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The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range

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Summary

1. Ectomycorrhizal (EM) fungi play key roles in forest ecosystems, but the potential effects of invasive plants on EM fungal communities have not been assessed. In this study, we tested whether the non-mycorrhizal herbaceous plant *Alliaria petiolata* (garlic mustard) can alter the abundance of EM fungal communities in North America.

2. In three forests in New England, USA, we compared EM root tip abundance in soils where *A. petiolata* had invaded to adjacent areas without a history of *A. petiolata* invasion. At one site, we also intensively sampled EM root tip abundance across the edges of *A. petiolata* patches to determine the spatial pattern of *A. petiolata* effects on EM fungi. In a glasshouse experiment, we experimentally invaded soils with *A. petiolata* and *Impatiens capensis*, a native species and compared EM fungal colonization of white pine (*Pinus strobus*) seedlings grown in both soils. We also measured the effect of the *A. petiolata* allelochemical benzyl isothiocyanate on the growth of three species of EM fungi in pure culture.

3. In the field, EM fungal root tip biomass was lower in invaded soils, with the strongest reductions observed in forests dominated by conifers. *Alliaria petiolata* invasion did not have a significant effect on total root biomass. The influence of *A. petiolata* on EM fungal abundance in the field was localized, with the strongest inhibition observed within 10 cm of the edge of *A. petiolata* patches.

4. Pine seedlings growing in soils that were experimentally invaded with *A. petiolata* also had lower EM fungal root tip biomass compared to uninvaded soils. The native species *I. capensis* caused similar reductions in EM fungal colonization. Growth of pure cultures of all three species of EM fungi was completely inhibited by benzyl isothiocyanate.

5. *Synthesis. Alliaria petiolata* inhibits the growth of EM fungi in forests of its introduced range. Changes in EM fungal communities caused by the invasion of *A. petiolata* may influence tree seedling establishment and biogeochemical cycling.

Key-words: *Alliaria petiolata*, ectomycorrhizal fungi, exotic plant invasion, garlic mustard, invasive species, mycorrhizal fungi, *Pinus strobus*

Introduction

As diverse and abundant constituents of forest microbial communities, ectomycorrhizal (EM) fungi play key roles in the functioning of forest ecosystems (Aerts 2002; Read *et al.* 2004). Direct and indirect effects of human activities can alter the composition and function of EM fungal communities by changing the abundance and composition of hosts and properties of the soil. For example, studies in a number of different ecosystems have shown that nutrient deposition can alter the composition of EM fungal communities, with species-specific responses to nutrient inputs (Peter *et al.* 2001; Lilleskov *et al.* 2002; Avis *et al.* 2003). Management practices such as timber harvesting and prescribed burning can also cause changes in the composition and function of EM fungal communities (Jones *et al.* 2003; Lazaruk *et al.* 2005).

The invasion of exotic plants into forests may be another way by which human activities can indirectly impact EM communities. Dominant native plant species have been shown to influence the abundance of EM fungi in the soil and on roots of neighbouring plants through the production of inhibitory compounds or from competitive interactions (Nilsson *et al.* 1993; Walker *et al.* 1999; McHugh & Gehring 2006). When an exotic plant becomes dominant in a forest

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community, it may also be capable of changing the composition and function of the EM fungal community using similar mechanisms. Although exotic plants have been shown to affect other components of native soil communities (reviewed in Wolfe & Klironomos 2005), the potential impact of an exotic plant invasion on EM fungi has not been assessed.

Allliaria petiolata (garlic mustard) is an exotic plant of temperate latitudes that has the potential to alter EM fungal communities. It is an herbaceous biennial in the family Brassicaceae that does not form mycorrhizal associations. It is one of the few plant species that has been able to successfully invade forest understorey plant communities of North America (Nuzzo 2000). In many forests *A. petiolata* can become the dominant understorey species, often forming distinct, high density patches (Winterer *et al*. 2005). Previous studies have shown that arbuscular mycorrhizal (AM) fungi are almost completely eliminated from the roots of tree seedlings growing in soils where *A. petiolata* had invaded (Roberts & Anderson 2001; Stinson *et al.* 2006). This species has the potential to degrade local mycorrhizal mutualisms leading to a positive feedback that favours its growth over those of native mycorrhizal-dependent species (Vogelsang *et al*. 2005; Reinhart & Callaway 2006). Previous work with *A. petiolata* was based on mycorrhizal associations resynthesized in the glasshouse. Therefore, the strength of the effects of *A. petiolata* on mycorrhizal fungi colonizing mature tree roots in the field is unknown. Moreover, these studies were in forests dominated by AM fungi, so responses of EM fungi to *A. petiolata* are unknown. Given that EM and AM fungi are phylogenetically distinct (James *et al.* 2006) and functionally divergent (Aerts 2002), their responses to *A. petiolata* invasion may be different.

