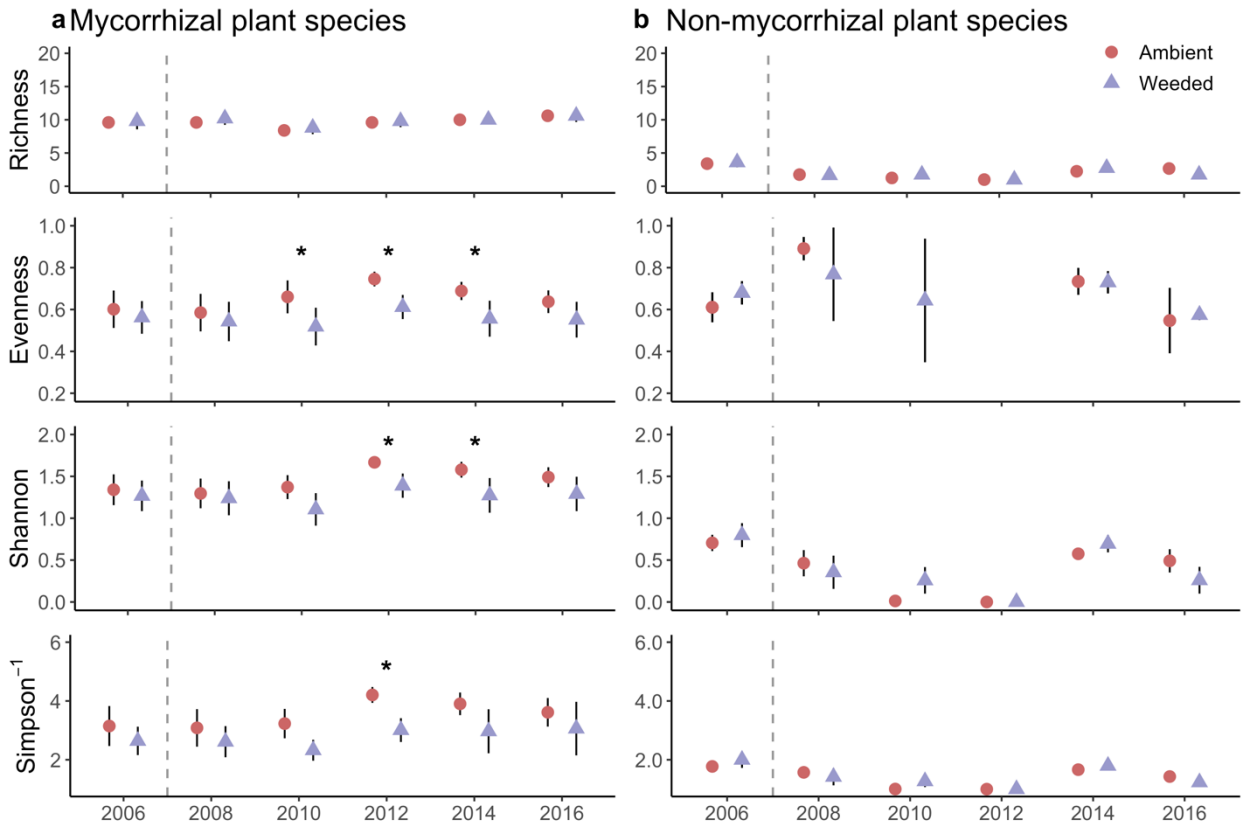


Figure 2.3 Species richness, evenness, Shannon diversity and Inverse Simpson diversity for a) mycorrhizal plant species, and b) non-mycorrhizal plant species. Points represent plot means for garlic mustard ambient (red circles) and garlic mustard weeded (purple triangles) treatments (2006-2016). Garlic mustard data was excluded from calculation of diversity metrics. Error bars are +/- 1 SE. Vertical dashed lines separate pre-garlic mustard weeded (2006) from post-garlic mustard weeded time points. The interaction and main effects of garlic mustard treatment and time were non-significant for species richness for both mycorrhizal and non-mycorrhizal plant species. The main effect of garlic mustard treatment was significantly different for evenness, Shannon and Inverse Simpson diversity for mycorrhizal plant species, but not the non-mycorrhizal plant species. Asterisks = significant pairwise comparisons at p = 0.05.

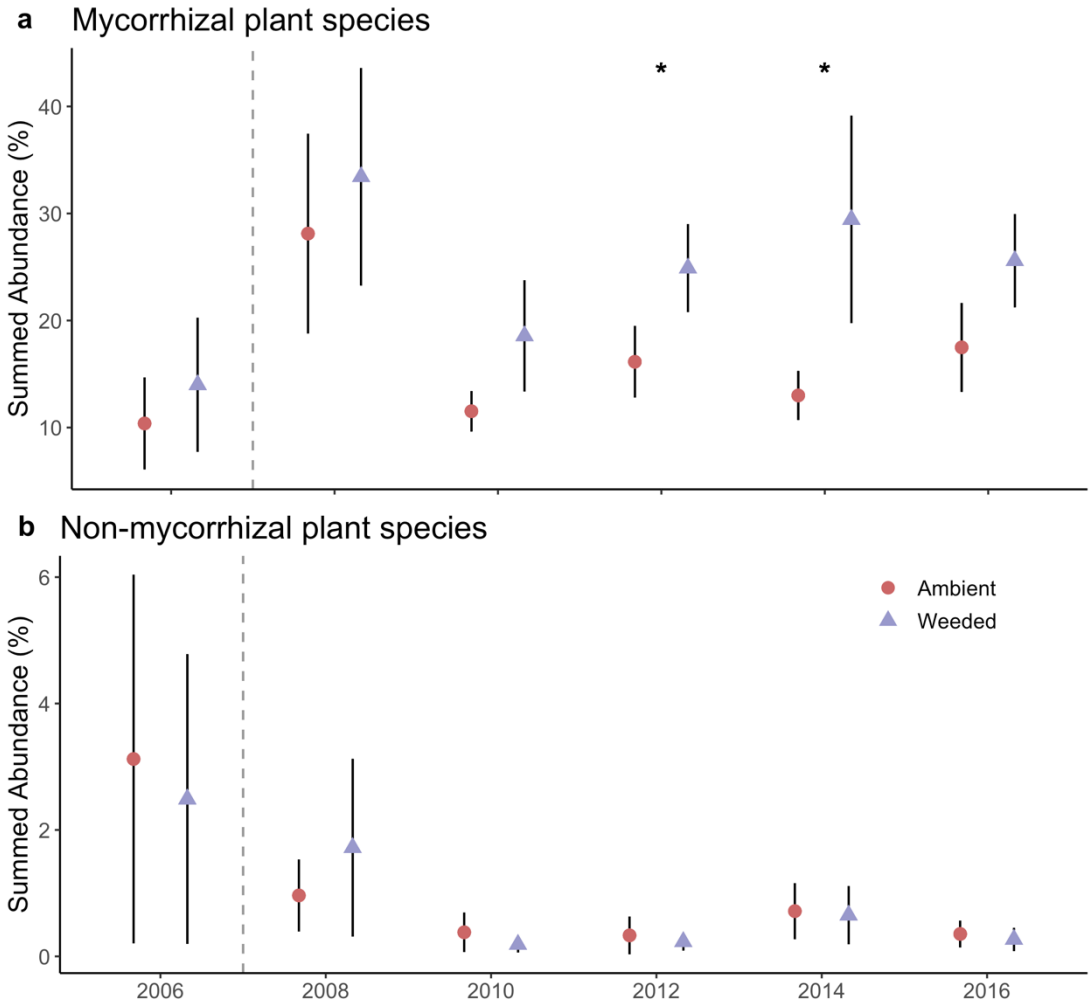


Figure 2.4 Effect of garlic mustard treatment over time on summed abundance of a) mycorrhizal plant species and b) non-mycorrhizal plant species. Points are plot means of summed abundance in garlic mustard ambient (red circles) and garlic mustard weeded (purple triangles) treatments (2006-2016). Garlic mustard abundance was excluded. Error bars are +/- 1 SE. Vertical dashed lines separate pre-garlic mustard weeded (2006) from post-garlic mustard weeded time points. Summed abundance between treatments was not significantly different in 2006 (pre-garlic mustard weeding). There was a significant main effect of the garlic mustard treatment on summed abundance of mycorrhizal plants, but no significant interaction between garlic mustard treatment and time. For non-mycorrhizal plant species, the main effect of treatment was not significant, while the main effect of time was significant; there was no significant interaction between garlic mustard treatment and time. Asterisks = significant pairwise comparisons at p = 0.05.

