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A review of garlic mustard (*Alliaria petiolata*, Brassicaceae) as an allelopathic plant

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Abstract. *Alliaria petiolata* is a widespread biennial herb from Eurasia that is one of the most recognizable invasive plants of forests in the eastern United States and southern Canada. After two decades of intensive study on its physiology, ecology, and impacts, this plant has come to be known in both the scientific and gray literature as an allelopathic plant capable of exerting negative, chemically mediated effects on plants and microbes in its environment. A critical review of the literature reveals that there is evidence both supporting and failing to support this assertion, and that conclusions can be affected greatly by the experimental approaches taken, the target species examined, the sources of allelopathic inputs, and environmental factors. The objective of this review is to provide a history of allelopathy research in *A. petiolata*, describing the various approaches that have been taken and conclusions drawn, and to summarize the current standing of *A. petiolata* as an allelopathic plant using the most ecologically relevant data on this phenomenon. Finally, we discuss the degree to which allelopathy, versus other mechanisms, may contribute to the invasive success of this plant.

Key words: allelochemicals, bacteria, glucosinolates, invasive plants, mycorrhizae

***Alliaria petiolata* as a Model Invasive Plant.** *Alliaria petiolata* (Cavara and Grande, Brassicaceae) "*Alliaria*" is a biennial herb from Eurasia that was first identified in North America in the 1860s (Nuzzo 1993). *Alliaria* has since become known as one of the most notorious invasive plants of forest understories and edges in the eastern United States and Canada, but it is also a common, but less intrusive, plant in its native range (Blossey *et al.* 2001). Studies of the ecology of this plant in North America first arose in the 1970s and many of the early studies were on the life history and reproductive ecology of this plant (*e.g.*, Cavers *et al.* 1979). As *Alliaria* was increasingly viewed as a problematic plant, attention turned to mechanisms of impact, including its ability to compete for resources (*e.g.*, Meekins and McCarthy 1999). Another mechanism of impact, for which *Alliaria* has become a "poster child," is the production of secondary metabolites that can exert negative effects on native plants, insects, and microbes (*e.g.*, Cipollini *et al.* 2012a), collectively referred to here as *allelopathy*. After two decades of intensive study on this phenomenon, support for the assertion that allelopathy is an important invasive mechanism in this plant remains mixed. In this review, we examine the history of allelopathy research in *Alliaria*, highlighting studies of its direct and indirect effects on plants and beneficial microbes, and summarize the current standing of this plant as an allelopathic plant using the most ecologically relevant data on this phenomenon.

Allelochemicals From *Alliaria petiolata* and Their Direct Effects on Plants. In order for a plant to exert allelopathic effects, it must produce compounds with bioactive effects that are capable of entering the environment around the plant and persisting long enough to have effects on neighboring organisms. Attesting to the bioactive properties of its tissues, *Alliaria* has long been used as a spice, having been found in 6,000-yr-old Neolithic cooking pots from northern Europe (Saul *et al.* 2013). It was likely originally brought to North America in the late 1800s for culinary or medicinal uses, but accidental introductions have likely occurred as well (Nuzzo 1993). Its utility as a spice is largely due to the presence of glucosinolates, a class of compounds characteristic of the Brassicaceae that provide the pungency to mustard and other food products from this family of plants (Drewnowski and Gomez-Carneros 2000). Glucosinolates and their derivatives have

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been long investigated for their weed-suppressive and antimicrobial activities (Shreiner and Koide 1993). By as early as 1845, the bioactive chemistry of roots and leaves of *Alliaria* was being investigated, revealing that *Alliaria* produced a "mustard oil" in its roots similar in nature to that of black mustard, *Brassica nigra* (Wertheim 1845). Interestingly, Wertheim indicated that *Alliaria* was a common weedy plant of gardens in Germany at the time. The chemical similarity between *Alliaria* and *B. nigra* is due to the presence of allyl isothiocyanate (AITC), a volatile compound that is liberated when the glucosinolate sinigrin is hydrolyzed by the enzyme myrosinase. Allyl isothiocyanate accounts for nearly half of the volatile content of fresh *Alliaria* leaves (Blazevic and Mastelic 2008). While present in many weedy mustards (*Brassica* spp., in particular) that have invaded open habitats in North America, sinigrin and AITC appear to be novel in the native North American flora (Agerbirk *et al*. 2010, Barto *et al.* 2010a). Later studies driven primarily by a desire to understand negative effects of this plant on some North American native insects have revealed a suite of other compounds in roots and leaves of *Alliaria*, including novel hydroxynitrile glucosides, such as alliarinoside, as well as a variety of flavonoids, flavones and their glycosidic derivatives, and cyanide and other volatile products (*e.g*., Haribal and Renwick 2001, Cipollini and Gruner 2007, Blazevic and Mastelic 2008, Frisch *et al*. 2014).

