Effects of *Alliaria petiolata* (garlic mustard; Brassicaceae) on mycorrhizal colonization and community structure in three herbaceous plants in a mixed deciduous forest[1]

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Herbaceous plant species are important components of forest ecosystems, and their persistence in forests may be affected by invasive plant species that reduce mycorrhizal colonization of plant roots. I examined the effect of the invasive plant *Alliaria petiolata* on arbuscular mycorrhizal fungi (AMF) colonizing the roots of three forest plant species. AMF root colonization and community structure was examined from plants that were growing either in the absence or presence of *Alliaria* under natural forest conditions. AMF root colonization varied among the plant species but was not significantly affected by *Alliaria*. With molecular methods, ~12 different taxa of AMF could be distinguished among the root samples, and these taxa belonged to the genera *Acaulospora* and *Glomus*, with *Glomus* dominating AMF communities. There were significant differences between the community of AMF colonizing roots of *Maianthemum racemosum* and *Trillium grandiflorum*, but only AMF communities of *Maianthemum* roots were significantly affected by *Alliaria*. Indicator species analysis found that an *Acaulospora* species type was a significant indicator of *Maianthemum* plants grown in the absence of *Alliaria*. These results suggest invasive plants like *Alliaria* may selectively suppress AMF fungi, and this suppression can affect AMF communities colonizing the roots of some native plant species.

Key words: *Alliaria petiolata*; arbuscular mycorrhizal fungi; *Arisaema triphyllum*; diversity; *Maianthemum racemosum*; terminal restriction fragment length polymorphisms; TRFLP; *Trillium grandiflorum*.

Herbaceous plants may comprise as much as 80% of the total plant richness in northern hardwood forests (Gilliam, 2007). These plant communities provide resources for animal and insect populations, can affect regeneration of forest trees, and affect soil microbes responsible for maintaining soil fertility (Zak et al., 1990; Westover et al., 1997; George and Bazzaz, 2003; Costa et al., 2006; Gilliam, 2007). In addition, herbaceous plants can mediate both carbon and nutrient cycling and effect the export of nutrients from forest ecosystems (Muller and Bormann, 1976; Tessier and Raynal, 2003; Gilliam, 2007). Consequently, herbaceous plant communities are important components of northern hardwood forests, and changes to these communities could have ecological significance. The composition, structure, and persistence of these communities are governed by the extent to which the physical limitation of the environment and biotic interactions promote or hinder individual plant growth and reproduction (Gleason, 1926; Crawley, 1997). One especially important biotic interaction is the one that forms between plants and mycorrhizal soil fungi.

Arbuscular mycorrhizal fungi (AMF) are soil fungi that form mutualistic relationships with plant roots. AMF colonize plant roots and extensively explore soil, acting as an extension of the plant root system. Therefore, AMF play a crucial role in plant nutrient acquisition, especially the acquisition of nitrogen and phosphorus (Smith and Read, 1997 and references therein). Numerous studies have demonstrated the importance of AMF colonization to plant growth and survivorship, including the survival of young life stages of forest trees (Hetrick et al., 1988; Smith and Read, 1997; Stinson et al., 2006). Plant root systems can be colonized by a diverse group of AMF (Helgason et al., 1999; Vandenkoornhuyse et al., 2003; Santos-González et al., 2007), each of which differs in functional attributes (Helgason et al., 2002). These differences in AMF functional attributes could lead to specialization in resource acquisition among different portions of the plant root system. The effects of AMF on maintaining plant community diversity and productivity (van der Heijden et al., 1998; Klironomos et al., 2000; Vogelsang et al., 2006) may, in part, be attributed to differences in the functional attributes of AMF taxa that increase resource acquisition and survivorship under natural conditions.

A number of human disturbances, including nitrogen enrichment (Egerton-Warburton and Allen, 2000; Egerton-Warburton et al., 2001), soil disturbance (Merryweather and Fitter, 1998), changes in land use and management (Vandenkoornhuyse et al., 2003), and changes in plant community composition (Vandenkoornhuyse et al., 2003; Santos-González et al., 2007) have been implicated in reducing AMF diversity and root colonization of plants and soil. Of increasing interest is the effect of invasive plant species on AMF in soil and how invasive plants can affect AMF diversity and the growth of native plants. *Alliaria petiolata* (garlic mustard; Brassicaceae) is a biennial herb native to Eurasia that is becoming a rampant invader in woodlands throughout eastern North America (Nuzzo, 1999). Whole-plant extracts of *Alliaria* contain secondary compounds, including glucosinalates and other allelopathic chemicals that have reported antifungal properties, that are exuded through roots and leaf litter (Roberts and Anderson, 2001). Previous studies have demonstrated that aqueous extracts of *Alliaria*
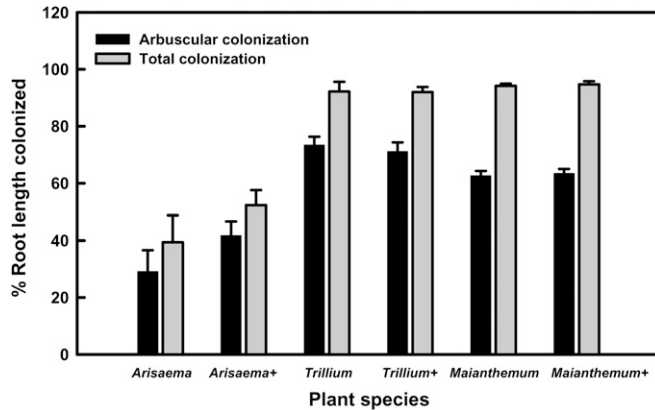
doi:10.3732/ajb.0800184

Fig. 1.   Arbuscular mycorrhizal colonization of roots of three forest plants growing in the absence or presence of *Alliaria*. Plants growing in the presence of *Alliaria* are denoted by a (+). Mean colonization and SE bars are shown.

tissue can reduce plant growth and mycorrhizal colonization (Roberts and Anderson, 2001; Stinson et al, 2006) under greenhouse conditions.