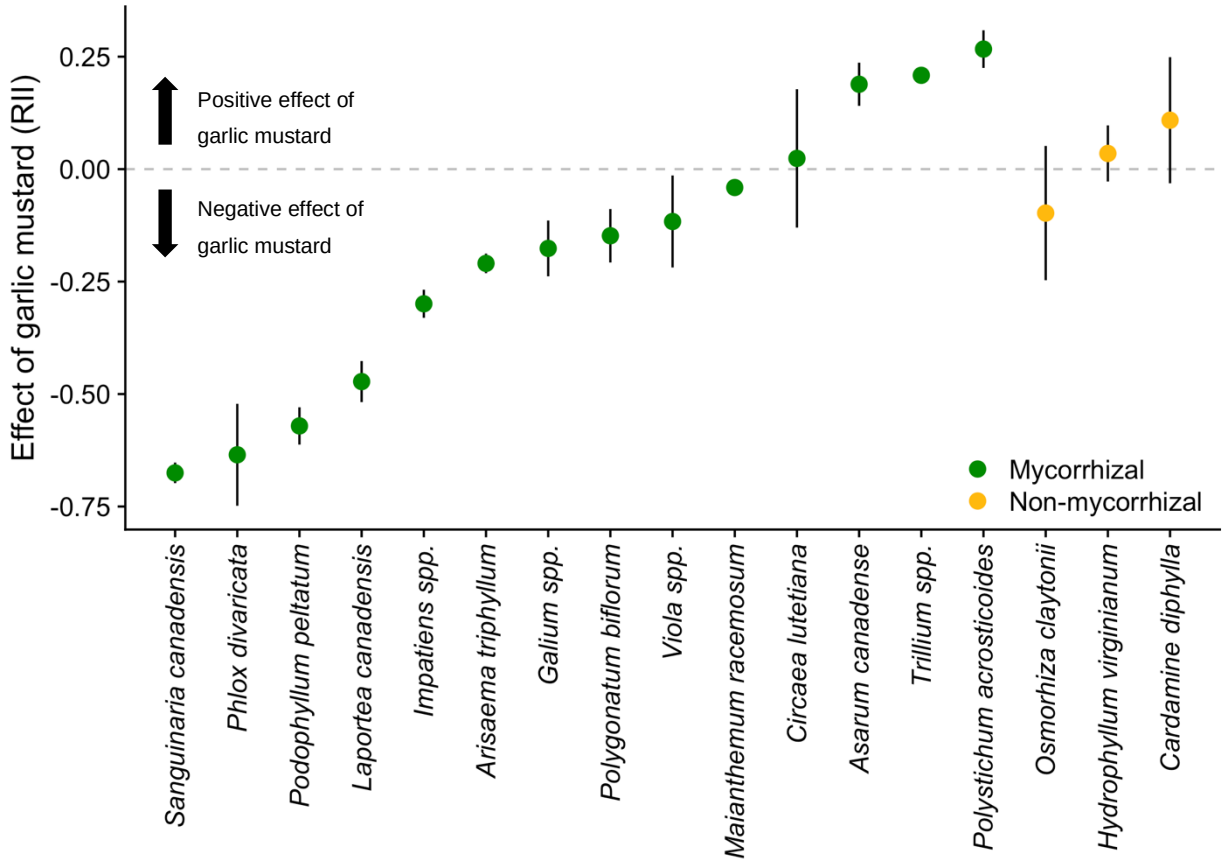


Figure 2.5 Relative interaction index (RII) of each plant species between garlic mustard ambient and garlic mustard weeded treatments. A negative RII indicates that a species relative abundance is higher in the weeded treatment than in the ambient treatment. Conversely, a positive RII indicates that a species has lower relative abundance in the ambient treatment. Points are the mean RII for each species across plots and years, error bars are +/- 1 SE.

CHAPTER 3 MUTUALISM DISRUPTION: PHYSIOLOGICAL MECHANISM OF INVASION IMPACT IS EVIDENT ACROSS HETEROGENEOUS ENVIRONMENTAL CONDITIONS AND VARYING INVASION INTENSITY

Abstract

The impact of a biological invasion on the native community is heterogeneous across the invasion landscape. Abiotic conditions, the abundance of the invasive species, and traits and composition of the native community with which invaders interact can all influence the impact of the invasive species. Scaling up known mechanisms driving invasive species' local impact on other plant species is important for predicting which regions and which native communities are vulnerable to particular invasive species. Invasive plants frequently exhibit allelopathic traits, which can be toxic to plants directly or toxic to mycorrhizal fungi that form symbiosis with their roots, and indirectly alter a plants performance by disrupting this mycorrhizal mutualism. I used species percent cover data collected in 186 forest plots collected from 1998 to 2018 across the state of Illinois to assess the whether the mutualism disruption hypothesis explains observed changes in native plant species across a range of garlic mustard (*Alliaria petiolata*) abundances and environmental conditions. A disproportionate negative effect of garlic mustard on the mycorrhizal component of the native plant community when compared to the component of the community that is non-mycorrhizal would provide support for the mutualism disruption hypothesis. Consistent with the mutualism disruption hypothesis, garlic mustard percent cover (abundance) was negatively correlated with abundance of mycorrhizal plant species, but not non-mycorrhizal plant species. Garlic mustard abundance predicted plant abundance and diversity as well or better than abiotic variables at the smallest sampling unit (quadrat), but this relationship was not significant when quadrats were aggregated across an entire site. I found that garlic mustard's impact on the plant community was highly localized but was equivalent to abiotic variables for predicting plant diversity at local scales. I showed that the invasion impact, mutualism disruption, is widely driving patterns of plant diversity across invasion intensities and heterogeneous environments. Therefore, the mutualism disruption hypothesis provides strong generalizable predictions of the impacts of mutualism-disrupting allelopathic invasive plants that hold up at large spatial scales.

Introduction

The potential ecological impact of any invasive species on populations, communities, or ecosystems is notoriously difficult to predict (Hulme et al. 2013, Ricciardi et al. 2013). In part, this is because of the considerable variation in the responses of native communities to invasions. Based on how the traits of the resident species within that community interact with those of the invader, the invader might be prevented from establishing (biotic resistance; Levine et al. 2003), establish but have negligible impact on the recipient community (naturalize; Macdougall et al. 2009), or establish and have a large impact (Hejda et al. 2009, Vilà et al. 2011, Pyšek et al. 2012). In addition, the abiotic conditions at a particular location may more or less match the invaders physiological optimum, and thus provide a competitive advantage to either invasive or native species, respectively (Kestrup and Ricciardi 2009, Ricciardi et al. 2013, Iacarella et al. 2015). However, our ability to predict the effect of varying biotic or abiotic conditions on invasion impacts can be limited if the mechanistic processes underlying those impacts are not well understood (Levine et al. 2003). Knowledge of mechanistic drivers of invasion impact will lead to more accurate predictions of which native species, communities, or geographic locations are more likely to be negatively affected by invasive species and will inform management decisions.