In this study, we characterized the interactions between *A. petiolata* and EM fungi through experiments in the field, glasshouse and laboratory. In the field, we measured the abundance of EM fungal root tips in three forests in plots with and without *A. petiolata*. We predicted that plots invaded by *A. petiolata* would have a lower abundance of EM fungal root tips. We also recorded the spatial extent of the effect of *A. petiolata* on EM fungi. Because the putative allelochemicals of *A. petiolata* are highly volatile (Vaughn & Berhow 1999), we predicted that the inhibitory effects of *A. petiolata* on EM fungi extend outside of *A. petiolata* patches. In a glasshouse experiment, we assessed the effect of the experimental invasion of *A. petiolata* and a native weedy plant species *Impatiens capensis* on growth and EM colonization of *Pinus strobus* (white pine) seedlings. We predicted that *A. petiolata* would reduce the growth and EM colonization of seedlings and would have a greater effect than the native plant species. To test for a direct link between the presence of *A. petiolata* and inhibition of EM fungi, we determined how benzyl isothiocyanate (BITC), an allelochemical found in tissues of *A. petiolata* (Vaughn & Berhow 1999), affected the growth of three EM fungal species in pure culture. We predicted that different EM fungal species would show different levels of growth inhibition in the presence of BITC.

Methods

EXPERIMENT 1: EFFECTS OF *ALLIARIA PETIOLATA* IN THREE FORESTS

We assessed the influence of *A. petiolata* invasion on EM fungi in the field at three different forests in New England, USA. We chose our sites based on an abundance of *A. petiolata* at the sites and dominance of these sites by EM tree species. The first site is a stand of *P. strobus* (hereafter called 'Pine Stand') in Falls Village, CT. The understorey at this site consists of *Trientalis borealis*, *Fragaria virginiana* and *Arisaema triphyllum*. The second site is a mixed deciduous conifer forest (hereafter called 'Mixed Forest') located in Salisbury, CT with *Tsuga canadensis*, *Quercus rubra* and *Populus deltoides* as the dominant EM trees and *Maianthemum canadense*, *Smilacina racemosa* and *Solidago* spp. as understorey herbaceous plant species. The third site is an urban forest (hereafter called 'Urban Forest') at the Arnold Arboretum in Boston, MA, where both native (*T. canadensis*) and exotic (*Picea abies*) conifers are the dominant tree species and the understorey consists of *Dennstaedtia punctilobula*, *Solidago flexicaulis* and *M. canadense*.

Within an area approximately 40×40 m^2 at each of the three sites, we established 1 m^2 plots in twelve locations: six plots were in areas invaded by *A. petiolata* and six plots were in adjacent areas without *A. petiolata*. Uninvaded plots were at least 2 m but not greater than 5 m from the nearest *A. petiolata* plant. To minimize major differences in soil characteristics uninvaded plots were placed near invaded plots. Areas invaded by *A. petiolata* had densities of at least 100 plants m^{-2}.

In the third week of June 2006, two cores (5 cm diameter to a depth of 5 cm) were taken from randomly selected locations within each plot. We chose this sampling depth because at these sites *A. petiolata* root density and EM root tip density are greatest within the top 5 cm of soil (B. Wolfe, personal observation). The cores were placed on ice in a cooler and moved to storage at 4 °C within 24 h.