Despite its use as a spice, some understanding of its bioactive chemistry, and its recognition as a weedy plant, no studies of allelochemically mediated ecological impacts of *Alliaria* are found in the literature until the 1990s. The first published study on allelopathy of *Alliaria* examined the effects of aqueous extracts of leaf and root tissue that were macerated in a vegetable juicer on germination and seedling growth of four nonnative commercial plant species (McCarthy and Hanson 1998). Little evidence of allelopathic inhibition of germination or growth was found, but it was likely that the vigorous tissue disruption and mixing of plant enzymes with their substrates resulted in a loss of bioactive allelochemicals from extracts, including toxic and volatile degradation products of glucosinolates that result from the action of myrosinase on parent glucosinolates. Soon after, Vaughan and Berhow (1999) reported strong effects of organic solvent extracts of whole *Alliaria* tissues containing allyl isothiocyanate (AITC) and benzyl isothiocyanate (BzITC), toxic degradation products of the two major glucosinolates that *Alliaria* produces, sinigrin and glucotropaeolin. Both compounds significantly inhibited radicle elongation of wheat (*Triticum aestivum*) and garden cress (*Lepidium sativum*).

Effects of either extracts of *Alliaria* or the purified compounds it contains have been found to vary with experimental conditions and target species. In tests of purified AITC and BzITC and their parent glucosinolates, *Lepidium sativum* (a member of the Brassicaceae) was more susceptible than *Triticum aestivum* to the effects of the parent glucosinolates (Vaughan and Berhow 1999), which may have been due to the possession of endogenous myrosinase by *L. sativum* that yielded bioactive degradation products from the parent glucosinolates. A subsequent study with *L. sativum* revealed that it was more tolerant to aqueous extracts of dried and ground *Alliaria* leaves than was lettuce, but it was more negatively affected by extracts of *Alliaria* than by extracts from a weedy member of its own genus, *Lepidium perfoliatum* (Aminidehaghi *et al.* 2006). Later studies (Cipollini *et al.* 2008, Cipollini *et al*. 2012) showed that *Arabidopsis thaliana* (a member of the Brassicaceae) suffered no negative growth and/or fitness effects from exposure to aqueous extracts of fresh *Alliaria* leaves, although it did from exposure to extracts of another putatively allelopathic plant, *Lonicera maackii*. Similarly, germination of *Brassica rapa* was reduced by extracts of fresh leaves of *L. maackii*, but not of leaves of *Alliaria* (Cipollini *et al.* 2012b). Conversely, Pisula and Meiners (2010) found that extracts from dried *Alliaria* leaves could inhibit germination of the seeds of radish, *Raphanus sativus* (a member of the Brassicaceae) at relatively low concentrations, but that extracts from dried *Lonicera japonica* leaves could not. Finally, in a study using aqueous extracts of seeds, germination percentage of *B. rapa* seeds was unaffected by exposure to *Alliaria* seed extracts, but germination was delayed (Barnum and Franks 2013). However, exposure of *B. rapa* seeds to its own seed extracts both reduced germination percentage and delayed germination to a greater degree than exposure to *Alliaria* extracts. Only one study has compared the relative impact of root versus leaf extracts, and found milder effects of root extracts than leaf extracts on germination of three target species (Cipollini and Flint 2013).