In addition, soil microbial communities in North America may be more sensitive to *Alliaria* than soil microbial communities in the plants native range that have coevolved with *Alliaria* secondary compounds (Callaway et al., 2008). Communities of soil fungi in North America may constitute a "naïve" community of organisms especially susceptible to the secondary chemistry of *Alliaria* (Callaway et al., 2008). The success of *Alliaria* in invading native plant communities may depend on allelopathy, which acts as a novel weapon to suppress AMF mutualists of resident plant species (Callaway and Ridenour, 2004; Vogelsang et al., 2004; Inderjit et al., 2006; Callaway et al., 2008). Here, I describe a study conducted in a mature, mixed hardwood forest to assess the impact of *Alliaria* on AMF colonization and community structure on the roots of three important native forest wildflowers: *Arisaema triphyllum* L. (Jack-in-the-pulpit; Araceae), *Maianthemum racemosum* (L.) Link subsp. *racemosum* (false solomon's seal; Liliaceae), and *Trillium grandiflorum* Michx. Salisb. (large-flowered trillium; Liliaceae). I chose to focus this study on these three species because they are extensively colonized by mycorrhizal fungi, are important components of many forest plant communities, and populations of these plants are on the decline in many places in eastern North America. I hypothesized that the roots of plants growing in the presence of *Alliaria* would have both reduced colonization and reduced diversity of AMF. Consequently, I used a combination of standard staining methods to assess root length colonized by AMF as well as molecular methods to describe how *Alliaria* affected the community of AMF colonizing plant roots in the field.

MATERIALS AND METHODS

Site description and plant sampling—The study site is a 16-ha forest known as Trillium Trail located in Allegheny County, Pennsylvania. This forest is a mature mixed mesophytic forest dominated by white and red oak (*Quercus alba* L. and *Q. rubra* L.), sugar maple (*Acer saccharum* Marshall), American beech (*Fagus grandifolia* Ehrh.), and tulip poplar (*Liriodendron tulipifera* L.), and the forest contains a diverse woody and herbaceous understory. The study site is characterized by moderately sloping ground (8–15% slope) and silt loam soils.

Garlic mustard (*Alliaria petiolata* Bieb.) began invading Trillium Trail from road edges in the early 1990s and has since become an important component of the forest understory. *Alliaria* is uniformly distributed throughout the site and grows in discrete patches within the forest. Sampling was conducted during early summer (mid-June) of the 2007 growing season. Native plant species were sampled from within patches of *Alliaria* and also from within adjacent areas that did not contain *Alliaria*. Areas that did not contain *Alliaria* were never more than 5 m from *Alliaria* patches. In this fashion, we were able to collect 10 plant pairs (*Alliaria*+ and *Alliaria*−) for each of three plant species: Jack-in-the-pulpit (*Arisaema triphyllum* L.), false solomon's seal [*Maianthemum racemosum* (L.) Link subsp. *racemosum*], and large-flowered trillium (*Trillium grandiflorum* Michx. Salisb.) (3 species × 2 treatments × 10 replicates per species). Plants were gently dug from the soil to limit disturbance of the root system. Soil was gently shaken from the plants, which were then placed in a cooler and transported to the laboratory. In the laboratory, all roots attached to the plant rhizome were excised 1.0 cm from the rhizome (approximately 500 mg wet mass root tissue), and rinsed in sterile distilled water to remove all visible soil. Roots were then placed in 70% ethanol and subsequently stored at 4°C until analysis.

Arbuscular mycorrhizal colonization of roots—Half of each plant's root system (250 mg wet mass, ~50-cm total root length) was used to estimate AMF colonization. Roots were stained with trypan blue in lactic acid using a modification of the procedure outlined by Kormanik and McGraw (1982). Roots were cleared in 5% KOH for 6 h at room temperature, stained at room temperature for 2 h, then destained overnight. Trypan blue is a common nonvital stain used to estimate AMF colonization (Dickson and Smith, 1998), and the no-heat modification of the standard procedure has been successfully used (Burke et al., 2002, 2003). Root samples were mounted on microscope slides, and colonization was determined using the slide mount method of McGonigle et al. (1990). A total of 150 intersections were scored for each plant sample except for *Arisaema triphyllum* for which only 100 intersections were scored because roots were short. The root length colonized by mycorrhizal hyphae, arbuscules/coils, and vesicles was determined according to the method of Brundrett et al. (1994), who considered hyphae to be mycorrhizal only if they were visually connected to vesicles or arbuscules. Because both *Trillium* and *Maianthemum* tended to form hyphal coils in roots more than arbuscules, we considered arbuscules and coils as the same structure for scoring AMF colonization.

DNA extraction and TRFLP analysis—DNA was extracted from the remaining root tissue of each sample. Roots weighing more than 250 mg fresh mass were placed in a 1.5-ml bead beating tube containing 300 mg of 400 µM glass beads (VWR, West Chester, Pennsylvania, USA), 300 mg 1-mm glass beads (Chemglass, Vineland, New Jersey, USA) and 750 ml 2% CTAB (cetyltrimethylammonium bromide). Samples were beaten for 90 s on a Precellys homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) at 6500 rpm. Approximately 500 ml of the extractant was then removed from the tube, and nucleic acids were purified by phenol/chloroform extraction, then precipitated with 20% polyethylene glycol 8000 in 2.5 M NaCl (Burke et al., 2005). All extracted DNA was suspended in Tris-EDTA buffer and stored at −20°C. DNA was diluted in PCR grade water, and a 20-fold dilution of the DNA was used for PCR. PCR was made using universal eukaryotic primer NS31 (Simon et al., 1992) and AMF-specific primer AM1 following general procedures in Helgason et al. (1998) except that only 30 cycles of PCR were performed. Primers were labeled with the fluorochromes 6-carboxyfluorescein (6FAM) (AM1) and 4,7,2′,4′,5′,7′-hexachloro-6-carboxyfluorescein (HEX) (NS31). I empirically determined that the restriction enzymes *Alu*I and *Hsp*92 (Promega, Madison, Wisconsin, USA) provided the largest number of distinct terminal restriction fragments (TRFs), so these enzymes were used to cut the labeled PCR product. Terminal restriction fragment length polymorphisms (TRFLPs) were analyzed at the Cornell Bioresource Center using an Applied BioSystems (Foster City, California, USA) 3730xl DNA Analyzer. Because *Arisaema* plants contained substantially less root tissue than either *Trillium* or *Maianthemum*, only six composite root samples for *Arisaema* (*N* = 3/treatment) could be analyzed with molecular methods as compared to 20 samples for both *Trillium* and *Maianthemum*.