Within 48 h of collection, coarse roots, including EM roots, were removed from each core and EM fungal root tips were separated from the coarse roots using a dissecting scope. Both coarse roots and EM root tips were dried for 36 h in a drying oven and were weighed to obtain total dry weight root biomass and total dry weight EM root biomass. Values for coarse root biomass per core, EM root tip biomass per core and standardized root tip biomass per core (EM root tip biomass/mg total root tip biomass) of the two cores from each plot were averaged to generate a mean value for each plot.

Two-way fixed factor ANOVAS were used to determine the effects of site and presence/absence of *A. petiolata* on (i) coarse root biomass, (ii) EM root tip biomass and (iii) standardized root tip biomass. To improve normality, standardized root tip biomass was $\log(x + 1)$ transformed prior to analysis. *Post hoc* comparisons were done using Tukey HSD tests.

EXPERIMENT 2: SPATIAL EXTENT OF EFFECTS IN THE FIELD

To identify the spatial extent of the influence of *A. petiolata* on EM fungi in the field, we took 5 cm wide and 5 cm deep cores along a 35 cm transect positioned across the edge of *A. petiolata* patches at the *P. strobus* stand of Experiment 1. We established one transect at each of seven different *A. petiolata* patches scattered throughout the stand. The seven patches were separated by a minimum distance of

5 m. Few other understorey species in New England forests form dense, monospecific patches similar to those formed by *A. petiolata*, therefore we were unable to perform a similar experiment at the edges of patches of a native plant species. Cores were processed as described for Experiment 1 above except that *A. petiolata* roots were also removed from the cores and dried and weighed. The roots of *A. petiolata* roots have a distinct morphology and odour and were easy to distinguish from other roots that occurred in the cores.

To test for a relationship between EM fungal root tip biomass and *A. petiolata* root biomass along the transects, we used a modified *t*-test with correction for spatial autocorrelation. This test calculates Pearson's product–moment correlation between two variables that have some degree of spatial autocorrelation using the Clifford, Richardson and Hemon (CRH) correction procedure (Clifford *et al.* 1989). Using this correction method, the effective sampling size of each variable is adjusted based on the level of spatial autocorrelation between samples. This test was performed using the 'modified *t*-test for correlation' procedure of PASSAGE (Michael S. Rosenberg, <http://www.passagesoftware.net>).

EXPERIMENT 3: EFFECTS OF EXPERIMENTAL INVASION ON PINE SEEDLINGS

To experimentally assess the effects of *A. petiolata* on EM fungi, we performed a soil conditioning experiment with *A. petiolata* and the native species *I. capensis*. We used *I. capensis* in this experiment as a contrast to *A. petiolata* because it is a forest understorey herb that grows in similar habitats to those invaded by *A. petiolata* (forest edges and gaps), but it is native to eastern North America. In New England, *I. capensis* frequently co-occurs with *A. petiolata* (B. Wolfe, personal observation). *I. capensis* forms associations with arbuscular mycorrhizal fungi, but not with EM fungi (Wang & Qiu 2006).

Soil was collected from two forest stands (hereafter referred to as Streamside and Red Eft) within the Harvard Forest (Petersham, MA). At these sites, the overstorey trees are *T. canadensis* and *P. strobus* and the herbaceous understorey included *Aster divaricatus*, *Aralia nudicaulis* and *D. punctilobula*. Neither *A. petiolata* nor *I. capensis* was growing at these sites, but both species are found within 1 km of each site at the Harvard Forest.

Soil from each site was homogenized separately on a sterile surface by breaking up large pieces of soil and mixing the soil to an even consistency by hand. Large rocks or woody debris were removed. A total of 60 1-L pots were filled with a 4 : 1 mix of field soil and sterile sand, 30 pots for each of the two forest soils. Each set of 30 pots were divided so that 10 pots were planted with three three-leaved basal rosettes of *A. petiolata* ('*Alliaria*'), 10 pots were planted with three five-leaved seedlings of *I. capensis* ('*Impatiens*') and 10 pots were left free of host plants ('Control'). Seedlings of both species were collected from multiple populations at the Harvard Forest. Soil was rinsed from the roots of the seedlings before being planted into the pots.