One interesting commonality in the studies cited so far is that many of them used model plants from the same family as *Alliaria* as a target for potential allelopathic effects, which could either enhance or obscure the appearance of effects. Few of these plants are particularly relevant to the ecology of *Alliaria* in introduced habitats. While still using extracts, Cipollini and Flint (2013) and Cipollini and Greenawalt (2016) extended earlier work by using ecologically relevant species. Leaf extracts of *Alliaria* significantly reduced germination of the native target species *Anemone virginiana*, *Blephilia hirsuta* and *Elymus hystrix*, while root extracts significantly affected *A. virginiana*, but not *B. hirsuta* and *E. hystrix* (Cipollini and Flint 2013). In Cipollini and Greenawalt (2016), however, germination of *E. hystrix* and another native, *Chamaecrista fasciculata* were not affected by leaf extracts of *Alliaria*. These disparate findings raise important questions about tissue handling (dried versus fresh tissues), extract preparation (maceration versus soaking, solvent versus aqueous extractions), and source of inputs (seeds versus leaves versus roots). Each of these choices can determine the relative "toxicity" of an extract preparation, but it is unclear in most studies how closely laboratory-prepared extracts represent natural allelochemical inputs from live and decaying roots and leaves in the field. In addition, relatively few extract studies have examined direct allelopathy with ecologically relevant target species, and those that have found that effects can vary by target species or experimental conditions (Cipollini and Flint 2013, Cipollini and Greenawalt 2016). For example, while Cipollini and Flint (2013) found direct effects of *Alliaria* leaf extracts on germination of *E. hystrix*, a grass native to invaded habitats, a subsequent study by Cipollini and Greenawalt (2016) found minimal effects on the same species. Differences may be attributable to the use of *Alliaria* collected from different sites and during different seasons, as the bioactive chemistry of *Alliaria* can vary with environmental conditions (Cipollini 2002, Hillstrom and Cipollini 2011, Cipollini and Lieurance 2012, Smith 2015, Smith and Reynolds 2015), as well as with population (Cipollini *et al*. 2005, Lankau *et al*. 2009, Hillstrom and Cipollini 2011) and season (Haribal and Renwick 2001). There is also increasing recognition of pathogenic microbes that may inhibit growth of *Alliaria* above- or belowground, which may in turn reduce allelopathic effects (*e.g*., Cipollini and Enright 2009).

Due to a number of limitations, extract bioassays may have a relatively limited value in predicting what might happen in field environments (Inderjit and Nielsen 2003); for example, allelochemicals rarely enter the environment in the form of a concentrated fresh leaf extract. However, because extract studies are often optimized to see effects, extract studies can reveal a species' potential to have allelopathic effects. While the observation of effects in the laboratory does not mean that effects will be important in the field, the lack of effects observed in the laboratory likely means that effects will be unimportant in the field. Extract bioassays can also more easily allow the comparison of invasive species to determine relative potential for phytotoxic effects. For example, Pisula and Meiners (2010), Cipollini *et al*. (2012b), Cipollini and Flint (2013), and Cipollini and Greenawalt (2016) compared the effects of 11, 3, 3, and 5 invasive species, respectively, in this manner. In most of the above studies, *Alliaria* generally was one of the strongest inhibitors, though effects were weaker in Cipollini and Greenawalt (2016). However, extracts are usually complex mixtures of plant secondary compounds, proteins, sugars, mineral nutrients, and microbes, among other things, which are variables that are infrequently controlled. Thus, even when effects are seen in extract studies, it can be difficult to pinpoint the mechanism of impact.

