Reduced Ectomycorrhizae on Oak near Invasive Garlic Mustard

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Abstract - Invasive plants may disrupt symbioses between plants and soil biota. We tested whether ectomycorrhizal fungi (ECM) associating with *Quercus rubra* (Northern Red Oak) seedlings was lower near invasive *Alliaria petiolata* (Garlic Mustard). We quantified ECM colonization, identified morphotypes, and compared composition in forests with contrasting Garlic Mustard densities. Seedlings had lower ECM colonization and diversity in a stand with dense Garlic Mustard than in a stand without Garlic Mustard. ECM composition also differed between stands. Seedlings in a stand with moderate density Garlic Mustard had marginally less ECM than those at the no Garlic Mustard site. These findings suggest that ECM association is negatively correlated with Garlic Mustard invasion. This association may result in lasting changes to forest communities and hinder site restoration following Garlic Mustard removal.

Introduction

Invasive species, those that spread rapidly outside their native range, are reported to be a major cause of species decline and loss of biodiversity (Wilcove et al. 1998) and result in an estimated $120 billion in environmental damages and loses in the United States each year, of which non-native plants are a major contributor (Pimental et al. 2005). Plant invasions have the potential to impact native plant communities through niche displacement, resource competition, allelopathy, alteration of nutrient cycling, hydrology, and fire regimes, and disruption of mutualistic relationships (Ehrenfeld 2003, Gordon 1998, Mack et al. 2000, Mooney and Cleland 2001, Orr et al. 2005, Stinson et al. 2006, Vitousek et al. 1997) .

Historically, the effects of invasive plants on below-ground mutualisms between native plants and soil microbes have not been well studied. Soil microbes, especially fungi, are important for decomposition and nutrient cycling, ultimately controlling the availability of some nutrients to plants and thus playing an integral role in ecosystem functioning (Chapin et al. 1997). Recently there has been increased research in this area, especially concerning relationships between invasive plants and beneficial soil mycorrhizal fungi (Burke 2008, Mooney and Cleland 2001, Reinhart and Callaway 2006, Roberts and Anderson 2001, Stinson et al. 2006, Wolfe et al. 2008).

The association of plants and mycorrhizal fungi is one of the most ubiquitous mutualisms on earth (Lilleskov et al. 2004, Peterson et al. 2004, Reinhart and Callaway 2006) involving an estimated 95% of all plants (Smith and Read 1997), including most woodland herbs (Whigham 2004). These fungi form mutualistic relationships with plants, in which they contribute to plant acquisition of water

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and nutrients, while utilizing the plant as a carbon source. While most land plants are symbiotic with endomycorrhizal fungi, which invade root cells, about 8000 (3%) seed plant species form associations with ectomycorrhizal (ECM) fungi (Taylor and Alexander 2005). ECM host plants, mostly woody perennials including trees such as *Betula* (Birch), *Fagus* (Beech), *Pinus* (Pine), and *Quercus* (Oak), are typically dominant components of woodlands (Smith and Read 1997, Taylor and Alexander 2005). The diversity of ECM fungi is quite high, with global diversity estimated to be 7000–10,000 species (Taylor and Alexander 2005). Plants forming ectomycorrhizal associations benefit by having greater access to mineral nutrients, increased nutrient absorption, protection from pathogens, and increased tolerance to environmental stresses such as water, salinity, pH, temperature, and heavy metal stress (Gupta et al. 2000). These benefits likely enhance host fitness, so long as costs due to carbon (photosynthate) losses are minimal.

Non-mycorrhizal plants, such as introduced members of the Brassicaceae (Mustard family), may negatively affect important relationships between soil fungi and native plants (Burke 2008, Callaway et al. 2008, Mooney and Cleland 2001, Schreiner and Koide 1993, Stinson et al. 2006, Wolfe et al. 2008), potentially resulting in the decline of native plant species. Members of the Mustard family contain a diversity of secondary compounds, many of which deter herbivory (Fahey et al. 2001, Freeland and Janzen 1974), including cyanide-containing compounds, flavonoids, and glucosinolates (Callaway et al. 2008, Cipollini and Gruner 2007, Fahey et al. 2001, Vaughn and Berhow 1999). Hydrolysis products from glucosinolates, as well as flavonoids, have been shown to be toxic to fungi, including mycorrhizal fungi (Callaway et al. 2008, Mayton et al. 1996, Wolfe et al. 2008).