A separate conditioning experiment used sterile soil to assess whether invasion by *A. petiolata* can have effects on forest soils that are unrelated to the presence of EM fungi, but that might contribute to growth effects on pine seedlings. Because this experiment started with sterile soil, it was also used to assess the background level of EM fungal contamination in this glasshouse. Twenty pots were filled with the soil mix from the Red Eft site after it had been autoclaved for 1 h at 120 °C. Ten of these sterile soil pots were conditioned with *A. petiolata* ('Sterile-*Alliaria*') and ten pots were not conditioned ('Sterile-Control'). All pots were maintained in the Harvard Forest Torrey Glasshouse at ambient temperature and with shade cloth to reduce ambient light.

After 2 months, the plants used to condition soil in both experiments began to senesce and were removed from the pots. Soil from each treatment was removed from pots, homogenized by thoroughly mixing by hand on a sterilized surface and then redistributed among the pots with the treatment. One, 3-week-old seedling of *P. strobus* that had been grown from surface sterilized seeds in sterile soil (seeds obtained from Sheffield's Seed Co., Inc., Locke, NY) was planted in each pot. By homogenizing the conditioned soil and redistributing it among the pots, we aimed to minimize the variability in soil communities across the pots within a treatment. Homogenization reduced our ability to determine the magnitude of the effects of soil conditioning on EM fungi, but allowed for an estimate of the direction of the effects to corroborate field observations.

Both experiments were kept under ambient light and temperature in the Biolabs Glasshouse at Harvard University for 7 months. Seedlings were harvested and the number of EM fungal root tips for each seedling was assessed by counting root tips with emanating hyphae and/or a developed mantle under a dissecting microscope. Shoot and roots were separated and dried and biomass was determined for each seedling.

ANOVAS were performed to detect significant differences in total seedling biomass and the number of EM fungal root tips per seedling between the two different forest soils and the different soil conditioning treatments. *Post hoc* comparisons were done using Tukey HSD tests. EM fungal root tip abundance was standardized between seedlings based on the below-ground root biomass of each seedling and is expressed as number of EM fungal root tips per mg dry weight of root.

EXPERIMENT 4: EFFECT OF BITC ON EM FUNGI

To determine if isothiocyanates found in tissues of *A. petiolata* may play a role in the inhibition of the growth of EM fungi, a pure extract of BITC obtained from Sigma Aldrich Corp. (St. Louis, MO) was added to modified Melin–Norkans media for a culture growth experiment. We used BITC because it has been shown in previous work to be found in high concentrations in the roots of *A. petiolata* (Vaughn & Berhow 1999) and because isothiocyanate compounds have been shown to inhibit the growth of other fungi in laboratory experiments (Drobnica *et al.* 1967; Sarwar *et al.* 1998; Troncoso *et al.* 2005). The concentration of BITC in our agar was 7.5 nmol g^{-1}. We used this concentration because it is within the range of measured total isothiocyanate concentrations in soils where brassicaceous crop species were grown (from 1 nmol g^{-1} to 90 nmol g^{-1} as reported in Morra & Kirkegaard 2002; Gimsing & Kirkegaard 2006). A volume of sterile water equal to the volume of BITC solution was added to the control media.

Three species of EM fungi were grown on replicate plates of the BITC media and control media: *Hebeloma crustuliniforme*, *Laccaria bicolor* and *Scleroderma cepa*. All fungi were isolated from sporocarps collected in North America by Plant Health Care, Inc. (Pittsburgh, PA). Each treatment was replicated seven times for each species for a total of 42 plates. Plates were placed in a completely randomized design in the dark at 22 °C.

Radial growth of mycelia from an 8 mm diameter plug placed in the centre of each Petri dish was measured every 7 days for 3 weeks from the date of inoculation. Data are expressed as a growth rate which was calculated as mm growth from edge of plug divided by number of weeks of experiment (3 weeks). An ANOVA was conducted to detect significant differences in growth rate with species and treatment as predictor variables.

Results

EXPERIMENT 1: EFFECTS OF *ALLIARIA PETIOLATA* IN THREE FORESTS

Total root biomass varied significantly across sites ($F_{2,30}$ = 6.61, P = 0.004), but there was no effect of *A. petiolata* or an interaction between site and presence/absence of *A. petiolata* on total root biomass (Fig. 1a; *A. petiolata*: $F_{1,30}$ = 0.01, P = 0.919; *A. petiolata* × Site: $F_{2,30}$ = 2.00, P = 0.153).