Like many processes in ecology, mechanisms of invasion impacts are likely scale-dependent, so the geographic scale of study may influence the observed strength of the mechanism (McGill 2010). Climate, for example, is understood to act at large spatial extents, influencing the region in which an invasive species might be able to establish and invade (Record et al. 2018). However, species interactions between the invasive species and the native community including herbivory, competition, or mutualisms may influence the abundance and impacts of an invader at small spatial extents (Wiens 2011). Spatial scale is also an important in quantifying and predicting community invasibility. There is a seemingly paradoxical relationship between invasions and biodiversity, that at large scales, high biodiversity is positively correlated with species invasions because at that scale, areas with high species diversity support more types of species, including invaders. At small scales however, sites with high biodiversity are resistant to invasions because there are few open niches and thus,

there is a negative relationship between species richness and invasion (Sax et al. 2002, Davies et al. 2005, Fridley et al. 2007, Powell et al. 2011, 2013).

A mechanistic understanding of invasive species impacts can improve how we anticipate, manage, and mitigate for these impacts. For example, the invasive annual grass, cheatgrass (*Bromus tectorum*), both increases fire frequency in habitats with historically low fire frequency by increasing connectivity of fine fuels and rapidly re-establishes post-fire, preventing establishment of non-fire adapted native species (Brooks et al. 2004, Bradley et al. 2018). These traits of cheatgrass lead to mechanistic predictions: Areas with native species that are not fire adapted and have low annual precipitation (i.e. the Great Basin) are likely to experience large impacts of cheatgrass invasion. Conversely, native species that are fire tolerant and locations that have low fire frequency (i.e. the Eastern US) may be less likely to experience large impacts of cheatgrass invasion, regardless of this invader's ability to establish in those locations. Clearly, understanding the mechanism of impact, the scale of influence of that mechanism, and climatic conditions conducive to the specific mechanism's functioning are important for predicting where and why invaders are expected to have high impact (Chambers et al. 2014).

The mutualism disruption hypothesis describes an indirect mechanism by which invasive species impact native species by negatively affecting native species' mutualistic partnerships (Hale and Kalisz 2012). The impact of biological invasions on mutualistic interactions is important to understand because mutualisms are vulnerable to the negative effects of invasive species (Aslan et al. 2013) but the structure of these interactions may buffer partners to perturbations such as invasion (Fricke et al. 2017). Disruption of mutualisms is common and tends to have a negative effect on native species (Christian 2001, Brown et al. 2002, Stinson et al. 2006, Traveset and Richardson 2006, 2014, Meinhardt and Gehring 2012, Riginos et al. 2015, Grove et al. 2017a, Rogers et al. 2017), but the full extent and patterns of the threat is unknown (White et al. 2006). An example of a widespread mutualism that is threatened by mutualism disruption is mycorrhizal associations between plant roots and mycorrhizal fungi (Grove et al. 2017a). Invasive plants can disrupt mycorrhizae by releasing secondary metabolites that are toxic to mycorrhizal fungi in the soil, thereby preventing

the exchange of nutrients and water for photosynthetically-derived carbon compounds between plant roots and their mycorrhizal fungi (Cipollini et al. 2012, Hale and Kalisz 2012, Zhang et al. 2020). Notably, there is evidence that many invasive plants may have antimicrobial allelopathic properties (Hale and Kalisz 2012, Kalisz et al. 2020), so this phenomenon may be widespread, although rigorous confirmation of allelopathy is needed for many individual species' cases.

However, the effect mutualism disruption has on the plant partner is not expected to be uniform across all plant species in an invaded community. Plant species differ in their dependence on mycorrhizal colonization for soil resource acquisition. Thus, if mycorrhizal mutualism disruption explains the impacts of an allelopathic invader, the response of a particular native plant species should depend on its requirement for and strength of mycorrhizal associations. Additionally, the abiotic and biotic environment in which the interaction takes place (Suding et al. 2008, Johnson 2010) likely influences the response of a particular plant species to an invader's disruption of its mycorrhizal mutualism. Differences in plant species composition and susceptibility of each community member to allelopathic mutualism disruption may lead to community-level responses to mutualism disruption (McCary et al. 2019). And indeed, allelopathic invasion showed no negative effects on a non-mycorrhizal plant (Cipollini et al. 2008), but has strong effects on mycorrhizal tree species (Stinson et al. 2007, Bainard et al. 2009, Meinhardt and Gehring 2012, Grove et al. 2017b). In a long-term experiment in which an allelopathic invader was either present at ambient levels or removed from the plots, the abundance of herbaceous mycorrhizal plant species increased when the invader was removed (Roche et al. 2020). However, non-mycorrhizal plant species were unaffected by invader presence (Roche et al. 2020).

Further, the impact of an invasive species is expected to scale with its abundance (Parker et al. 1999, Sofaer et al. 2018). Although high abundances of any one species is expected to decrease abundance of other species, invasive species tend to have higher per capita effects than native species possibly due to their lack of evolutionary history with the native community (Pearse et al. 2019). This means that at high abundance, an invasive species has a more negative impact on the rest of the community than a native species would at that same abundance (Pearse et al. 2019).

Since invasions naturally exhibit a wide range of invader abundance, it is critical for predictions and management decisions that the effect of invasion is established across an invader abundance gradient (Yokomizo et al. 2009).

Experimental studies can expose and provide strong support for the mechanistic causes of invasive species impact, but these studies are typically limited in geographic scope. It is rare for a well-supported mechanism of invasion impact to be assessed across different abiotic and biotic conditions observed across the invaded range (Theoharides and Dukes 2007, Goldstein and Suding 2014). In this study I build on evidence from experimental studies with *Alliaria petiolata* (Brassicaceae, Bieb., Cavara & Grande, hereafter garlic mustard) to examine the extent to which garlic mustard affects native plant communities across a large geographic area. Garlic mustard releases allelopathic secondary metabolites that are toxic to soil microbes including AM fungi (Callaway et al. 2008, Cantor et al. 2011). At small scales, mutualism disruption by garlic mustard shifts the plant community with disproportionate negative impacts on plants that associate with mycorrhizal fungi (Stinson et al. 2007, Roche et al. 2020). I therefore expect that garlic mustard's impacts on plant communities at large scales will follow predictions based on the mutualism disruption hypothesis (i.e. impacts only on the portions of the plant community that depend on mycorrhizal fungi). I test the impact of garlic mustard on plant communities across time and space using long-term and large-scale plant survey data and ask three main questions. 1) How does the abundance of garlic mustard translate to its impact on abundance of other plant species? 2) Does garlic mustard alter plant communities at large geographic and temporal scales in a way that is consistent with mutualism disruption when accounting for varying abiotic conditions? 3) How strong is the effect of garlic mustard invasion on diversity of plant communities in relation to abiotic variables? I predict that within a site, the abundance of focal species that form mycorrhizal associations would be lower when garlic mustard abundance is high in a way that is consistent with mutualism disruption, whereas abundance of non-mycorrhizal plant species would not respond to increases in garlic mustard abundance. Across space, I expect abundance and diversity of mycorrhizal plant species to decrease with increasing garlic mustard abundance when accounting for variable biotic and abiotic conditions across a broad geographic scale,

whereas non-mycorrhizal species would not change in relation to increasing garlic mustard abundance. Since species interactions are anticipated to have effects at smaller spatial scales than climate, I expect the effects of garlic mustard on abundance and diversity of other plant species to be stronger than those of abiotic variables.