AMF TRFLP sequence database—The TRFLP sequence database (was created from two sources of environmental DNA: (1) roots harvested from *Trillium* and *Maianthemum*; and (2) forest soil collected at the Trillium Trail site. DNA was extracted and AMF amplified with unlabeled primers as noted before. The PCR product was separated by gel electrophoresis on a 1% agarose

TABLE 1. Results of two-way ANOVA for root colonization by arbuscular mycorrhizal fungi (AMF) in presence and absence of *Alliaria*. Results for arbuscular colonization (AC), vesicular colonization (VC), and total root length colonized by AMF (Total AMF) are shown. Boldfaced *P*-values indicate significant contrasts.

Dependent variable	Source of variation (df)	SS	MS	*F*	*P*
AC	Plant (2)	1.628	0.814	41.427	**<0.001**
	Alliaria (1)	0.0305	0.0305	1.553	0.218
	Plant × *Alliaria* (2)	0.0802	0.0401	2.042	0.140
	Residual (52)	1.022	0.0197		
	Total (57)	2.756	0.0484		
VC	Plant (2)	0.0273	0.0136	3.389	**0.041**
	Alliaria (1)	0.00449	0.00449	1.117	0.295
	Plant × *Alliaria* (2)	0.00531	0.00265	0.660	0.521
	Residual (52)	0.209	0.00402		
	Total (57)	0.246	0.00432		
Total AMF	Plant (2)	4.400	2.200	72.363	**<0.001**
	Alliaria (1)	0.0294	0.0294	0.969	0.330
	Plant × *Alliaria* (2)	0.0652	0.0326	1.072	0.350
	Residual (52)	1.581	0.0304		
	Total (57)	6.071	0.107		

gel in Tris-acetate-EDTA (TAE) buffer. The PCR products were excised from the gel and purified using a PCR purification kit (Promega, Madison, Wisconsin, USA) using the manufacturer's instructions. PCR product was then cloned using a pGEM-T Easy Vector System (Promega) using the manufacturers instructions. Fifty randomly selected colonies from each type of environmental DNA were incubated overnight at 37°C in Luria-Bertani (LB) medium, and plasmids were harvested using a Wizard Plus SV Minipreps DNA Purification System (Promega). A total of 150 clones (50 clones each from *Trillium* and *Maianthemum* roots, 50 clones from soil DNA) were recovered and screened for distinct TRFs. Clones were used for PCR using labeled NS31 and AM1 as noted, and the PCR product was then used for TRFLP. Clones that represented distinct TRFs were sequenced using a Dye Primer Cycling Kit (Applied Biosystems). A total of 100 clones were sequenced following this procedure to capture distinct TRFs. Sequencing was completed through the Cornell Bioresource Center using an Applied BioSystems 3730xl DNA Analyzer. Generated sequences were compared to EMBL/GenBank/DDBJ database entries using the FASTA program (European Bioinformatics Institute, Hinxton, UK, http://www.ebi.ac.uk) to determine the putative identity of AMF.

Data and statistical analysis—Differences in the percentages of root length colonized among the samples were analyzed by two-way ANOVA using procedures in the program SigmaStat version 3.5 (Systat Software, Richmond, California, USA). All data were arcsine transformed prior to statistical analysis. I used TRFLP fingerprints (both *Alu*I and *Hsp*92 profiles) for determining species type identity and proportional abundance within root samples. AMF species types were considered present in a root system if peaks distinct to that species type were identified in both the *Alu*I and *Hsp*92 TRFLP profiles. The areas under these distinct peaks were used to calculate the proportional abundance of AMF species types in the core (Burke et al., 2005, 2006). In some instances, AMF species types shared TRFs. This fact necessitated lumping together some AMF species types based on TRFLP patterns. However, this should provide a more conservative estimate of species richness and community diversity among the samples. Evenness (equitability) and Shannon diversity (*H′*) were calculated using procedures available through the program PC-ORD 4 (MjM Software, Gleneden Beach, Oregon, USA). Differences in root colonization and community diversity among samples were analyzed by a two-way ANOVA using procedures in the program SigmaStat 3.5. To determine if differences existed in AMF root communities among the plant species and in the presence of *Alliaria*, I used multiresponse permutation procedures (MRPP) and indicator species analysis available through PC-ORD 4. MRPP is a nonparametric, multivariate procedure for testing the hypothesis of no difference between groups. The test statistic (*T*) explains the separation between the groups in multidimensional space; the more negative the *T*-value, the greater the separation between the groups. The *P*-value describes how well the separation between groups is likely due to chance alone, and *A* represents the chance-corrected within group agreement and is a measure of the effect size (McCune and Grace, 2002). For both MRPP and indicator species analysis, the propor-
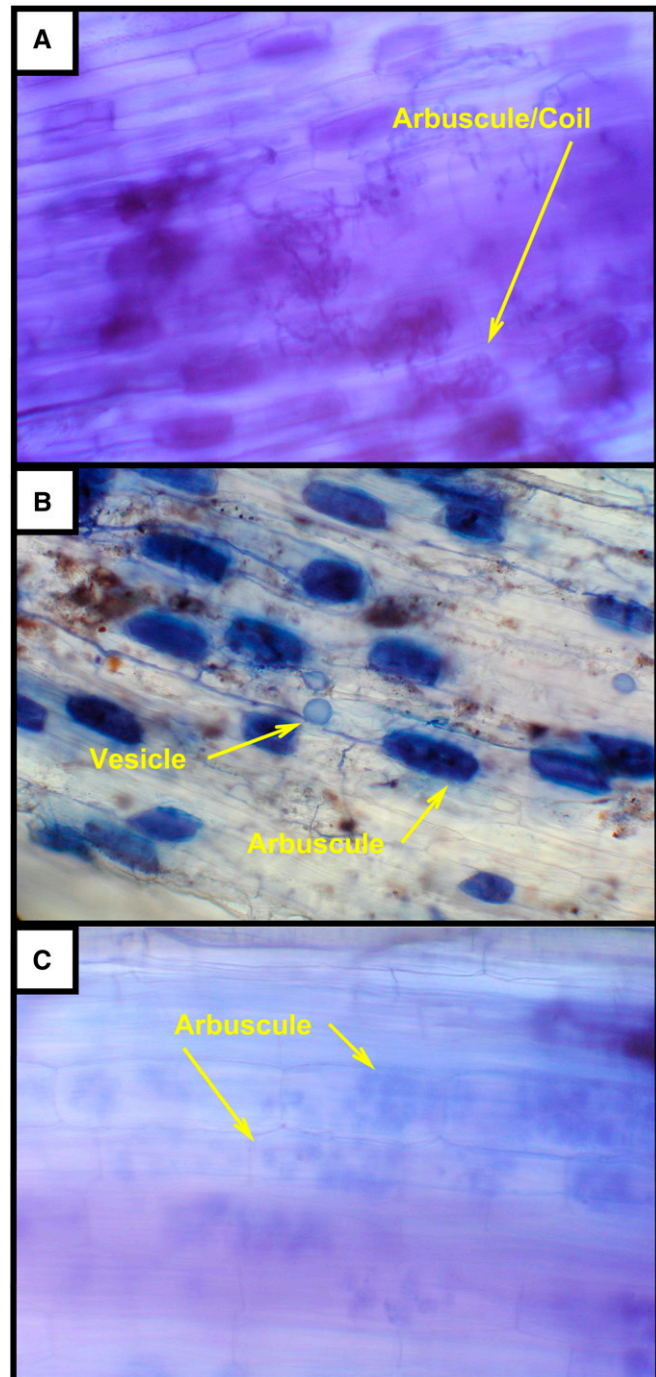
Fig. 2. Root sections stained with trypan blue showing arbuscular mycorrhizal structures. Roots were observed at a magnification of 200× for assessment of AMF colonization. (A) *Trillium*, hyphal coiling within cortical cells. (B) *Maianthemum*, vesicles and darkly stained cells containing arbuscules/coils. (C) *Arisaema*, lightly stained arbuscules in cortical cells.