EM fungal root tip biomass was different across sites ($F_{2,30}$ = 6.79, P = 0.004) and was significantly affected by the presence/absence of *A. petiolata* ($F_{1,30}$ = 10.61, P = 0.003) with significantly lower EM fungal abundance in invaded soils at the Pine Stand and in the Urban Forest (Tukey HSD tests, P < 0.05; Fig. 1b). The interaction between site and presence/absence of *A. petiolata* for EM fungal biomass was not significant ($F_{2,30}$ = 0.70, P = 0.504).

When EM fungal root tip biomass was standardized to reflect differences in total root biomass in each core, there was no significant effect of invasion by *A. petiolata* ($F_{1,30}$ = 3.05, P = 0.091), but the pattern of higher EM abundance in uninvaded soils remained (Fig. 1c). Neither site nor the interaction between site and presence/absence of *A. petiolata* had a significant effect on standardized root tip biomass (Site: $F_{1,30}$ = 1.37, P = 0.270; Site × *A. petiolata*: $F_{2,30}$ = 0.07, P = 0.930).

EXPERIMENT 2: SPATIAL EXTENT OF EFFECTS IN THE FIELD

Alliaria petiolata root biomass decreased while EM fungal root tip biomass increased moving along the transects from inside to outside *A. petiolata* patches. After taking into account spatial autocorrelation between sampling points, there was a significant negative correlation between *A. petiolata* root biomass and EM fungal root tip biomass (CRH corrected r = –0.285, P = 0.012). EM fungal root tip biomass was approximately 75% higher 20 cm outside of the *A. petiolata* patch compared to inside the *A. petiolata* patch (Fig. 2).

EXPERIMENT 3: EFFECTS OF EXPERIMENTAL INVASION ON PINE SEEDLINGS

Total biomass of the *P. strobus* seedlings was not affected by site ($F_{1,54}$ = 0.62, P = 0.436), but was affected by soil conditioning ($F_{2,54}$ = 6.63, P = 0.003; Fig. 3a). According to Tukey HSD tests, seedling biomass was significantly lower in both the *Impatiens* and *Alliaria* treated soils at the Streamside site. There was no significant site × treatment interaction ($F_{2,54}$ = 0.91, P = 0.409).

For EM root colonization, there was a significant effect of soil conditioning ($F_{2,54}$ = 4.46, P = 0.016). Across both sites, EM root colonization was lower in the *Impatiens* and *Alliaria* conditioned soils, but this reduction was only significant for *Alliaria* conditioned soils at the Red Eft site according to Tukey HSD tests (Fig. 3b). There was no significant effect of site or a significant interaction between site and treatment (Site: $F_{1,54}$ = 1.50, P = 0.226; Site × Treatment: $F_{2,54}$ = 0.06, P = 0.946).