Evidence for Allelopathic Effects From Soil Conditioning and Field Studies. To add ecological realism, Prati and Bossdorf (2004) introduced a soil conditioning approach to allelopathy studies in *Alliaria* by growing this plant in soil and subsequently using the soil alone (presumably enriched with root exudates and likely an altered microbial community) in pot studies. This approach does not isolate allelopathic effects that are due specifically to secondary compounds, but it better reflects the way in which *Alliaria* roots affect the soil as it is growing in nature. It largely removes the potential allelochemical impact of leaves, however, which may be important in the field due to leaf leaching by precipitation and degradation of senescing leaves. Prati and Bossdorf (2004) also used activated carbon in their study, which had become a popular control in studies of allelopathy due to its ability to absorb organic compounds from soils and to ameliorate allelopathic effects (Ridenour and Callaway 2001). While putative allelochemicals were not measured, nor were effects mediated by nutrients or microbes considered, seed germination of two *Geum* species was generally lower in soils conditioned by *Alliaria* than in unconditioned soils, and it generally increased with the addition of activated carbon (activated carbon alone had no direct effect on seed germination in unconditioned soils). By using one *Geum* species from Europe and one from North America, and *Alliaria* populations collected from both continents, this study also added a biogeographical perspective. In particular, the North American *Geum* species appeared to benefit more from activated carbon treatment overall than the European species, suggesting the increased sensitivity to *Alliaria* in a naïve competitor. In addition, the European *Geum* species only responded positively to activated carbon treatment in soil conditioned with European *Alliaria*, suggesting that there were weaker allelopathic effects from populations of *Alliaria* found in North America.

Soil conditioning and field studies have indicated that allelopathic effects of *Alliaria* can vary across populations or with the degree of competition experienced by *Alliaria* or its neighbors. Lankau *et al*. (2009) showed that the allelopathic effects of soil conditioned by *Alliaria* growth on biomass of three tree species generally decreased with the age of the *Alliaria* population used to condition the soil, which correlated with declining root glucosinolate levels with population age. They suggested that the costs and benefits of allelochemical production favored the loss of allelopathic potential in older populations of *Alliaria* where *Alliaria* was the dominant species and mostly competing with itself. While the negative correlation between *Alliaria* root glucosinolates and population age was relatively strong in this study, correlations between either of these factors and target tree biomass were weak, suggesting that soil concentrations would have been important to measure in order to implicate them in age-dependent allelopathic effects. Interestingly, the growth of *Platanus occidentalis* appeared to decline in this study in response to increasing age (and root glucosinolate level) of the *Alliaria* populations, but this same tree species did not respond significantly to the presence of *Alliaria* in live soils in another pot study (Lankau 2009), in which root glucosinolate levels also did not correlate with tree growth. Barto *et al.* (2010b) showed that root and leaf extracts of *Alliaria* could reduce seed germination and growth of *Impatiens pallida*, an effect that could be ameliorated by activated carbon. When applied at high doses to the soil, effects of these extracts also followed a plant density-dependent pattern of phytotoxicity on growth of *I. pallida* in a pot study, in which plants growing at low densities suffered greater per capita negative effects than plants growing at higher densities. However, when applied at concentrations expected in field soils, effects of *Alliaria* extracts on *I. pallida* growth were not distinguishable from the effect of resource competition alone (Barto and Cipollini 2009a). Lankau (2012) showed that *Alliaria* root glucosinolate levels were higher in plants from areas with high competitor densities, but that a native competitor, *Pilea pumila*, appeared to be evolving resistance to allelopathic effects of *Alliaria*. These results concur with results from the first study to suggest the evolution of resistance of a native plant, *Impatiens capensis*, to the presence of *Alliaria* in its invasive range, though allelopathy was not explicitly considered (Cipollini and Hurley 2009). Such evolution would be expected if allelopathy by *Alliaria* has exerted selective pressure on competing plant populations for a sufficient amount of time.

In the first study to address allelopathic effects of *Alliaria* in the field with the use of activated carbon as a tool to ameliorate allelopathy, Cipollini *et al.* (2008b) demonstrated a beneficial effect of activated carbon placed in the root zone on growth of the annual, *I. capensis*, transplanted near *Alliaria*. Lankau (2009) later used activated carbon to reveal that direct allelopathic effects of *Alliaria* on *P. occidentalis* growth in a pot study were only apparent in sterilized soils, suggesting that the microbial community of live soils played an important role in degrading allelochemicals. It was also only in sterilized soils where negative correlations between glucosinolate concentrations of *Alliaria* roots and biomass production of *P. occidentalis* were observed. However, it is important to note that studies using activated carbon as a manipulative tool must be interpreted with caution due to potentially confounding effects, including alterations in nutrient dynamics, that activated carbon addition may produce (Lau *et al.* 2008). Like studies using tissue extracts, most studies using either soil conditioning approaches or that were performed in the field and coupled with activated carbon addition have indicated the potential for allelopathic effects of *Alliaria* to be important. However, these studies were not able to isolate the mechanism(s) of impact and whether effects were direct or indirect.