tional peak area of each identified species type was used as an indicator of abundance within each sample. A total of 1000 randomizations were used for the Monte Carlo test during indicator species analysis. Because AMF communities can be affected by a number of interacting environmental variables, we considered *P* < 0.1 significant for comparison of communities among plants from *Alliaria*+ and *Alliaria*– sites. Because of the low number of root samples available for community analysis, *Arisaema* roots were not used for MRPP or

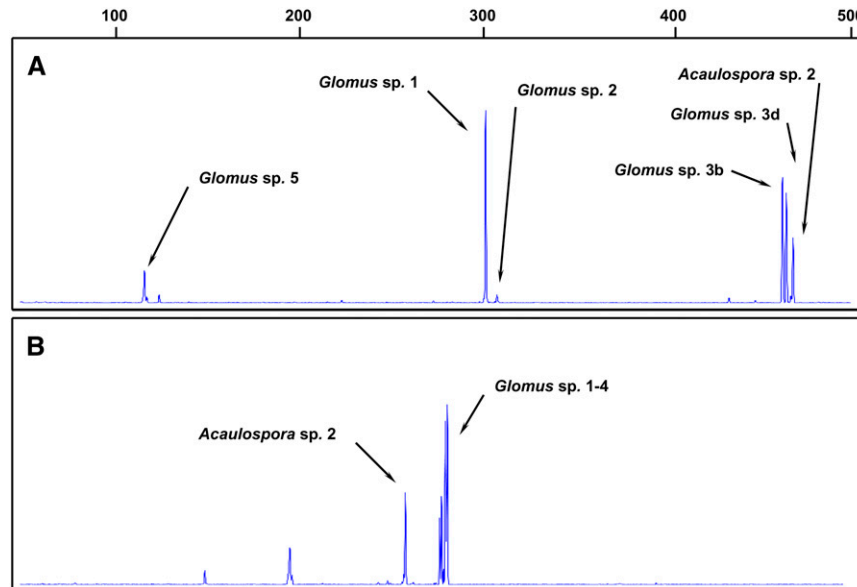Fig. 3. Terminal RFLP (TRFLP) profiles of the arbuscular mycorrhizal community found in the root system of an individual *Maianthemum* plant growing in the absence of *Alliaria*. The *x*-axis represents the size of the TRF in base pairs, while the *y*-axis represents integrated fluorescence units on a scale of 0–3000. (A) Profile generated with the restriction enzyme *Alu*I showing major peaks contributed by AMF taxa identified through sequence cloning. (B) Profile generated with the restriction enzyme *Hsp*92 showing major AMF taxa.

indicator species analysis. Similarities of recovered AMF sequences to known sequences were obtained by comparison to EMBL/GenBank/DDBJ database entries using the FASTA program. The taxonomic affinity of some recovered sequences was shown using a multiple sequence alignment created with the program Clustal X 1.83 (Thompson et al., 1997), and this alignment was used to construct a distance tree by the neighbor-joining method (Appendix S1, see Supplemental Data with the online version of this article). All sequence data between primers NS31 and AM1 were used for alignment purposes.
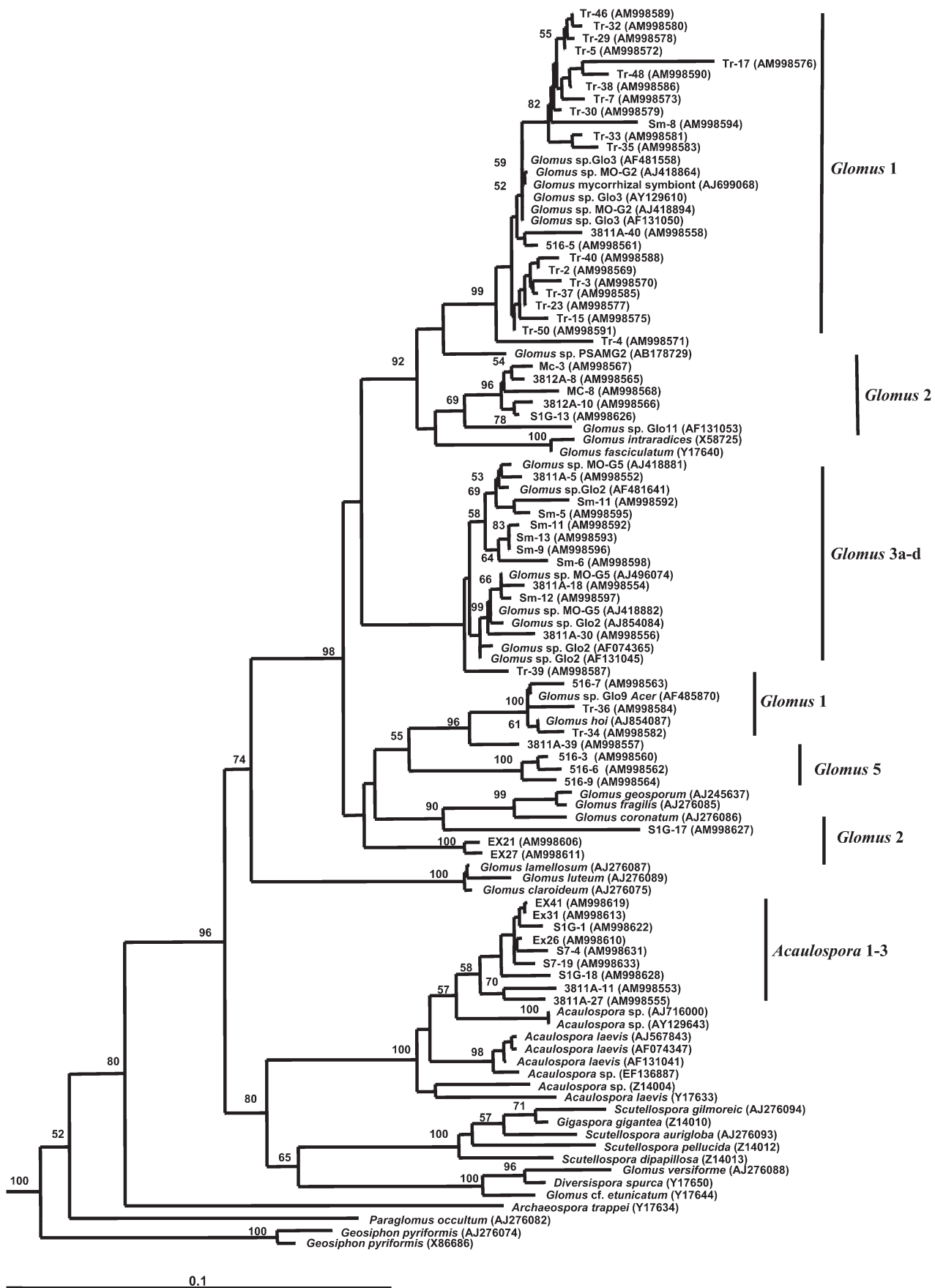
RESULTS