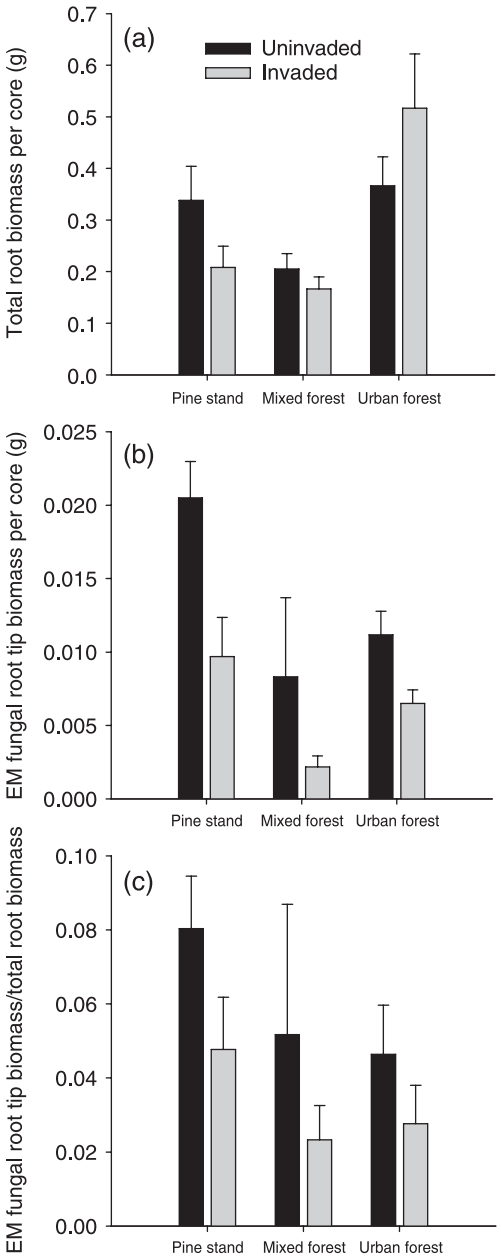

Fig. 1. Effects of *A. petiolata* invasion on root biomass and ectomycorrhizal (EM) root tip biomass in three forests in New England: a *Pinus strobus* stand ('Pine Stand'), a mixed conifer-hardwood forest ('Mixed Forest'), and an urban conifer forest ('Urban Forest'). (a) Total coarse root biomass per core in uninvaded and invaded soils. (b) Total EM root biomass per core in uninvaded and invaded soils. (c) EM root tip biomass per milligram of total root biomass per core. Error bars are ± one SE of the mean. See text for statistics.

For the sterile soil experiment, there was no significant difference in the growth of pine seedlings in the Sterile-Control vs. Sterile-*Alliaria* treatments (Table 1; $F_{1,18}$ = 0.78, P = 0.388). Seven of the 20 seedlings in the sterile soils had

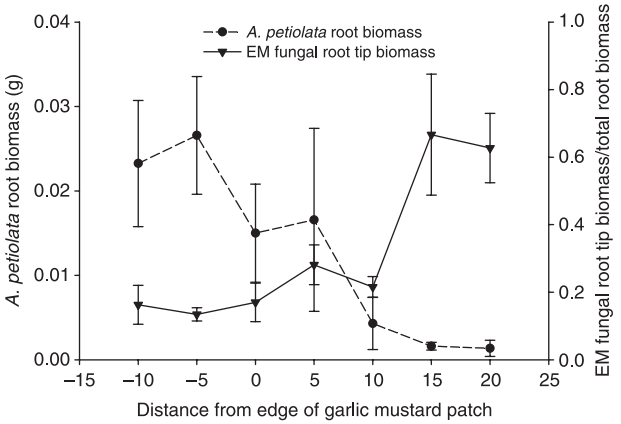

Fig. 2. *Alliaria petiolata* (garlic mustard) root biomass and ectomycorrhizal root tip biomass along transects across the edges of *A. petiolata* patches. Cores were sampled beginning 10 cm inside garlic mustard patches and were sampled to 20 cm outside of garlic mustard patches. '0 cm' on the *x*-axis represents the garlic mustard patch edge. The dotted line represents mean *A. petiolata* root biomass per core and the solid line represents ectomycorrhizal root tip biomass per total root biomass per core. Error bars are ± one SE of the mean. See text for statistics.

Table 1. Total biomass and ectomycorrhizal colonization of pine seedlings grown in sterile forest soil without conditioning ('Sterile-Control') and with conditioning by *Alliaria petiolata* ('Sterile-*Alliaria*'). Values in parentheses are ± one SE

	Total biomass (g)	EM colonization*
Sterile-Control	0.149 (0.03)	0.025 (0.01)
Sterile-*Alliaria*	0.112 (0.03)	0.043 (0.02)

*Number of EM root tips^{-1} mg seedling root biomass.

Table 2. Mean growth rate (± one SE) of three species of ectomycorrhizal fungi grown on media supplemented with water (Control) or with benzyl isothiocyanate (BITC)

	Growth rate (mm^{-1} week)	
EM species	Control	BITC
Hebeloma crustuliniforme	0.696 (0.12)	0.000 (0.00)
Laccaria bicolor	7.340 (0.46)	0.000 (0.00)
Scleroderma cepa	4.161 (0.94)	0.000 (0.00)

signs of EM fungal colonization, but the background level of colonization across all sterile seedlings was low (3.6 ± 1.4 root tips per seedling compared to 101.86 ± 12.4 root tips per seedling in the control live soils).