A species capable of disrupting mutualistic associations may affect composition of plant communities highly dependent on mycorrhizae, while potentially enhancing its own spread through alteration of soil microbial communities and positive feedback (Bever 2003). Here we investigate whether an introduced, invasive forb, *Alliaria petiolata* (M. Bieb) Cavara and Grande (Brassicaceae, Garlic Mustard), potentially impacts mycorrhizae in a natural forest setting.

Originally from Eurasia, Garlic Mustard is now widely recognized as an important invader (Rodgers et al. 2008a). It was first documented in native communities in 1869 on Long Island, NY and has since spread to much of the Midwest and northeastern United States as well as some western states (Nuzzo 1991). As of 2010, Garlic Mustard has been documented in 36 US states, and 5 Canadian provinces (NRCS 2010). Garlic Mustard is an obligate biennial producing overwintering rosettes in the first year, blooming from early spring through July of the second year, and producing fruit from June through September, after which the plant dies (Anderson et al. 1996, Baskin and Baskin 1992, Byers and Quinn 1998, Cavers et al. 1979). Garlic Mustard is adapted for generalist pollinators, is capable of self-pollination (Anderson et al. 1996, Cruden et al. 1996), and produces as many as 9500 to 107,000 seeds per m^2 (Anderson et al. 1996, Cavers et al. 1979, Nuzzo 1993), which remain viable in the soil for up to 5 years (Baskin and Baskin 1992, Nuzzo 2000). These characteristics allow a single Garlic Mustard plant to successfully found a new population (Cruden et al. 1996) that can grow

rapidly (Meekins and McCarthy 2002). While disturbance is associated with new Garlic Mustard establishment (Bartuszevige et al. 2007), it is not a prerequisite for successful establishment and survival (Meekins and McCarthy 2001). Once established, Garlic Mustard is difficult to eradicate (Nuzzo 2000, Slaughter et al. 2007) and becomes a permanent part of the forest community (Nuzzo 1999). Garlic Mustard, while being outcompeted by *Acer negundo* L. (Box Elder), has been shown to outcompete seedlings of another native tree, *Quercus prinus* L. (Chestnut Oak), compete equally with some native annuals such as *Impatiens capensis* Meerb. (Jewel Weed) (Meekins and McCarthy 1999), and reduce seed germination of the native perennial *Geum laciniatum* Murray (Rough Avens) (Prati and Bossdorf 2004). McCarthy (1997) also found that removal of Garlic Mustard resulted in an increase in cover of annuals, tree seedlings, and vines over the course of three years. A five-year study evaluating the response of native plant communities following herbicide treatment of Garlic Mustard found that this treatment did not affect species richness or diversity, but it did increase cover of spring ephemerals and graminoids (Carlson and Gorchov 2004, Hochstedler et al. 2007). Stinson et al. (2007) found that native species varied in their response to Garlic Mustard density, but in general, species diversity was lower at higher Garlic Mustard density and increased upon Garlic Mustard removal. More importantly they found that of all functional groups investigated, tree seedlings had the most negative relationship with Garlic Mustard density and responded most positively to partial removal of Garlic Mustard (Stinson et al. 2007).

Recent studies link impacts of Garlic Mustard on native plant communities, at least in part, to the disruption of mutualistic relationships between soil fungi and plants. Garlic Mustard negatively affects spore germination and interferes with the formation of mutualisms between endomycorrhizal fungi and plants, including native hardwood canopy trees (Roberts and Anderson 2001, Stinson et al. 2006), while being correlated with a reduction in host plant vigor (Callaway et al. 2008). Burke (2008) suggests that fungal community structure may also be altered in the presence of Garlic Mustard. These are phenomena not observed in its native region (Callaway et al. 2008). Further evidence that effects are mediated by mycorrhizae comes from Cipollini et al. (2008), who found that Garlic Mustard extracts did not affect growth or reproduction of non-mycorrhizal *Arabidopsis thaliana* (L.) Heynh. (Mouse Ear Cress). While Garlic Mustard's effects on endomycorrhizae have been investigated (Callaway et al. 2008, Roberts and Anderson 2001, Stinson et al. 2006), less is known of its effects on ECM. Wolfe et al. (2008) recently reported that soil cores from three forest stands had fewer ECM roots in plots invaded by Garlic Mustard compared to non-invaded plots, but proportional ECM biomass to total root biomass did not differ significantly. A glasshouse experiment also showed that ECM colonization of *Pinus strobus* L. (Eastern White Pine) was reduced in soils conditioned with Garlic Mustard (Wolfe et al. 2008). Here we investigate whether Garlic Mustard is associated with low ECM infection of hardwood seedlings on the forest floor.