Root colonization—Total root length colonized ranged from a mean of 39 ± 9% in *Arisaema* to 94 ± 1% in *Maianthemum* (Fig. 1). Total root length colonized by arbuscules or coils ranged from a mean of 29 ± 7% in *Arisaema* to 71 ± 3% in *Trillium* (Fig. 1). Total vesicular colonization was always less than 1% for all plant species. ANOVA indicated that there were significant differences among the plant species for AMF root colonization (Table 1), but that the presence of *Alliaria* had no significant effect on AMF root colonization. Roots of all species were heavily colonized, with extensive hyphal coiling in *Trillium* (Figs. 1 and 2).

AMF TRFLP and sequence database—I recovered 78 sequences that had high similarity to database entries (Appendix S2; see Supplemental Data with the online version of this article). The remaining 22 sequences had only low similarity (<80%) to existing database entries. Of the 78 sequences with high similarity, nine matched non-AMF sequences. Two sequences had high similarity to *Penicillium* sp. (99% similarity), three sequences had high similarity to the ectomycorrhizal genus *Russula* (97–99% similarity), and the remaining four sequences matched other soil fungi. Of the 69 sequences that matched AMF, most sequences had similarity to species in the genus *Glomus* (86% of total AMF), while some sequences matched the genus *Acaulospora* (14% of total AMF) (Figs. 3, 4).

I used several restriction enzymes (*Alu*I, *Hinf*I, *Hsp*92, *Mbo*I, *Rsa*I, *Taq*I) for TRFLP on the recovered clones and found that restriction enzyme *Alu*I and *Hsp*92 provided the largest number of diagnostic restriction fragments. Although restriction enzyme *Hinf*I generated a large number of distinct TRFs using the labeled forward primer (all TRFs with the reverse primer had less than 20 bp), some *Glomus* species types shared *Hinf*I TRFs with the genus *Acaulospora*, making separation between these groups difficult. Consequently, I used fragments associated with the reverse primer AM1 using *Alu*I and *Hsp*92 to determine the presence of AMF species types within our samples. TRFs of non-AMF were generally not detected in root sample TRFLP profiles except for the genus *Russula* that generated a TRF of 461 and 286 base pairs with *Alu*I and *Hsp*92, respectively, and was detected in five of the 46 root samples used for TRFLP.

TRFLP community analysis—AMF species-type richness was not significantly affected by plant species or *Alliaria* (Fig. 5, Table 2). There was a significant interaction of plant species with *Alliaria* for evenness, and individual species contrasts indicated that AMF species evenness was significantly affected by *Alliaria* in *Arisaema* but not in *Maianthemum* or *Trillium* (pairwise multiple comparison, Holm–Sidak method, $P = 0.033$). Shannon diversity was not affected by either plant species or *Alliaria* (Fig. 5, Table 2). I did observe a statistically significant interaction between plant species and *Alliaria* on diversity, but individual species contrasts indicated that Shannon diversity was significantly affected only for *Arisaema* in the presence of *Alliaria* (pairwise multiple comparison, Holm–Sidak method, $P = 0.034$).

MRPP analysis of *Maianthemum* and *Trillium* indicated that significant differences existed in the community structure of AMF colonizing plant root systems (Table 3). Multiple comparisons of specific contrasts found that AMF communities in *Maianthemum* and *Trillium* grown without *Alliaria* were