EXPERIMENT 4: EFFECT OF BITC ON EM FUNGI

The allelochemical treatment had a significant effect on growth rate ($F_{1,36}$ = 135.24, P < 0.001). None of the fungi inoculated on the BITC agar plates showed signs of growth after 3 weeks but the fungi on the control plates did grow and had growth rates typical of this type of media (Table 2).

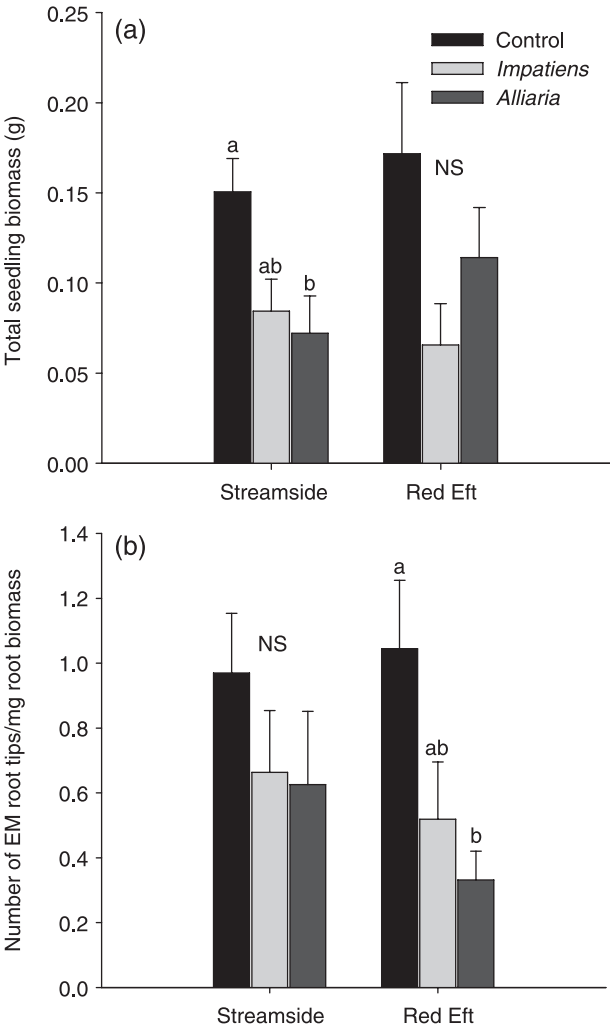

Fig. 3. Effects of soil conditioning by *Alliaria petiolata* and *Impatiens capensis* on *Pinus strobus* seedling biomass and ectomycorrhizal root colonization. Soils from two sites (Streamside and Red Eft) were conditioned with no plant (**Control**), *I. capensis* (***Impatiens***) or *A. petiolata* (***Alliaria***). (a) Total biomass of *P. strobus* seedlings at the end of the experiment. (b) Ectomycorrhizal colonization of *P. strobus* seedlings at the end of the experiment. Error bars are ± one SE of the mean. Different letters above bars indicate significant differences among means within a site according to Tukey HSD tests (P < 0.05). 'NS' = no significant differences among means within a site.

Discussion

Because EM fungi play key roles in forest ecosystems, there is a growing interest in identifying mechanisms that can lead to changes in the structure and function of EM communities. Previous studies have shown that human activities such as nutrient enrichment and timber harvesting have impacted EM fungal communities (Lilleskov *et al.* 2002; Avis *et al.* 2003; Jones *et al.* 2003). In this work we have shown that the

invasion of a non-native plant species can also alter associations between EM fungi and native plants. We add to a growing body of literature which demonstrates that exotic plant species can modify soil communities (Wolfe & Klironomos 2005; Van der Putten *et al.* 2007).

Previous work focused on the effects of *A. petiolata* on mycorrhizal propagules in the soil or resynthesized mycorrhizal associations of seedlings in the glasshouse (Roberts & Anderson 2001; Stinson *et al.* 2006). This work provides a context for understanding how seedling regeneration may be affected by *A. petiolata* invasion, although it does not directly indicate whether the mycorrhizal associations of canopy trees in forests are affected by the invasion of *A. petiolata*. Our results from Experiments 1 and 2 show that *A. petiolata* can cause significant declines in the mycorrhizal colonization of mature roots of canopy trees in the field. Given that a large amount of the nutrients in an EM tree are acquired through EM fungi (Simard *et al.* 2002; Hobbie and Hobbie 2006), these reductions in EM colonization may have functional implications for the nutrient uptake of overstorey trees.