Allelochemical Effects of *Alliaria* on Mycorrhizal Fungi. Since the Brassicaceae generally lack associations with mycorrhizal fungi (Shreiner and Koide 1993), a great deal of attention has been given to the ability of *Alliaria* to allelopathically inhibit mycorrhizal fungi of competing plants. This would allow *Alliaria* to inhibit mutualistic microbes of its neighbors, possibly gaining a competitive advantage with no negative effects on itself, a possibility first raised by Vaughn and Berhow (1999). A simple laboratory study demonstrated that leaf extracts of *Alliaria* were able to inhibit spore germination of *Gigaspora rosea*, an arbuscular mycorrhizal fungal (AMF) species (Roberts and Anderson 2001). In this study, the density of *Alliaria* plants growing naturally in the field was also negatively correlated to mycorrhizal inoculum potential of the soils, which could have been due to (unmeasured) increases in allelochemical concentrations in soils, or simply due to a decline in suitable hosts with increases in *Alliaria* density caused by resource competition.

Stinson *et al*. (2006) subsequently demonstrated lowered mycorrhizal infection rates in several North American tree species exposed to both aqueous *Alliaria* extracts and to soils conditioned by *Alliaria*, with a resultant indirect negative effect on tree growth. Wolfe *et al*. (2008) similarly found lowered ectomycorrhizal fungal (EMF) infection rates of pine trees in areas invaded by *Alliaria* in the field. Callaway *et al*. (2008) used a combination of soil conditioning, leaf extracts, and a range of naturally co-occurring plant species to show that allelopathic effects of *Alliaria* increased with the extent of mycorrhizal dependence of the target plant species, and that North American species generally suffered greater negative effects than their congeneric European counterparts. Importantly, this study used the ratio of tissue concentrations to soil concentrations of glucosinolates reported in studies of *Brassica* green manures (*e.g*., Brown and Morra 1997) to estimate field-relevant soil concentrations of *Alliaria* allelochemicals to utilize in experiments. Using this approach, and the ability to produce leaf extracts with different classes of metabolites, AMF from North American soils lacking an evolutionary history with *Alliaria* were shown to be more susceptible to negative effects of ecologically relevant levels of *Alliaria* extracts than AMF from European soils. Both glucosinolate and flavonoid extracts of *Alliaria* leaves were partly responsible for allelopathic effects on AMF spores, and the mixture of the two worked synergistically to inhibit the germination of spores present in soils with no history of *Alliaria*. This study provided some of the best support for the novel weapons hypothesis (Callaway and Ridenour 2004) to date for this or any other plant, and was the first to implicate metabolites other than glucosinolates in allelopathic effects of *Alliaria*.

Koch *et al*. (2011) showed that extracts of *Alliaria* leaves containing both glucosinolates and flavonoids could inhibit colony growth of *Glomus intraradices*, an AMF species, in carrot root cultures. However, Barto *et al.* (2010b) found that if *I. pallida* plants had established a relationship with AMF prior to contact with *Alliaria* extracts, then vegetative growth was unaffected by exposure to extracts. This was true even though some negative effects on AMF colonization could be seen in glass root-viewing chambers where AMF colonization could be monitored using epifluorescence microscopy of live roots through time. This indicates that spore germination and the early development of mycorrhizal symbioses are critical stages that may be affected by *Alliaria*, but that healthy mycorrhizal relationships may protect against allelopathic effects of *Alliaria*. Hale *et al.* (2011) found that soil respiration was reduced in the presence of *Alliaria* in a pot study, ostensibly due to a reduction in mycorrhizal community function. There was a concomitant reduction in physiological function in *Maianthemum racemosum*, potentially reflecting the cost of disruption of mycorrhizal mutualisms. This effect may have been amplified in this study, however, due to the direct incorporation of fresh leaves to soils, which is unlikely to be a common route of entry of allelochemicals to soils in the field.