Tr-46 (AM998589)
Tr-32 (AM998580)
Tr-29 (AM998578)
Tr-5 (AM998572)
Tr-17 (AM998576)
Tr-48 (AM998590)
Tr-38 (AM998586)
Tr-7 (AM998573)
Tr-30 (AM998579)
Sm-8 (AM998594)
Tr-33 (AM998581)
Tr-35 (AM998583)
Glomus sp.Glo3 (AF481558)
Glomus sp. MO-G2 (AJ418864)
Glomus mycorrhizal symbiont (AJ699068)
Glomus sp. Glo3 (AY129610)
Glomus sp. MO-G2 (AJ418894)
Glomus sp. Glo3 (AF131050)
3811A-40 (AM998558)
516-5 (AM998561)
Tr-40 (AM998588)
Tr-2 (AM998569)
Tr-3 (AM998570)
Tr-37 (AM998585)
Tr-23 (AM998577)
Tr-15 (AM998575)
Tr-50 (AM998591)
Tr-4 (AM998571)
Glomus sp. PSAMG2 (AB178729)
Mc-3 (AM998567)
3812A-8 (AM998565)
MC-8 (AM998568)
3812A-10 (AM998566)
S1G-13 (AM998626)
Glomus sp. Glo11 (AF131053)
Glomus intraradices (X58725)
Glomus fasciculatum (Y17640)
Glomus sp. MO-G5 (AJ418881)
3811A-5 (AM998552)
Glomus sp.Glo2 (AF481641)
Sm-11 (AM998592)
Sm-5 (AM998595)
Sm-11 (AM998592)
Sm-13 (AM998593)
Sm-9 (AM998596)
Sm-6 (AM998598)
Glomus sp. MO-G5 (AJ496074)
3811A-18 (AM998554)
Sm-12 (AM998597)
Glomus sp. MO-G5 (AJ418882)
Glomus sp. Glo2 (AJ854084)
3811A-30 (AM998556)
Glomus sp. Glo2 (AF074365)
Glomus sp. Glo2 (AF131045)
Tr-39 (AM998587)
516-7 (AM998563)
Glomus sp. Glo9 *Acer* (AF485870)
Tr-36 (AM998584)
Glomus hoi (AJ854087)
Tr-34 (AM998582)
3811A-39 (AM998557)
516-3 (AM998560)
516-6 (AM998562)
516-9 (AM998564)
Glomus geosporum (AJ245637)
Glomus fragilis (AJ276085)
Glomus coronatum (AJ276086)
S1G-17 (AM998627)
EX21 (AM998606)
EX27 (AM998611)
Glomus lamellosum (AJ276087)
Glomus luteum (AJ276089)
Glomus claroideum (AJ276075)
EX41 (AM998619)
Ex31 (AM998613)
S1G-1 (AM998622)
Ex26 (AM998610)
S7-4 (AM998631)
S7-19 (AM998633)
S1G-18 (AM998628)
3811A-11 (AM998553)
3811A-27 (AM998555)
Acaulospora sp. (AJ716000)
Acaulospora sp. (AY129643)
Acaulospora laevis (AJ567843)
Acaulospora laevis (AF074347)
Acaulospora laevis (AF131041)
Acaulospora sp. (EF136887)
Acaulospora sp. (Z14004)
Acaulospora laevis (Y17633)
Scutellospora gilmoreic (AJ276094)
Gigaspora gigantea (Z14010)
Scutellospora aurigloba (AJ276093)
Scutellospora pellucida (Z14012)
Scutellospora dipapillosa (Z14013)
Glomus versiforme (AJ276088)
Diversispora spurca (Y17650)
Glomus cf. *etunicatum* (Y17644)
Archaeospora trappei (Y17634)
Paraglomus occultum (AJ276082)
Geosiphon pyriformis (AJ276074)
Geosiphon pyriformis (X86686)

Glomus 1

Glomus 2

Glomus 3a-d

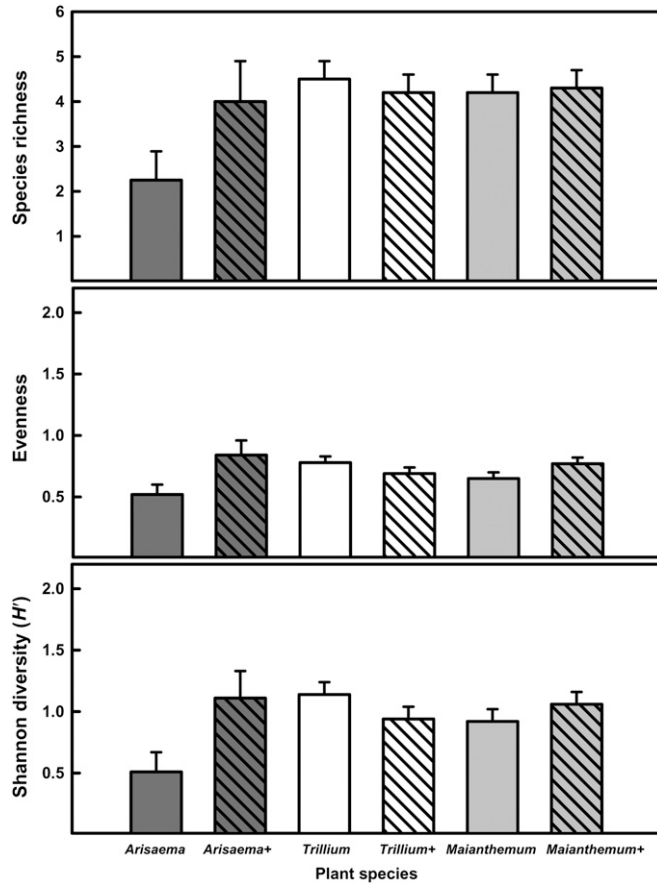*Glomus* 1

Glomus 5

Glomus 2

Acaulospora 1-3

0.1

Fig. 5.   Arbuscular mycorrhizal community diversity in roots of three forest plants growing in the absence or presence of *Alliaria*. Plants growing in the presence of *Alliaria* are denoted by a (+). Means and SE bars are shown. Species richness is the number of taxa detected in the root systems.

TABLE 2.   The results of two-way ANOVA for measures of community diversity of arbuscular mycorrhyzal fungi (AMF) in the presence and absence of *Alliaria*. A *P*-value less than 0.1 (boldfaced) was considered significant for these analyses. Richness equals the total number of AMF species detected in the plant root system; evenness (equitability) and Shannon diversity (*H′*) are shown.

Dependent variable	Source of variation (df)	SS	MS	*F*	*P*
Richness	Plant (2)	6.597	3.299	2.044	0.143
	Alliaria (1)	2.089	2.089	1.295	0.262
	Plant × *Alliaria* (2)	4.426	2.213	1.371	0.265
	Residual (40)	64.550	1.614		
	Total (45)	80.457	1.788		
Evenness	Plant (2)	0.0128	0.00638	0.227	0.798
	Alliaria (1)	0.104	0.104	3.710	**0.061**
	Plant × *Alliaria* (2)	0.223	0.111	3.969	**0.027**
	Residual (40)	1.123	0.0281		
	Total (45)	1.424	0.0316		
H′	Plant (2)	0.233	0.117	1.169	0.321
	Alliaria (1)	0.266	0.266	2.666	0.110
	Plant × *Alliaria* (2)	0.750	0.375	3.759	**0.032**
	Residual (40)	3.992	0.0998		
	Total (45)	5.297	0.118		

status, and improved plant growth and fitness (Merryweather and Fitter, 1996; Lapointe and Molard, 1997; Helgason et al., 2002). Consequently, any biotic or abiotic factor that reduces mycorrhizal colonization or diversity in soil can have a negative impact on the growth and persistence of herbaceous plants in forest ecosystems. In this study, I examined the effect of *Alliaria petiolata* on mycorrhizal colonization and mycorrhizal community structure in three important forest plants under natural conditions. *Alliaria* did not significantly affect the root length colonized by mycorrhizal fungi although I was able to detect some effects of *Alliaria* on mycorrhizal community structure in at least one plant species, *Maianthemum racemosum*.