Methods

Study species

Garlic mustard is an invasive biennial herb that exhibits allelopathic properties and impacts native plants by disrupting plant-mycorrhizal mutualisms (Stinson et al. 2006, Callaway et al. 2008, Wolfe et al. 2008, Barto et al. 2011, Hale et al. 2011, 2016, Brouwer et al. 2015, Bialic-Murphy et al. 2019). It is a problematic invader of North American forest understories and can form dense stands even in relatively intact forests (Rodgers et al. 2008). Garlic mustard, like many species in the Brassicaceae, produces glucosinolates as secondary metabolites in all of its tissues (Vaughn and Berhow 1999). Glucosinolates are toxic to soil microbes, including arbuscular- and ecto-mycorrhizal fungi (Stinson et al. 2006, Callaway et al. 2008, Cantor et al. 2011). Since a large majority of forest understory species form associations with mycorrhizal fungi (Smith and Read 2008), garlic mustard poses a severe threat to forests in its invaded range.

Long-term observational dataset

I used data from the Illinois Critical Trends Assessment Program (CTAP) of the Illinois Natural History Survey, University of Illinois at Urbana-Champaign (Molano-Flores 2002) to assess the effects of garlic mustard and mutualism disruption on plant communities across the state of Illinois. This program was established to quantify the condition and ecological trends of four habitat types (forests, wetlands, grasslands, and streams) and three major groups of organisms (plants, birds, and insects) in Illinois. I used plant survey data from the 186 forest plots collected from 1998 to 2018. CTAP forest plots are resampled on a 5-year rotation, meaning that approximately 1/5 of the plots are visited in each year. If a site no longer met the CTAP criteria for inclusion in the monitoring program when revisited, a new site within the same township is established to replace it. Data from replacement sites are not merged with the original

sites that no longer meet sampling criteria. Details of site selection and sampling methods are outlined in Molano-Flores (2002). I used species-specific ground cover data (<1 m in height) for my study because it has been established that garlic mustard's mutualism disruption can have negative effects on forbs and tree seedlings that form mycorrhizal associations (Stinson et al. 2006, Callaway et al. 2008, Cipollini et al. 2008, Roche et al. 2020). At each site, ground cover sampling occurred at 10 1 m x 1 m quadrats every 5 meters along 3 permanent 50 m transects. Ordinal cover classes (<1%, 1-5%, 5-25%, 25-50%, 50-75%, 75-95% and 95-100%) were recorded for each plant species in each quadrat. For each site, I analyzed data both at the quadrat and site level, by averaging percent cover of each species across all 30 quadrats to create a site-level measure of percent cover per species. I included known absence data only for those species that had occurred in a site or quadrat at least one sampling time point to account for losses or additions of species over time. For all species in the dataset, I determined the mycorrhizal status of each species (whether or not they form mycorrhizal associations, and if so, with what type of mycorrhizal fungi) using the well-resolved plant family associations (Brundrett and Tedersoo 2019). I assigned plant species to one of four mycorrhizal categories following Brundrett and Tedersoo (2019): arbuscular mycorrhizal (AM), facultative arbuscular mycorrhizal (fac AM), ectomycorrhizal (EM), and non-mycorrhizal (NM). I was able to successfully assign a mycorrhizal status to 624 of the 679 species in the CTAP forest sites dataset.

Mean-centered abundance

To test whether a plant's mycorrhizal status influenced the effect of increasing garlic mustard abundance within a site or quadrat, I used linear mixed effects models. I only included the 69 sites at which garlic mustard was observed at least once (Figure 3.1), and that had been sampled more than once. Because each site was sampled at least twice, I centered the percent cover of each focal species around the site- or quadrat-level mean abundance for that species. Mean-centering data reveals changes in percent cover of focal species in a site over time (van de Pol and Wright 2009). It is also useful to look for changes in abundance of each species within a site to isolate the effects of garlic mustard abundance over time from any effects, such as abiotic and biotic conditions, that differ over space. Since biotic effects are predicted to be stronger

at smaller scales (Wiens 2011), I performed the analyses both with data averaged across quadrats within an approximately 75 m^2 site (site-level analysis) and with 1 m^2 quadrat level data. For both scales, I used mean-centered focal species percent cover as the response variable, with a fixed effect of the interaction between garlic mustard percent cover and focal species mycorrhizal status and focal species and site (for site-level analysis) or quadrat within site (for quadrat-level analysis) as random effects.

Diversity over space

To assess the effects of garlic mustard across space and the effect of abiotic variables on plant diversity metrics relative to the impact of garlic mustard abundance, I gathered 19 bio-climatic data from CHELSA (Climatologies at High resolution for the Earth's Land Surface Areas) at 30 arc second resolution (Karger et al. 2017) and soil variables including soil moisture at 5 arc minute resolution (Willmott et al. 1985), NPP at 10 arc minute resolution (Foley et al. 1996), soil carbon and pH at 5 arc minute resolution (IGBP-DIS), and percent soil clay at 30 arc second resolution (Hengl et al. 2014). From those datasets, I extracted values for each abiotic variable at each site. In these analyses, I used all sites regardless of whether garlic mustard had been observed, but that had been sampled four or more times. To test for effects of garlic mustard abundance, I used both the garlic mustard abundance at a sampling date and garlic mustard abundance at the previous sampling date (i.e. 5 years prior). I used both the current and previous garlic mustard abundance to account for lag effects of garlic mustard abundance in previous years. Since CTAP sampling is repeated at 5-year intervals the annual abundance of garlic mustard in intervening years is unknown. I calculated pairwise Pearson correlations between all abiotic variables and removed variables that were highly correlated (>0.9). I included 17 total abiotic variables in my analyses as well as garlic mustard abundance and previous garlic mustard abundance (see Figure 3.3). I calculated summed abundance, species richness, Pielou's evenness, Shannon diversity and inverse Simpson diversity for all species, and for each mycorrhizal status separately. For each model, I ran backwards model selection to find the best fit model, sequentially eliminating non-significant predictors. I ran all models with site level and with quadrat level data. I included site or quadrat within site as random effects to control for non-independence of repeated sampling over time. Since

there was low abundance of ectomycorrhizal and non-mycorrhizal plant species, I was unable to fit models for those mycorrhizal groupings at the quadrat level.

Statistical analyses

All statistical analyses were conducted using R version 3.6.2 (R Core Team 2020). Linear models were fit using the glmmTMB package version 0.2.3 (Brooks et al. 2017) and model fits were assessed in DHARMa version 0.2.6 (Hartig 2016). I calculated diversity metrics using the vegan package version 2.5-6 (Oksanen et al. 2019). Pearson correlations were calculated using stats package in base R.

Results

Mean-centered abundance

At the site level, an increase in garlic mustard abundance was not associated with a decrease in percent cover of other plant species (Figure 3.2a; $\chi^2(1) = 0.57$, $P = 0.5$). As garlic mustard abundance increased in a quadrat, the abundance of other plants decreased (Figure 3.2b; $\chi^2(1) = 24.58$, $P < 0.001$). However, the effect of garlic mustard on the abundance of other plants within quadrats depended marginally on the mycorrhizal status of those plants (Figure 3.2c; interaction between garlic mustard abundance and mycorrhizal status: $\chi^2(3) = 6.17$, $P = 0.10$). As garlic mustard increased in abundance in a quadrat, the abundance of AM, facultative AM and EM plant species decreased, but the abundance of other non-mycorrhizal plants increased (Figure 3.2c).