Barto *et al*. (2011) showed that AMF infection rates of sugar maple growing near *Alliaria* plants were reduced more greatly in Ohio than in Massachusetts, with populations of *Alliaria* being presumably younger in Ohio. Arbuscular mycorrhizal fungal community composition in the rhizosphere of sugar maple also changed in response to the presence of *Alliaria* in this study. Lankau (2010a) later showed that effects of *Alliaria* populations on mycorrhizal fungal richness and community structure in the rhizosphere of *Quercus rubra* were correlated with concentrations of glucosinolates and alliarinoside in the roots of *Alliaria*. Importantly, as *Alliaria* increasingly altered mycorrhizal community structure, growth of *Q. rubra* and another native tree increasingly declined. However, correlations between the effect of *Alliaria* and population age were not as apparent as in the 2009 study. While links between mycorrhizal community structure and native plant performance may be unclear, alterations in mycorrhizal community structure induced by the presence of *Alliaria* persisted for at least six years after its removal from a forested habitat (Lankau *et al.* 2014).

While some studies have attempted to expose competing plants or AMF to ecologically realistic concentrations of putative allelochemicals from *Alliaria* through soil conditioning or extract dilution, only two studies have assessed concentrations of putative allelochemicals in soils affected by *Alliaria*. Barto and Cipollini (2009b) were unable to extract glucosinolates from field soils under *Alliaria*, but they were able to capture some potentially bioactive flavonoid derivatives through biomimetic extraction using polydimethylsiloxane (PDMS) tubing, a technique developed to capture allelochemicals in soils by Weidenhamer (2005). However, compounds were only detectable at a few time points during the season and half-lives of most of these compounds were on the order of hours when they were applied to nonsterile field soils. Thus, the potential impact of these metabolites would vary seasonally, and they would likely need regular replenishment in the field to be influential. Frisch *et al.* (2014) has shown that most of the bioactive glucosinolates and alliarinoside in *Alliaria* leaves are metabolized by endogenous plant enzymes (*e.g.*, glucosidases, such as myrosinase) within hours in leaf homogenates, and many of the products are volatile. This has implications for the expected longevity of bioactive compounds in the environment, as well as implications for the stability of extracts used in allelopathy studies. Despite these issues, Cantor *et al.* (2011) were able to detect AITC (the volatile derivative of sinigrin) in field soils at levels that were sufficient to inhibit spore germination of *Glomus clarum*, an AMF species, in laboratory bioassays. This was important because no studies implicating glucosinolates in allelopathic effects of *Alliaria* in either field or pot studies had confirmed the presence of the parent compounds or their derivatives in the soil. Nonetheless, the transient nature of their detection and their degradation rates indicate that if glucosinolates or other metabolites are partly responsible for allelopathic effects of *Alliaria*, then they must generally work in low concentrations and would be expected to vary in their contribution to allelopathic effects throughout the season. To that end, only minor reductions on fungal hyphal abundance were noted in *Alliaria*-invaded plots in the field where AITC could be detected.