This study compares characteristics of ECM colonization of seedlings of a native ECM host, *Quercus rubra* L. (Northern Red Oak), in forested sites

of contrasting Garlic Mustard density. Our hypothesis was that ECM infection of Northern Red Oak is negatively associated with Garlic Mustard. We predicted that Northern Red Oak grown in sites with Garlic Mustard would have fewer ECM infected root tips than in sites without Garlic Mustard. Furthermore, because it is likely that some fungi are more tolerant of Garlic Mustard, we predicted a lower diversity of fungi-forming ectomycorrhizae in invaded sites. A negative relationship between Garlic Mustard on Northern Red Oak mycorrhizal colonization would have implications on the regeneration of oaks, and perhaps other ECM host trees, possibly contributing to the decline of these trees in eastern deciduous forests (Abrams 1992, Gribko et al. 2002, Lorimer et al. 1994) and a change in the composition of temperate forest communities.

Field Site Description

This two-approach study was conducted in three forested sites in southwestern Ohio. One approach we took, involving seeds planted in the field, was conducted at Reinhart Preserve (Butler County; 39°31′22″N, 84°42′28″W), a Miami University Natural Area, and Bradford-Felter Tanglewood Preserve (hereafter referred to as Tanglewood) (Hamilton County; 39°11′18″N, 84°33′24″W), a property of the Cincinnati Park Board. Reinhart is contiguous with a larger natural area, Bachelor Reserve, and both are managed as a natural preserve. The land-use history of this area was row-crop farming and grazing, but after 1938, these activities declined dramatically, and now successional areas and forests cover 80% of the immediate land area (Medley and Krisco 2007). The specific area where this study was conducted is closed-canopy forest, and aerial photographs reveal that this area has been tree-covered since at least 1938. Tanglewood comprises a series of separate land acquisitions through donations and Nature Conservancy transfers beginning in 1938, with a large tract added in 1978, and smaller portions added in the early 1980s, for a total of 71.2 hectares (Cincinnati Park Board 2008, TNC 2008). Tanglewood is kept in its natural state, and aerial photographs reveal this site has been dominated by hardwood forests since at least 1938.

Sites used for the second approach of this project, involving naturally occurring seedlings, were Reinhart Preserve, described above, and Kramer Woods (39°31′46″N, 84°42′59″W), a Miami University Natural Area which is a 4.9-ha old regrowth stand of at least 100 years that was donated to the university in 1989 (Medley 1996).

The tree canopy of all these sites is dominated by *Acer saccharum* Marsh (Sugar Maple). Co-dominants are *Fraxinus* spp. (Ash) and *Prunus serotina* Ehrh. (Wild Black Cherry) at Reinhart; Northern Red Oak and *Fraxinus americana* L. (White Ash) at Tanglewood; and *Liriodendron tulipifera* L. (Tulip Tree), ash, and *Fagus grandifolia* Ehrh. (American Beech) at Kramer (Table 1). At all three sites, ectomycorrhizal host tree species comprise about 25% of the canopy (based on importance value; Table 1) and are presumed to have ectomycorrhizal fungi present in the soil (Dickie et al. 2002). The soils of Reinhart and Kramer consist of mostly moderately eroded Hennepin-Miamian (HeE2) series silt loams with 18–25% slopes, and the soils at Tanglewood are mostly Eden (EcE) silty clay loam with 25–40% slopes (NRCS 2007).