Diversity over space

Garlic mustard was more often a significant predictor of abundance and diversity using quadrat-level data in comparison to site-level data. Increases in garlic mustard abundance had a consistently negative effect on abundance and diversity of the recipient community for quadrat-level data (Figure 3.3). Garlic mustard had a negative effect on site-level EM plant richness, Shannon diversity and inverse Simpson diversity (Figure 3.3), and previous garlic mustard abundance impacted abundance of both site-level all plant species and AM plant species. Climate and soil variables were important predictors at both spatial scales, however the variables that were important differ

between site- and quadrat-level data (Figure 3.3). Importantly, garlic mustard never affected the abundance of non-mycorrhizal plant species at the site level. With quadrat level data, garlic mustard abundance was often an important predictor of abundance and diversity within the community. With quadrat-level data for all species, garlic mustard was the most important predictor of abundance and richness, but % soil clay was more important for Shannon diversity and multiple variables (% soil clay, precipitation seasonality, and soil moisture) were all more important than garlic mustard abundance for all species inverse Simpson diversity. Only annual mean temperature was a better predictor of AM species abundance than garlic mustard abundance. AM plant richness and Shannon diversity were best predicted by garlic mustard abundance, but it was not in the best model for AM plant evenness. Multiple variables (mean temperature of the wettest quarter, soil clay, precipitation seasonality, and soil moisture) were better predictors of AM plant inverse Simpson diversity than garlic mustard abundance. Finally, garlic mustard abundance was an important predictor of facultative AM plant evenness, but less so than annual mean temperature, annual precipitation, precipitation of the wettest quarter and soil clay. Previous garlic mustard abundance increased abundance of all plant species and facultative AM plant species, and Shannon diversity of AM plant species. It was not possible to assess the effect of garlic mustard on non-mycorrhizal plant species with quadrat level data due to low richness and abundance of this group.

Discussion

Uncovering mechanistic drivers of invasion-mediated ecological change are critical for mitigating the ecological impact of invasive species on native communities (Levine et al. 2003). In this study, I explored a well-established mechanism of invader impact, mutualism disruption, to assess invader effects across a range of its abundance, and across heterogeneous geographic space. These are critical steps towards predicting where, and in which communities, invasive species such as garlic mustard will have a negative effect. I show that as garlic mustard abundance increased within a sampling quadrat (1 m x1 m), its impact on abundance of other plant species also increases. I also showed that garlic mustard abundance was as important as abiotic variables in

predicting plant diversity indices. My results provide support for the hypothesis that the impacts of invasive species are scale-dependent (Powell et al. 2011, 2013). The effect of garlic mustard was consistently much stronger when I analyzed data at the quadrat level rather than with site-level data. These results indicate that garlic mustard's impacts are apparent at small spatial extents, where an increase in garlic mustard abundance can increase garlic mustard's impact on the plant community. Thus, my analyses also provide overall support for the mutualism disruption hypothesis. While I was unable to determine if there are abiotic conditions that contribute to a higher impact of garlic mustard (an interaction between garlic mustard abundance and abiotic variables), I have shown that localized garlic mustard abundance (within 1 m^2) has a strong negative effect on abundance and diversity of mycorrhizal plant species using quadrat-level data both within a site and across sites.

When assessing changes in abundance of other plant species within a site or quadrat, high garlic mustard abundance is generally associated with a decrease in abundance of other species. While it is expected that increase in a dominant plant species would lead to decreases in abundances of other plant species, it is notable that a signature of an impact of garlic mustard abundance is detectable across a large geographic area. In addition, population growth rates of garlic mustard populations are predicted to span from below 1 to above 1 across the state of Illinois, and are limited in their southern distribution by high summer temperatures in particular (Merow et al. 2017),. This abiotic governor on the invasion of garlic mustard likely also affects the potency of garlic mustard impacts near the southern range limit in Illinois. Within the state of Illinois, garlic mustard populations may vary in age (i.e. year of site invasion, Lankau et al. 2009), and allelochemical concentrations (Lankau et al. 2009, Smith 2015), and thus differ among sites in their per-capita allelopathic effects on native species. In addition, microsite effects are known to influence garlic mustard invasion success (Anderson et al. 2019). The impact of each garlic mustard population on the native community it is invading is thus likely to respond to different combination of abiotic characteristics. Despite these expected differences in per capita effects of garlic mustard individuals, abundance of garlic mustard populations, and environmental conditions on native species, I still detect a signal of a disproportionate impact by garlic

mustard on the plant species that associate with mycorrhizal fungi. This result suggests that mutualism disruption widely plays a role in garlic mustard's impact on native plant communities.

My results from this analysis, showing the effect of garlic mustard across space, are consistent with results of the analysis that isolated changes within a site. Garlic mustard generally has a negative effect on quadrat-level abundance and diversity when accounting for variation in environmental conditions over space. The effects of garlic mustard on the plant community were highly localized, and rarely detected when analyzing site-level data. Unfortunately, I was unable to fully assess the mutualism disruption hypothesis using quadrat level data because of low total abundances of ectomycorrhizal and non-mycorrhizal plant species at the quadrat level. It is possible that mechanisms such as competition for light or soil resources may explain some of my results. However, the disproportionate effect on mycorrhizal plant taxa is consistent with the mutualism disruption hypothesis.

Data from the Illinois Critical Trends Assessment Program provides 30 years of plant cover observations, with repeated measurements at hundreds of sites across the state. Long-term data such as these are invaluable for recognizing patterns that simply cannot be captured with short-term experiments more common in ecological studies (Blossey 1999, D'Antonio and Flory 2017). However, there is a necessary trade-off that comes with such sampling breadth. I acknowledge that it is not possible to unequivocally establish mechanistic cause from observational data. Further, I cannot untangle whether garlic mustard is present and abundant in locations that have low diversity because it has caused those changes, or because those locations have open niche space due to low local diversity (Davies et al. 2005, Fridley et al. 2007). Yet previous work has experimentally established signatures of mutualism disruption on plant communities (Stinson et al. 2007, Roche et al. 2020), and my results are consistent with previous studies. Another unavoidable limitation of this study is that because sites were sampled every five years, I was unable to capture interannual variation in species abundance. Annual sampling intervals are likely important for a biennial invader, like garlic mustard, because the location of patches of the invader are known to move between years within a site (Anderson et al. 2019). In addition, the

effects of garlic mustard on soil conditions may be long-lived (Lankau et al. 2014, Anthony et al. 2019) and the impacts of changing soil conditions may only become apparent through other plant species responses after a lag period (Crooks 2005, Brouwer et al. 2015). Therefore, my study reflects ~instantaneous responses (within the same year) to garlic mustard abundance, and I establish that abundance of garlic mustard from five years prior is rarely important, but for the most part I am unable to assess the effects of previous garlic mustard abundance on native species.