The levels of AMF encountered in all three plants species were comparable to levels reported by other investigators under natural conditions (Brundrett and Kendrick, 1990a; Demars, 1996) with extensive hyphal coiling in *Maianthemum* and *Trillium* (Brundrett and Kendrick, 1990b). Although I did not observe any effects of *Alliaria* on root colonization, I did observe significant differences in root colonization among the three plant species, with *Arisaema* having the lowest levels of root colonization. These differences may be due, in part, to the different growth cycle of *Arisaema* roots which grow and senescence seasonally, whereas *Maianthemum* and *Trillium* have a perennial root system with longer-lived roots (Brundrett and Kendrick, 1990a); however, sampling was conducted in mid-June, during which time the *Arisaema* root system should have reached maximum levels of AMF colonization, so sampling time is unlikely to have affected these results (Brundrett and Kendrick, 1990a). On the other hand, sampling was made prior to *Alliaria* death and senescence and could have affected our root colonization results. Although secondary chemicals (i.e., allelochemicals) have been reported from the roots of *Alliaria*, senescence and degradation of *Alliaria* leaf litter may be an

significantly different and indicator species analysis suggests that species type *Acaulospora* 2 and *Glomus* 1 were significant indicators of *Maianthemum* communities (Fig. 6; Table 4). Multiple comparisons using MRPP also found that AMF communities in *Maianthemum* grown in the presence of *Alliaria* were significantly different than AMF communities in *Maianthemum* without *Alliaria* (Table 3). Indicator species analysis found that species type *Acaulospora* 2 was a significant indicator of *Maianthemum* grown without *Alliaria*. AMF communities in *Trillium* did not appear to be affected by *Alliaria* (Table 3).

DISCUSSION

The roots of herbaceous forest plants are commonly colonized by mycorrhizal fungi (Brundrett and Kendrick, 1990a; Demars, 1996), and these fungi benefit the plants they colonize through increased nutrient gain, improvement in plant water

←

Fig. 4.   Neighbor joining tree showing the taxonomic affinities of selected arbuscular mycorrhizal fungi (AMF) sequences recovered in this study. An *Acremonium*-like hyphomycete (AB108787) was used an outgroup (not shown). The number at each branch indicates percentage of bootstrap replicates of 1000 that support that branch. Values less than 50% are not shown. All clones used for the alignment were recovered from Trillium Trail except for clone series 3811, 3812, and 516, which were recovered from beech–maple forests at the Holden Arboretum.

TABLE 3. Comparison of arbuscular mycorrhizal communities under *Trillium grandiflorum* (*Tg*) and *Maianthemum racemosum* (*Mr*) using MRPP analysis. Plants grown in the presence of *Alliaria* are indicated by a +. The Sørenson distance was used as the measure of within-group distance. The test statistic (*T*) explains the separation between the groups in multidimensional space; the more negative the *T*-value, the greater the separation between the groups. The *P*-value describes how well the separation between groups is likely due to chance alone (boldface indicates significant contrasts), and *A* represents the chance-corrected within group agreement and is a measure of the effect size.

| | Mean within-group distance | | | | |
Groups	Group 1	Group 2	*A*	*T*	*P*
Tg vs. *Tg*+	0.642	0.443	0.018	−0.793	0.186
Mr vs. *Mr*+	0.544	0.610	0.054	−2.527	**0.020**
Tg vs. *Mr*	0.642	0.544	0.033	−1.475	**0.085**
Tg vs *Mr*+	0.433	0.610	0.026	−1.190	0.118

important source of AMF inhibiting substances under natural conditions (Roberts and Anderson, 2001; Stinson et al., 2006). Concentrations of glucosinalates, one of the primary secondary chemicals produced by *Alliaria* and implicated as an important AMF inhibitory substance (Roberts and Anderson, 2001; Stinson et al., 2006), vary at the field site with highest detectable levels in late summer following plant senescence (Data not shown). AMF colonization could be more impacted later in the season when the highest levels of *Alliaria* secondary chemicals are expected to be present in soil. Other studies have reported on the seasonal production of plant secondary compounds and how the effects of these compounds on other plant species may vary temporally and spatially (Blair et al., 2005; Inderjit et al., 2006). In addition, previous greenhouse studies using *Alliaria* plant extracts (Roberts and Anderson, 2001; Stinson et al., 2006) may have subjected soil communities to much higher levels of secondary chemicals than present under field condi-

tions. Lower concentrations and seasonal variation in secondary chemicals under field conditions could partly explain the lack of difference in root colonization in the presence of *Alliaria* in this study.