Despite the evidence that *Alliaria* or its extracts can have either direct or indirect allelopathic effects, a number of studies have not found major allelopathic effects of *Alliaria* on plants or their fungal partners. In some cases, the negative effects of *Alliaria* seen on some variables in a study have been emphasized over the lack of effect on others. For example, Burke (2008) found little effect of *Alliaria* presence on either AMF infection rates of three forest herbs or AMF community structure in a field study. Barto and Cipollini (2009a) and Barto *et al.* (2010b) showed that *Alliaria* leaf extracts can have direct negative effects on germination and growth of *I. pallida* in the laboratory. Extracts had no effect on growth or AMF infection rates of *I. pallida*, however, if the plants were colonized by AMF before exposure to *Alliaria* extracts (Barto *et al.* (2010b). Koch *et al.* (2011) found little effect of *Alliaria*-conditioned soils or extracts on AMF species richness or community structure in a pot study, despite showing that *Alliaria* extracts could reduce AMF colony growth in culture. Lankau (2010b) showed that effects of *Alliaria* on AMF community composition in the field appear to change with the age of *Alliaria* populations. In particular, AMF species richness declined and community structure changed when moving from young to medium-aged populations, but AMF species richness rebounded in older populations of *Alliaria*. Different conclusions about the effect of *Alliaria* on mycorrhizae and subsequent indirect effects on plant competitors could be related to variation in allelochemical quantities and profiles of *Alliaria* populations at different field sites (Lankau *et al*. 2009, Lankau 2010a). In most studies of the direct and indirect effects of *Alliaria* on mycorrhizae, the relative impact of *Alliaria* versus other interacting species has also not been determined. Cipollini and Greenawalt (2016) found fewer effects of *Alliaria* leaf extracts on mycorrhizal infection of *E. hystrix* than extracts of four other invasive species. However, Brouwer, Hale, and Kalisz (2015) found more negative effects on carbon storage in tissues of *Maianthemum racemosum* from treatment by leaves of *Alliaria* than by leaves of *Hesperis matronalis*, a related nonnative species considered to be less invasive than *Alliaria*. They also found that vital rates improved for this plant in the field in areas where *Alliaria* had been removed for several years. While implicating negative effects on mycorrhizae, infection rates and community structure were not examined. So, while the negative impact of *Alliaria* on mycorrhizal relationships has been often invoked, its effects, and their importance, may be no greater than that of some other (nonrelated) invasive species. No studies have directly compared the effect of *Alliaria* on mycorrhizae with that of related, but native, mustard species.

Allelochemical Effects of *Alliaria* on Bacteria and Bacterial Communities. Given its nonmycorrhizal status, potential impacts of *Alliaria* on mycorrhizal fungi have been a worthy target of attention. In contrast, impacts of *Alliaria* on soil bacteria have been much less well studied and can be difficult to interpret as an allelopathic mechanism, in part because negative impacts of *Alliaria* on some beneficial bacteria could harm its own growth. Nonetheless, a few studies have now examined impacts of *Alliaria* on either individual bacteria or bacterial communities. Burke and Chan (2010) found that while seasonal differences existed, bacterial richness, evenness, and diversity were similar in soils under *Alliaria* and under two native forest herbs. Instead, they suggested the physicochemical properties of the soil, and changes therein across seasons, were more important than plant identity in determining bacterial community structure. In contrast, Lankau (2010b) found that bacterial communities experienced significant shifts in richness and in the abundance of species sensitive to its effects after *Alliaria* invasion, but they tend to recover to the native condition in areas where *Alliaria* has been present for a long time, including the restoration of sensitive species.

In a recent study, Portales-Reyes *et al*. (2015) examined whether *Alliaria* extracts or synthetic AITC and BzITC were capable of disrupting interactions between a native legume, *Amphicarpaea bracteata*, and its mutualistic rhizobia. They found that BzITC applied at expected field concentrations reduced rhizobia growth rate in the laboratory, but its application had no effect on nodulation in the greenhouse when rhizobia were grown with their host plants. Allyl isothiocyanate did not affect either the plants or rhizobia in isolation, but plants grown with rhizobia in the presence of AITC showed reduced nodulation. Despite finding some impacts of synthetic glucosinolate derivatives, they found no effects of aqueous extracts of fresh leaves of *Alliaria* on plant performance or nodulation. In addition, the amount of biomass accumulated by plants exposed to AITC (that suffered reduced nodulation) was not significantly different from that accumulated by control plants (with typical levels of nodulation). This study should be commended for being the first to consider the potential impact of *Alliaria* on a native legume and its rhizobia. However, the lack of effect of *Alliaria* extracts and lack of the effect of reduced nodulation on plant growth are likely more important results than the minor impact of purified AITC on nodulation that was highlighted. This finding clearly warrants more research into its significance.