One major criticism of community ecology is that context dependency plays such a large role in determining outcomes that it is impossible to uncover generalizable theories (Lawton 1999). Indeed, conclusions surrounding the effects of garlic mustard on plant communities often vary widely by site (e.g. Stinson et al. 2007, Davis et al. 2015, Anderson et al. 2019, Roche et al. 2020). However, my analyses have shown that disruption of mycorrhizal mutualisms, which is known to have a physiological impact at the individual level on native mycorrhizal plant species, has effects that are generally detectable across 186 total sites that feature variation in environmental conditions and invasion intensities. I found that mutualism disruption generally explains patterns of plant diversity and abundance in response to allelopathic plant invasion across large scales. Many invasive plants exhibit allelopathic traits (Kalisz et al. 2020, Zhang et al. 2020), and have the potential to impact native plant communities via mutualism disruption (Hale and Kalisz 2012, Grove et al. 2017a). However, because the effects of these allelopathic species are rarely assessed in field conditions (Zhang et al. 2020), mycorrhizal mutualism disruption is likely an underestimated threat to global plant communities.

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Appendix

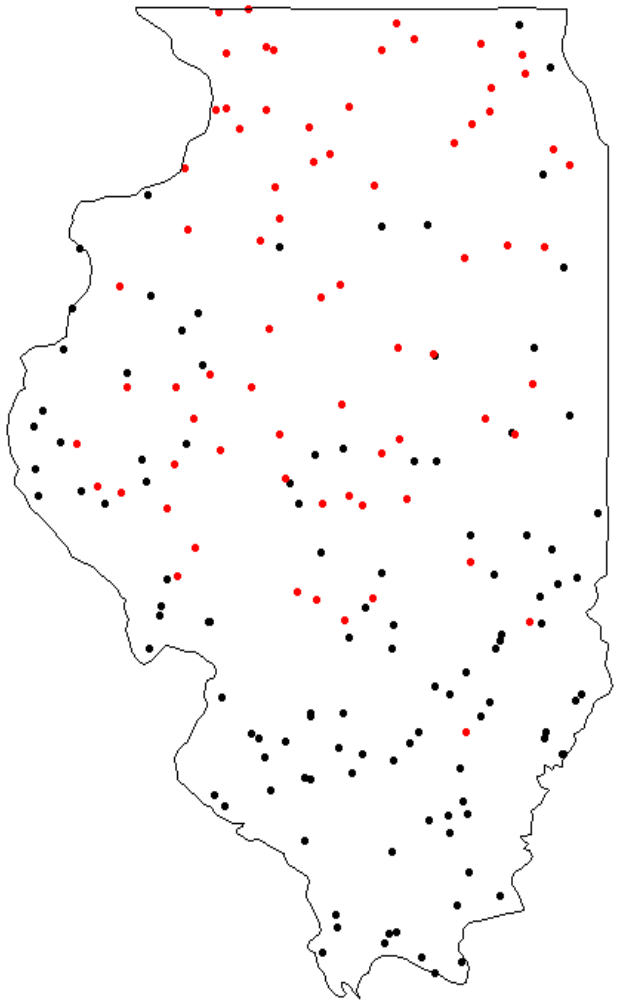


Figure 3.1 Map of 186 CTAP sites across Illinois. Each dot is a Forest CTAP monitoring site where data were collected from 1998 to 2018. Black dots indicate sites at which garlic mustard has never been observed. Red dots indicate sites that have at least one observation of garlic mustard.

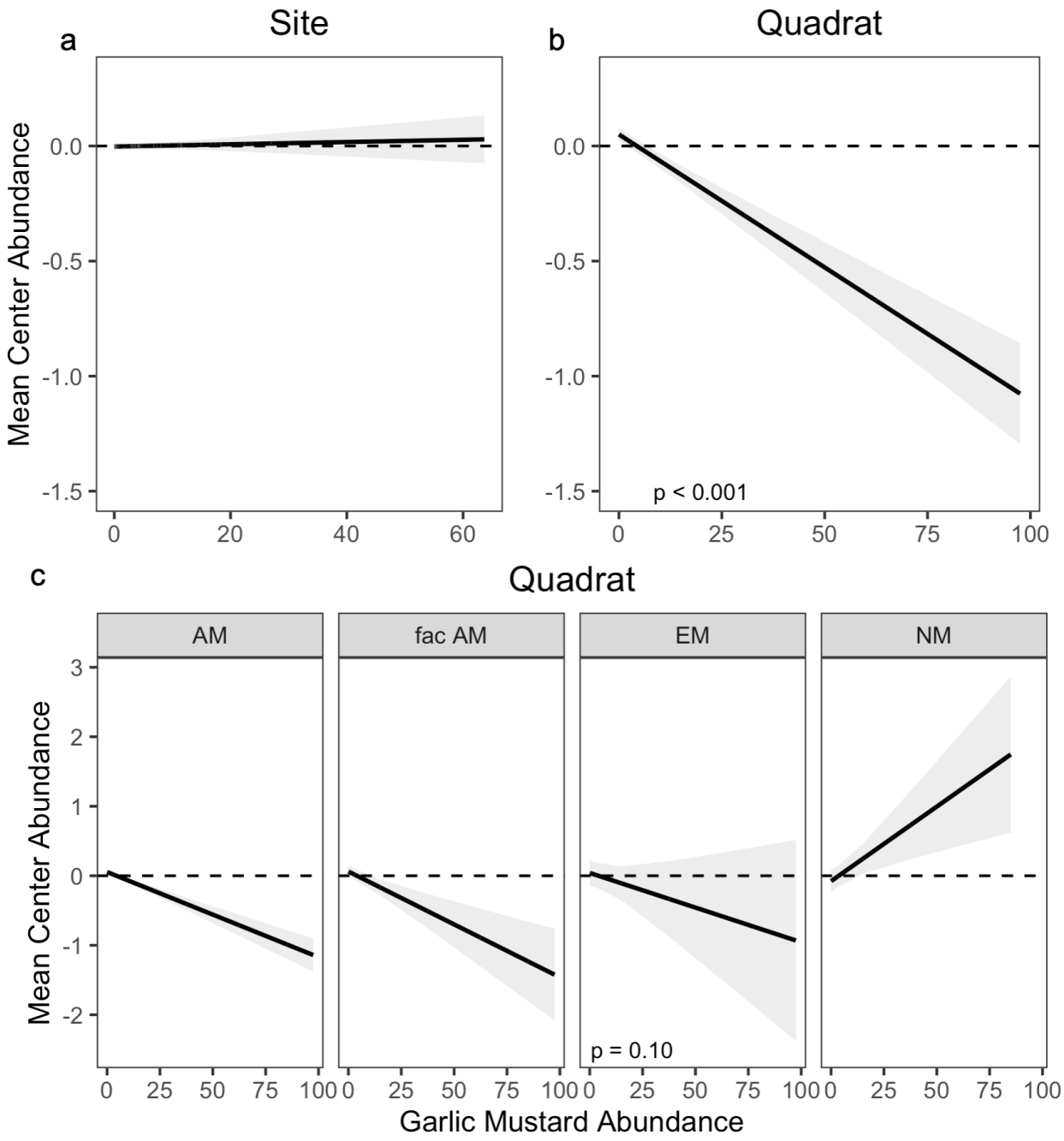


Figure 3.2 Impact of garlic mustard abundance on the mean-centered abundance of other plant species at the a) site or b-c) quadrat level over time. Panels a and b show the main effect of garlic mustard abundance. Garlic mustard has a strong negative effect on abundance of other plant species using quadrat-level data (b, c) , but not site-level data (a). Panel c shows the results of a marginally significant interaction between garlic mustard abundance and mycorrhizal status: arbuscular mycorrhizal (AM),

faculatatively arbuscular mycorrhizal (fac AM), ectomycorrhizal (EM), and non-mycorrhizal (NM). A mean centered abundance below 0 indicates that the abundance of that species is below the mean for that site or quadrat. Solid black lines are fitted from model outputs, surrounded by standard error in gray. Dashed black lines are at 0 indicating a positive effect of garlic mustard above the line and a negative effect of garlic mustard below the line. P-value in panel c refers to the interaction between garlic mustard abundance and mycorrhizal status for the entire panel.