Molecular analysis of plant roots and soil allowed for the construction of a database of AMF present at the field site that enabled me to identify AMF in plant roots. The majority of recovered sequences belonged to the genus *Glomus* with some sequences representing the genus *Acaulospora* also present. As was the case in our study, the genus *Glomus* was found to dominate clone libraries from tropical forest tree seedlings and grassland plant communities with as many as 95% of recovered clones belonging to this genus (Husband et al., 2002; Santos-González et al., 2007). On the other hand, only 27% of AMF sequences recovered from temperate forest soils belonged to the genus *Glomus* with the majority of sequences showing similarity to the genus *Acaulospora* (Helgason et al., 1998). Together, these studies suggest that forests may have site-specific communities of AMF, which are determined by biotic and abiotic conditions. For example, soils at our field site are finely textured, which may explain the lack of *Gigaspora* in our samples because this genus may prefer soils with low clay content (Lekberg et al., 2007). In total, I was able to detect 12 different AMF species types, but I was not able to distinguish clearly among the species types using TRFLP. Consequently, some species types that clearly differed in sequence alignment had to be combined into one species type for analytical purposes. However, my results compare favorably with the nine AMF species types that were colonizing the roots of *Hyacinthoides nonscripta* and identified through sequencing (Helgason et al., 1999). In a more recent study, Santos-González et al. (2007) identified 19 AMF species types colonizing the roots of two grassland forbs, and Vandenkoornhuyse et al. (2003) found much higher levels of AMF richness (between 15 and 30 based on TRF richness) than in this study. Nonetheless, my data may not be atypical for a
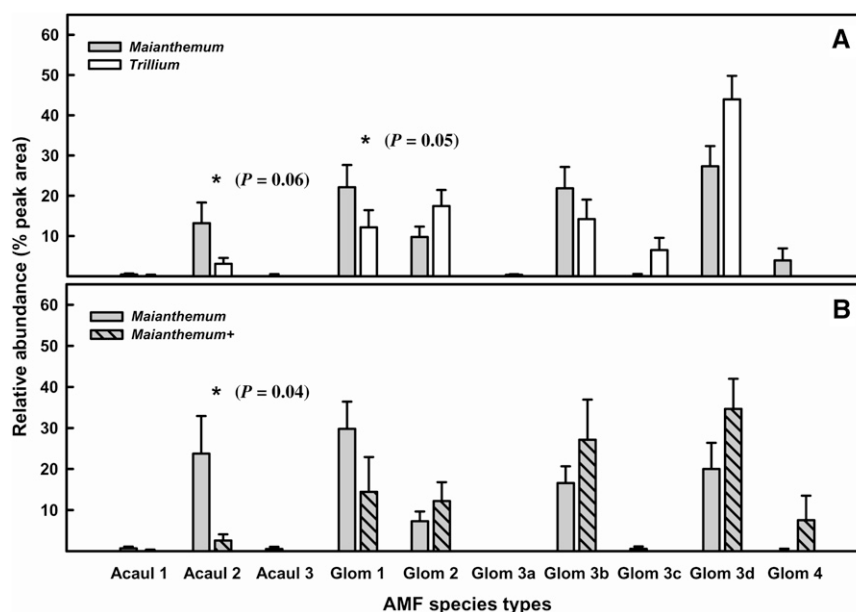

Fig. 6. Distribution of arbuscular mycorrhizal fungi (AMF) taxa among plant–treatment combinations. AMF taxa that are significant indicators of the plant–treatment combinations are denoted with an asterisk (*P*-value) (also see Table 4). Mean relative abundance (peak area) is shown with the SE bar. (A) Relative abundance of AMF taxa between *Maianthemum* and *Trillium* grown in the absence of *Alliaria*. (B) Relative abundance of AMF taxa between *Maianthemum* grown in the absence and presence (+) of *Alliaria*.

Table 4. Indicator species analysis comparing *Trillium grandiflorum* and *Maianthemum racemosum* (*Mr*) grown in the absence of *Alliaria* and comparing *Maianthemum* and *Maianthemum+Alliaria*. A total of 1000 randomizations were used for the Monte Carlo test. Only those taxa that are significant indicators of one of the two groups are shown.

AMF species type	Max group[a]	Indicator value[b]	Mean	SD	*P*
Trillium vs. *Maianthemum*					
Acaulospora 2	*Mr*	58.1	39.6	10.26	0.056
Glomus 1	*Mr*	61.0	45.3	8.44	0.053
Maianthemum vs *Maianthemum+Alliaria*					
Acaulospora 2	*Mr*	63.0	40.8	10.73	0.039

[a] Group (i.e., plant species) with the highest indicator value for those AMF taxa.

[b] Calculated using the relative frequency and relative abundance of the species encountered. The indicator value for a species will be high within a group if that species has higher relative frequency and relative abundance for that group than for other groups.

forest herbaceous community. Coadaptational specificity between plants and AMF taxa could result in a correspondence between plant species richness and AMF richness in natural systems and partly explain the higher AMF richness in grassland as compared to forests (van der Heijden et al., 2004).

I found that AMF community structure was significantly different between *Maianthemum* and *Trillium* and that *Maianthemum* communities were significantly impacted by *Alliaria*. Previous studies have observed differences in the AMF community among different plant species (Helgason et al., 2002; Vandenkoornhuyse et al., 2003; Santos-González et al., 2007), and in forest trees of different ages (Husband et al., 2002). Diversity measures were generally unaffected by either plant species or *Alliaria*, suggesting that AMF community structure changes between the plant species, but that diversity itself (e.g., AMF richness) remains unchanged. It is important to note, however, that standard diversity measures using TRFs may be insensitive to all but the largest changes in community structure (Hartmann and Widmer, 2006), and this insensitivity of TRFLP could explain the lack of differences in diversity in this study.

Indicator species analysis suggested that the community changes I observed may, in part, be related to changes in the presence of some AMF species types. AMF species types *Acaulospora* 2 and *Glomus* 1 were significant indicators of *Maianthemum*, and the *Acaulospora* 2 species type was also affected by *Alliaria* when colonizing *Maianthemum*. AMF taxa are more prevalent in some plant species than in others (Santos-González et al., 2007), but the associations between forest plants and AMF could also be controlled by the composition of the forest overstory community (Helgason et al., 1998), the season, and soil characteristics (Husband et al., 2002; Lekberg et al., 2007; Santos-González et al., 2007). In this study where we sampled in one forest at one time of the year, changes in the community of AMF are more likely specific to the plant species examined and to the presence of *Alliaria*. Although only one of *Maianthemum*'s AMF colonists may have been affected by *Alliaria*, AMF taxa can provide specific benefits to the host plant (van der Heijden et al., 1998; Hart and Klironomos, 2002; Helgason et al., 2002; Hart and Reader, 2002a, b; Koch et al., 2004). Therefore, any change in the community of AMF on *Maianthemum* could have important ramifications for root system function, especially given that *Acaulospora* 2 was a very important colonizer of the *Maianthemum* root system.

To my knowledge, this study is the first to address the potential effects of *Alliaria* on the mycorrhizal communities of native forest plants under field conditions, but my results come with some caveats. Although care was taken to collect plant pairs growing in the presence and absence of *Alliaria* within a short distance of each other, it is likely that the distribution of AMF is determined in part by the heterogeneity of the soil environment. Microsite differences in abiotic conditions between collection patches could have masked the effects of *Alliaria* on AMF communities especially given the small number of plants examined. In addition, my study was also confined to one particular forest with generally uniform overstory vegetation and land use history. Future studies should include a more comprehensive examination of AMF communities from many forest types invaded by *Alliaria*, especially because overstory vegetation can affect AMF communities. Nevertheless, although my results may be conservative, they do suggest that *Alliaria* can affect AMF community composition in the roots of some forest plants and that these effects may be specific to the AMF species present in the forest soil.

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