Placing Allelopathic Effects of *Alliaria* in Context. A large number of studies of allelopathy of *Alliaria* are limited by the fact that they are conducted under greenhouse or laboratory conditions, making the ability to extend results to the field difficult. However, when taken together, direct and indirect allelopathic effects of *Alliaria* on plants and their associated microbial mutualisms seem possible under some circumstances, but several studies have found little apparent allelochemical impact of *Alliaria* on either plants or their microbial partners. When allelopathic effects have been found in the most ecologically realistic scenarios, their magnitude depends on plant density, age and allelopathic potential of the *Alliaria* population, the evolutionary history of the soils and their microbial communities with *Alliaria*, and the dependence of target plants on mutualistic association with microbes. Few studies have followed the response of plants and microbial communities through the invasion process of *Alliaria*, but one study has demonstrated that changes in *Alliaria* densities and responses of the native plant community appeared to be largely independent (Davis *et al.* 2014). Unfortunately, only a few studies have attempted to place allelochemical-mediated impacts of *Alliaria* in the context of other mechanisms of competition or impact; thus, the relative importance of allelopathy as a mechanism of invasive success in *Alliaria* has been difficult to gauge. In one study that examined multiple impacts, Cipollini *et al.* (2008) showed that adding activated carbon to the rhizosphere improved the growth of *I. capensis* plants that were transplanted near *Alliaria* plants (presumably by ameliorating allelopathic effects), but removing the aboveground biomass of the competing *Alliaria* plants had similar positive impacts on growth of *I. capensis*. A recent study by Poon and Maherali (2015) weighed the relative importance of allelopathy and resource competition as mechanisms by which *Alliaria* may influence plants and their mycorrhizae. Specifically, they tested the hypothesis that suppression of mycorrhizal relationships by *Alliaria*, if it were important, would have more severe consequences under low resource conditions. These authors used the soil conditioning approach to produce soils in which *Alliaria* had been previously grown or not. Then, they grew 27 mycorrhizal tree, forb, and grass species from the introduced range of *Alliaria* in those soils, with and without competition with *Alliaria*. Previous growth of *Alliaria* in the soils reduced nitrogen and phosphorus availability by $>$ 50% and 17%, respectively, and reduced mycorrhizal colonization of competitor species by $>$ 50%. However, competition with *Alliaria* suppressed the biomass of 70% of competing species in control soil, but only 26% of competing species in soil with a previous history of *Alliaria* growth. Interestingly, in an interaction that has been rarely examined in the history of allelopathy studies, biomass of *Alliaria* itself was reduced by 56% in soils with a history of *Alliaria* growth, while the average reduction in biomass in competitor species was only 15% in soils with a history of *Alliaria* growth. Since *Alliaria* is nonmycorrhizal, this form of negative plant-soil feedback was likely mainly due to nutrient depletion. The authors concluded that, although mycorrhizal colonization was clearly suppressed by prior growth of *Alliaria* in most species in this study, the negative effect of nutrient depletion by *Alliaria* had larger impacts on competing species than the negative effect of suppressing mycorrhizal colonization. Importantly, this study was also the first to show that growth of *Alliaria* in field soils clearly has more detrimental effects on itself than on most other species, despite reducing mycorrhizal colonization of those species and depleting soil nutrients available to them.

The study of the direct and indirect allelopathic effects of *Alliaria* on plants and beneficial microbes is approaching its 20th year. A critical review of the evidence that has accumulated from laboratory and field studies suggests that this plant could be allelopathic under some circumstances in the field, but other mechanisms of impact, such as competition for nutrients, are perhaps more important. Studies isolating the role that allelopathy plays in the ecology of this plant in the field are lacking, and whether allelopathic effects, when seen, benefit the fitness of garlic mustard is still unclear. Opportunities exist to utilize variation in allelochemical production that exists in *Alliaria* populations, or changes induced by environmental conditions, coupled with better chemical capture and analytical techniques, to more clearly link allelochemical production by *Alliaria* to negative impacts on native communities and to the fitness of *Alliaria* itself. However, there is a growing body of literature on the relative lack of impact of *Alliaria* on resident plant or microbial communities, especially in areas that have been invaded for some time (Lankau 2010b, Davis *et al.* 2012, Davis *et al.* 2014, Davis *et al.* 2015). Impacts of *Alliaria*, whether due to allelopathy or other mechanisms, might be observed at the invasion front of this plant before it negatively impacts its own success, where soils and competing plants and microbes are still naïve to this plant, and before natural controls on its populations take root.

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