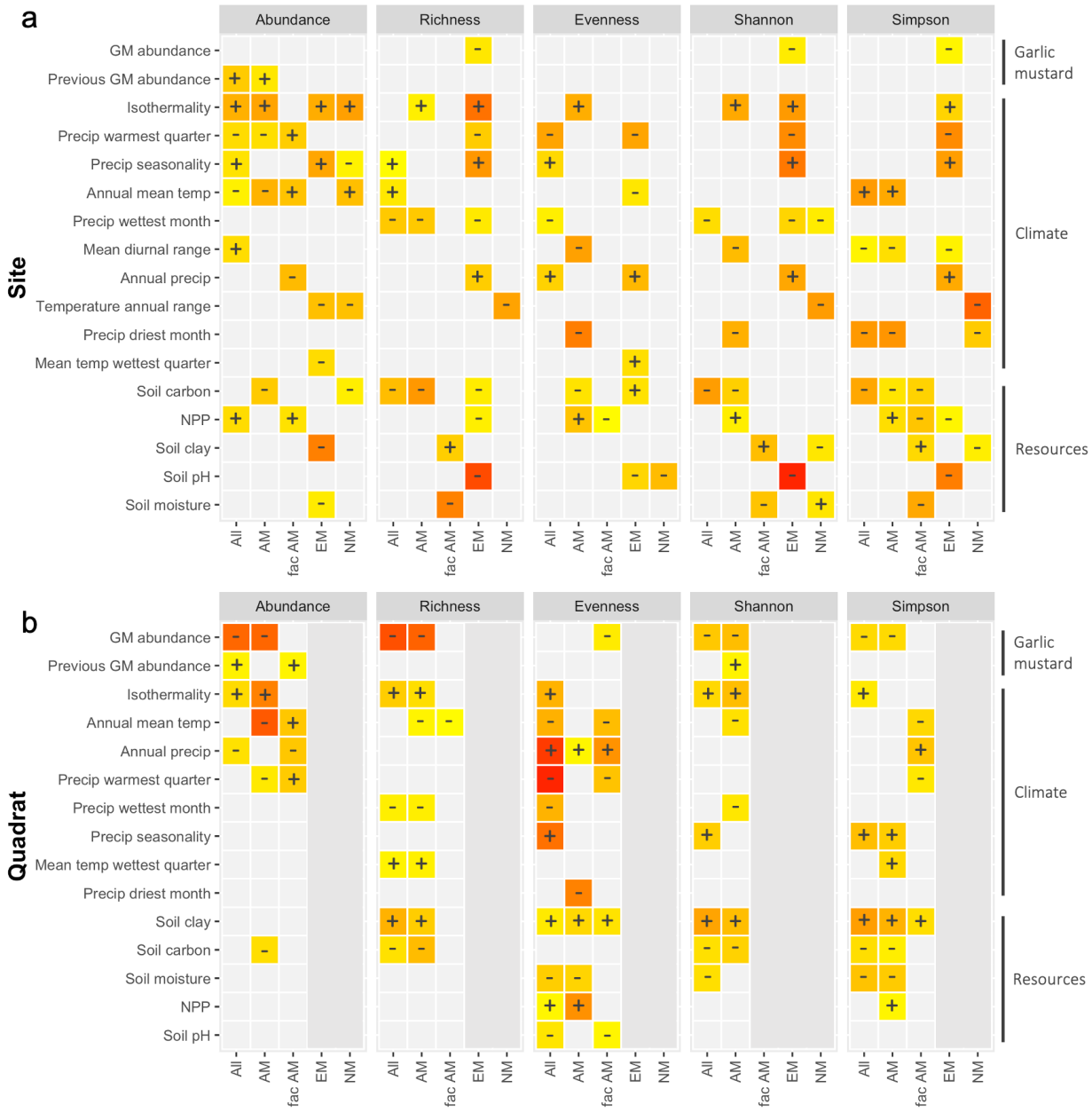


Figure 3.3 Heatmap showing χ^2 values from best models for five plant diversity metrics (abundance, evenness, richness, Shannon diversity and Simpson diversity) for five mycorrhizal status groups of plant species (all, arbuscular mycorrhizal (AM), facultative arbuscular mycorrhizal (fac AM) , ectomycorrhizal (EM), and non-mycorrhizal (NM)) at the a) site level and b) quadrat level. Dark red colors indicate higher χ^2 values and more important predictors, lighter yellow colors indicate lower χ^2 values and therefore less important predictors for the corresponding diversity metric and mycorrhizal group.

Variables are organized by type (garlic mustard variables, climate variables, and soil variables), and within each type are organized by number of times that variable was included in the best model. Variables higher in the heatmap within the variable type were included in more of the best models. Light gray squares indicate that that variable was not included in the best model. Darker gray bars in panel b indicate that there was insufficient data for a variable to be included in an analysis.

CONCLUSION

Ecological communities typically respond to stresses posed by global change in a hierarchical process, first by altered individual physiology, then changes in abundance of individuals in a population, which can compound to alter species presence in a community (Smith et al. 2009). Alternatively, species could respond via evolutionary rescue or shifting physiology and express resilience to global change stresses (Hawkes and Keitt 2015). However, different species within these communities, even when cooccurring, can respond differently to global change stressors.

When these stressors are due to the impacts of invasive species, they are often difficult to evaluate because they are often subtle, and effects at one scale may cascade to other scales in unexpected ways. Mechanistic hypotheses are useful for developing predictions of the impact of invasive species, but these hypotheses are difficult to uncover, and may have divergent effects at different scales (Levine et al. 2003, Suding et al. 2008). The field of invasion biology has made advancements in uncovering potential mechanisms of impacts of many invasive species (Ricciardi et al. 2013, Simberloff et al. 2013), the most well-studied being competition and predation. However, disruption of mutualistic interactions is a potentially widespread process by which invasive species impact native communities (Traveset and Richardson 2006, 2014, Aslan et al. 2013, Grove et al. 2017)

The mutualism disruption hypothesis is an example of a well-documented mechanistic hypothesis (Hale and Kalisz 2012, Aslan et al. 2013, Traveset and Richardson 2014) that can be used to scale up the effects of an invasive species from a mechanistic understanding to realized community level impacts. Many invasive plants exhibit allelopathic properties and have the potential to negatively impact native plant communities by disrupting mycorrhizal mutualisms (Kalisz et al. 2020, Zhang et al. 2020). The physiological and population level effects of mycorrhizal mutualism disruption on native plant species are well established (Stinson et al. 2006, Meinhardt and Gehring 2012, Hale et al. 2016, Bialic-Murphy et al. 2019). However, there are gaps in this literature on allelopathic plant invasion that link the AM fungal community belowground with previously observed effects on native plant physiology and populations, and then from plant populations to plant community responses. These links show how disruption of mutualisms scales across levels of ecological organization,

which informs general predictions of how plant communities are expected to respond to allelopathic plant invasion. Local land managers tasked with mitigating the effects of allelopathic plant invasion can potentially identify communities such as those with a high proportion of mycorrhizal native plant species that may be vulnerable to declines from allelopathic invasion, and focus efforts to reduce the abundance of the mutualism-disrupting allelopathic plant invader.