The interactions between *A. petiolata* in the field were variable across sites, with the most pronounced decrease in EM fungal abundance at the two sites most dominated by EM tree species and with the highest overall levels of EM fungal colonization (Pine Stand and Urban Forest). These results suggest that the impacts of *A. petiolata* on EM fungi may be controlled in part by the composition of the host tree species. Other factors that may contribute to the observed idiosyncratic responses include composition of the fungal communities at different sites, differences in the density of *A. petiolata* and the time since *A. petiolata* invasion.

The spatial pattern of the inhibitory effect of *A. petiolata* was apparent when samples were taken across the edges of *A. petiolata* patches. The sharp increase in EM fungal abundance at distances beyond 10 cm of *A. petiolata* patch edges suggests that the effects of *A. petiolata* on EM fungi are highly localized. As *A. petiolata* invades forests, uninvaded areas of soil may serve as reservoirs of EM fungal inoculum. Because *A. petiolata* populations are often composed of many distinct patches (Winterer *et al.* 2005), *A. petiolata* has the potential to alter the spatial structure of EM fungal communities (Boerner *et al.* 1996) and cause gaps in otherwise continuous EM fungal communities.

Although the anti-fungal properties of *A. petiolata* are incompletely understood (Stinson *et al.* 2006), our results with BITC provide a direct link between an allelochemical that is abundant in the roots of *A. petiolata* and the inhibition of mycorrhizal fungi. However, other compounds may also play a role in the inhibition of fungal growth in the rhizosphere. Cipollini & Gruner (2007) recently found high concentrations of cyanide compounds in *A. petiolata*, and these compounds may contribute to the reduction in EM abundance observed in our study. Future work is needed to determine the exact *in situ* biochemical processes that lead to reductions in mycorrhizal fungi where *A. petiolata* establishes.

Recent work suggests that *A. petiolata* may be a successful invader because it possesses 'novel weapons' that are unique to plant communities in its introduced range (Callaway *et al.* 2008). In the case of *A. petiolata,* the 'novel weapons' may be allelochemicals that can inhibit mycorrhizal associations of native plants (Stinson *et al.* 2006; Callaway *et al.* 2008). Although we did not specifically test the 'novel weapons hypothesis', it is interesting to note that in our glasshouse experiment, both *A. petiolata* and the native species *I. capensis* inhibited EM colonization of *Pinus* seedlings. *Impatiens* spp. can produce numerous anti-fungal compounds (Lee *et al.* 1999; Yang *et al.* 2001) and these may be responsible for the inhibition of EM fungi. Other studies have shown that native plants can also have inhibitory effects on EM fungi of native tree species via putative allelochemicals (Nilsson *et al.* 1993; Yamasaki *et al.* 1998; Walker *et al.* 1999). The ability of forest understorey plants to inhibit EM fungi may not be novel in North America, but the biochemical process by which *A. petiolata* inhibits EM fungi may be novel in these forest communities.

In conclusion, we have shown that invasion of *A. petiolata*, both in the field and experimentally in the glasshouse, is associated with decreased levels of EM fungal colonization. These effects were apparent across several different sites, suggesting that this is a widespread phenomenon. We took a below-ground perspective on the EM fungal community and did not consider the production of sporocarps at our sites. Future work should explore whether *A. petiolata* invasion inhibits the production of fungal fruiting bodies, as *A. petiolata* can invade sites with populations of valued edible fungi including chanterelles (*Cantharellus* spp.) and morels (*Morchella* spp.) (B. Wolfe, personal observation). With accumulating evidence that *A. petiolata* alters both arbuscular (Roberts & Anderson 2001; Stinson *et al.* 2006) and EM fungi (this work), future studies should focus on the impacts of this plant on saprotrophic fungi which play important roles in decomposition and the biogeochemistry of forest ecosystems (Dighton 2003).

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