Garlic Mustard is nearly absent at Reinhart, at low density at Kramer, and at very high density at Tanglewood. The density of Garlic Mustard in the Tanglewood study site averaged 6.7 second-year plants/m^2 and 33.4 first-year plants/m^2 in the summer of 2007, and this invasive had 100% cover in some areas (Castellano 2008). Although coverage was not quantified for other understory plants in this study, we observed that both Reinhart and Kramer had higher abundance of native understory herbs and tree saplings than Tanglewood, which was only sparsely populated with native herbs and nearly devoid of tree seedlings. This sparsity was especially true of oak seedlings, of which none were found near the Tanglewood study plot (S.M. Castellano, pers. observ.).

Materials and Methods

Out-planted seeds/seedlings

This portion of the project was conducted to investigate if seedlings planted at a site without Garlic Mustard (Reinhart) and a high density Garlic Mustard site (Tanglewood) differ in their proportion of ECM root tips and root-fungal community composition.

Planting design. Northern Red Oak acorns, collected in Richland County, OH (Lynn Brinley, NN Seed Co., Mansfield. OH, pers. comm.), were purchased from NN Seed Co. Prior to planting, we discarded any acorns with weevil holes and those that floated, indicating non-viability (Goodman and Mattson 1980). Usable acorns were surface sterilized in a 10% bleach solution for approximately 10 minutes. We planted 49 acorns at each site in early winter of 2006 (26–27 Dec. 2006 at Tanglewood and 4 Jan. 2007 at Reinhart) to allow for required cold stratification in situ (Goodman and Mattson 1980, Young and Young 1992). Single acorns were planted in mineral soil so that each was covered by approximately 0.5 cm of firmed soil, and covered with a thin layer of leaf litter (Sander 1990, Young and Young 1992). Each acorn was enclosed by a wire, vinyl-coated, mesh

Table 1. Importance values of common trees at Reinhart Preserve, Kramer Woods (A. Maye and D. Gorchov, Miami University, Oxford, OH, unpubl. data), and Bradford-Felter Tanglewood Preserve (Castellano 2008). Importance values were calculated as ∑ (relative basal area, relative density, and relative frequency)/3 for each stand. Names marked with (*) are species forming ECM associations. Data were collected near study areas and do not represent composition of entire stands.

	Reinhart	Kramer	Tanglewood
Sugar Maple	18.1	38.7	54.1
Ash	10.5	14.3	10.9
Wild Black Cherry	10.2	1.5	0.0
Northern Red Oak*	9.5	7.0	17.5
Tulip Tree	8.2	14.9	0.0
Chinkapin Oak*	6.2	0.0	1.8
American Beech*	2.1	14.2	0.0
Black Walnut	4.1	5.3	0.0
Black Maple	0.0	0.0	5.8
Other ECM Trees*	11.3	2.7	5.3
Total ECM Trees	29.0	23.9	24.6

cage (mesh size 1.27 x 1.27 cm) to protect against seed predation and herbivory, from both above and below-ground. Plots, one at each site, were 15- x 15-m squares with acorns planted at each intersection of 7 rows and 7 columns, spaced 2 m apart.

Acorns that failed to germinate by May 2007 were replaced with greenhouse-grown seedlings. These were derived from excess acorns that were cold-stratified and germinated in moistened vermiculite at approximately 4 °C during the winter of 2006 (Young and Young 1992) and planted in coarse perlite in "cone tube" pots. Seedlings were regularly watered with a very dilute (approximately 1 part fertilizer per 100 parts water) 21-7-7 fertilizer mix. Prior to planting in the field, 10 seedlings were randomly selected for destructive sampling to ensure that ECM colonization did not occur in the greenhouse, since some ECM species, such as members of the Thelephoraceae, are common greenhouse contaminates (Walker et al. 2005). Fine roots were inspected under a dissecting microscope for evidence of fungal hyphal or mantle development; none showed any indication of ECM colonization. A total of 31 oaks were planted at Tanglewood (24 May 2007), and 25 oaks were planted at Reinhart (25 May 2007), so that each site had a total (field-germinated plus bare-root-planted) of 49 living seedlings.