Overall, I observed community shifts in both the plant and fungal communities in response to allelopathic plant invasion, but in different ways. In garlic mustard ambient treatments, root AM fungal richness increased, but mycorrhizal plant abundance decreased. In the context of allelopathic plant invasion, the interplay between the plant and AM fungal communities may be buffering the impact on plant communities if the increase in AM fungal community is providing reduced mutualism services to plants that otherwise have depleted mutualisms, or exacerbating the impact if the invaded AM fungal community is parasitizing plants that are starved for soil resources.

A review of garlic mustard as an allelopathic plant recently concluded that although there are scenarios in which garlic mustard impacts native plants via disruption of mycorrhizal mutualists, other mechanisms such as competition for resources may be more generalizable (Cipollini and Cipollini 2016). These authors called for studies in natural systems to evaluate the importance of these two mechanisms. My work, in natural communities showed that plant communities, in general, respond to garlic mustard as predicted by the mutualism disruption hypothesis. Further, the plant community responses are consistent across numerous research sites with heterogeneous environmental conditions across the state of Illinois. Therefore, although there may be other hypotheses (i.e. direct competition) explaining some of garlic mustard's impact, indirect effects on plant communities mediated by the mycorrhizal fungal community is the dominant means by which garlic mustard impacts the plant communities in my study.

My findings throughout this dissertation advance our understanding of the consequences of allelopathic plant invasion and mutualism disruption. I took advantage of a rare opportunity to scale up a well-supported mechanistic hypothesis grounded in physiological responses to explain the impacts of plant invasions and assess its effects

on multiple components of the native community. First, I found that there was higher diversity of AM fungi in native plant roots exposed to garlic mustard. Since these native plant hosts decline in abundance and diversity when garlic mustard is present (Stinson et al. 2007, Brouwer et al. 2015, Hale et al. 2016, Roche et al. 2020), this suggests that under conditions created by garlic mustard invasion, native plants may tolerate colonization by AM fungi that are not as beneficial to plant function. If these results are generalizable to other sites, my results foreshadow future declines in native mycorrhizal plant species in communities invaded by garlic mustard or other allelopathic invaders that affect plant-fungal mutualisms. Second, I found experimental evidence for the effects of mutualism disruption on the plant community. Importantly, mycorrhizal, but not non-mycorrhizal, plant species were negatively affected by garlic mustard presence. These results confirm that garlic mustard invasion changes plant communities, and that the effects on native plant communities are primarily mediated by mutualism disruption. Finally, I used long-term and large-scale observational data to show that the impact of garlic mustard invasion on plant communities via mutualism disruption occurs across invasion intensity and environmental conditions. Together, my results connect the above- and below-ground community processes involved in the impacts of allelopathic plant invasion that scale from the microbial community within a plant to responses across large, heterogeneous geographic areas. These results highlight the importance of mechanistic processes such as mutualism disruption for understanding large scale patterns in response to biological invasions.

An important next step to understand the process by which plant communities with different compositions respond to allelopathic invasion is to experimentally test whether an addition of garlic mustard to plant communities with different mycorrhizal traits would follow trajectories that are predictable based on the mutualism disruption hypothesis (i.e. declines in abundance of species that associate with and depend upon mycorrhizal fungi for normal function). Since artificial introductions of invasive species in natural communities are not feasible, mesocosm experiments could be conducted. Experimental communities with different densities of mycorrhizal and non-mycorrhizal native plant species, or using plant species with a range of mycorrhizal dependency, could be grown with a range of garlic mustard densities. An experiment such as this

would explicitly test for the effects that I observed in natural plant communities (Roche et al. 2020) across differing plant communities, while keeping other abiotic conditions constant.

In addition, the consequences of mycorrhizal mutualism disruption do not occur in a vacuum. An important new direction would be to assess the effects of belowground mutualism disruption on other mutualistic interactions. Belowground communities are known to affect floral attractiveness through their effects on resource acquisition and allocation (Barber and Soper Gorden 2015). And since mycorrhizal mutualism disruption has negative effects on plant physiology and carbon gain (Brouwer et al. 2015, Hale et al. 2016), resources for allocation to floral attractiveness may be limited, particularly in perennial plants. Declines in allocation to floral attraction could lead to a change in pollinator visitation due to a change in native community floral displays (Cahill et al. 2008). Further, high densities of garlic mustard in invaded sites could compete for pollination services (Brown et al. 2002). Additionally, declines in floral resources could directly limit populations of floral visitors due to decreased nutritive benefit from the plant (Barber and Soper Gorden 2015).

My dissertation explores the impacts of garlic mustard both above- and below-ground. I uncovered the ways in which the AM fungal community responds to garlic mustard invasion. I scaled up the effects of mutualism disruption to the plant community and showed plant community responses that are consistent with mutualism disruption at varying garlic mustard abundances and environmental conditions. These results are critical for advancing predictions of the impacts of plant invasions across the invaded range, which naturally varies in recipient community composition, invasion intensity, and environmental conditions.

Scaling up ecological processes to inform large scale predictions is one of the major goals of ecological research (Simberloff 2004, Denny and Benedetti-Cecchi 2012, Chave 2013). In my dissertation, I scaled the effects of allelopathic plant invasion from the known physiological and population-level effects on plant populations to effects at the local plant and AM fungal community level, to regional-level consequences for plant communities. Community ecology is notorious for having immense complexity, leading to few generalizable laws (Lawton 1999, Simberloff 2004). Research on mechanisms of

invasion provides unique opportunities to understand how communities are structured and predict when and where invaders will succeed. With a deeper understanding of the mechanisms at play, my research on mutualism disruption allowed me to propose specific hypotheses regarding the differing responses of the mycorrhizal and non-mycorrhizal portions of the plant community across scales. Scaling up hypotheses using long-term and large-scale data opens up opportunities to understand how species interactions underlie generalizable patterns of impacts of biological invasions.

Furthermore, mutualism disruption is a potentially common driver of impacts of invasive species, with the vast majority of organisms forming mutualistic relationships, many of which are threatened by biological invasions (Traveset and Richardson 2006, 2014, Aslan et al. 2013, Grove et al. 2017). Specifically threatened by mycorrhizal mutualism disruption are the ~80% of land plants that associate with mycorrhizal fungi (Smith and Read 2008). In addition, allelopathy is a common trait of invaders, as it is estimated that 72% of invasive plants have allelopathic potential (Kalisz et al. 2020). Careful examination of allelopathic potential of many individual species is needed (Fitter 2003, Inderjit and Callaway 2003, Blair et al. 2009). Also, it is still necessary to determine whether the allelopathic properties of each invasive plant act directly (i.e. on a native plant in the invaded range) or indirectly (i.e. on the mutualist or through changes in soil characteristics) which then affect native plant species (Zhang et al. 2020). Because of the ubiquity of both mycorrhizal plants vulnerable to mycorrhizal mutualism disruption, and the prevalence of allelopathic plant invaders capable of mycorrhizal mutualism disruption, the threats to plant communities are potentially vast. The mutualism disruption hypothesis is a generalizable mechanism explaining patterns of plant invasion impacts that clearly predicts directional shifts in native plant communities worldwide.

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VITA

Morgan D. Roche earned her Bachelor of Arts in Biology and Anthropology in 2015 from Bucknell University in Lewisburg, PA. While there, she grew an interest in plant ecology, and began to pursue a career in science. In 2015, Morgan began her graduate education in the Department of Ecology and Evolutionary Biology at the University of Tennessee, Knoxville. During this time, she undertook research projects at the intersection of invasion biology and community ecology, which took her across the United States, including Knoxville, TN, Pittsburgh, PA, and Fort Collins, CO. When not doing ecology, you will find Morgan cooking great food, running around town, or curled up with her cat.