Our intention was to allow all the seedlings to grow throughout one growing season, and harvest them in the late fall of 2007. However, due to drought conditions during the summer and fall of 2007, seedling mortality and water stress occurred on both sites. In an effort to "rescue" samples from dry conditions, approximately half of the living seedlings on each site were harvested early in the fall (11 Sept. 2007 and 25 Sept. 2007), specifically those appearing to be most affected by the drought. The remainder of the seedlings were left and harvested in the spring of 2008 (13–14 May 2008). Prior to root-tip sampling, seedlings were stored and cleaned as described below.

*Soil analysis***.** In the spring of 2008, we collected ten soil samples, using systematic sampling, from the A horizon up to a maximum depth of 20 cm from each site. Composite samples were made for each site, air dried at ambient temperature (Jones 2001), and shipped to Spectrum Analytic, Inc. (Washington Courthouse, OH) for general nutrient testing, including available phosphorus and nitrogen. Lab methods included the ion selective electrode method for N analysis and the Mehlich 3 method for other nutrients (Vernon Pabst, Spectrum Analytic, pers. comm.).

Naturally occurring seedlings

This approach investigated whether the abundance or composition of ectomycorrhizal fungi colonizing naturally occurring Northern Red Oak seedlings differed between a low-density Garlic Mustard site (Kramer), and a site with no Garlic Mustard (Reinhart). A total of 19 "Garlic Mustard present" seedlings were collected from Kramer and 20 "Garlic Mustard absent" seedlings from Reinhart, in November of 2006. Although we did not determine the age of the seedlings, all collected seedlings were approximately 30 cm or less in height.

Collection criteria. A seedling considered to be "Garlic Mustard absent" had no trace of Garlic Mustard foliage, dead or alive, within a 2-m radius. This condition

was easily met as we found no Garlic Mustard within the Reinhart plot and we noticed only a few individual Garlic Mustard plants some distance from the study area. A seedling considered for the "Garlic Mustard present" group had at least one fruiting Garlic Mustard stem within 20 cm and at least 4 fruiting stalks within 50 cm. Second-year plants were used in the criteria because they indicate Garlic Mustard was present on site for at least 2 years, allowing more time to effect a change in mycorrhizal activity. On both sites, Northern Red Oak seedlings collected were not closer than 1.5 m to another collected seedling, to ensure independence of samples. Seedlings were stored and cleaned as described below.

Seedling cleaning and storage

Following collection, the seedlings and their intact root balls were stored in open plastic bags at approximately 4 ºC until they were analyzed. Seedling root balls were moistened as needed to prevent root and fungus desiccation (O'Dell et al. 1998).

Prior to analysis, seedlings were soaked in distilled water to loosen adhering soil and carefully washed over a wire-mesh screen to remove coarse and loosely adhered soil and organic material. Lateral roots were cut from the taproot, and finer cleaning was done in distilled water under a dissecting microscope using forceps, fine metal probes, and squirt bottle (O'Dell et al. 1998). Once clean, roots were stored in distilled water at 4 ºC, until analysis (Dickie and Reich 2005, Visser 1995).

Root-tip sampling

Fine roots were cut into 3–4 cm pieces and unbroken, living root tips <1 mm were evaluated for the presence of ECM colonization. For out-planted seedlings, 250 root tips were randomly selected from each seedling for scoring. In the event that an individual did not have 250 root tips, all live root tips were scored. For naturally occurring seedlings, 10 segments (3–4 cm) of fine roots were randomly selected from each seedling. All living, unbroken tips on these lengths were scored, for an average of 233 tips per plant.

Root-tip analysis

The formation of a fungal mantle defined ECM colonization, and colonization rate of a seedling was determined as the proportion of ECM colonized tips to all root tips sampled; all ECM root tips were counted as individual tips regardless of whether they were individual monopoid mycorrhizae or part of larger clusters (Dickie and Reich 2005, Dickie et al. 2005). Ectomycorrhizae found on the fine roots were identified to morphotype based on the color, growth pattern, and texture of the fungal mantle (Agerer 1993, Goodman et al. 1998). Abundance of each morphotype was quantified, and ECM samples of each were immediately frozen, and stored at -80 °C for subsequent DNA extraction and molecular identification (see below).

Comparison of ectomycorrhizal colonization

For out-planted seedlings, the proportion of fine root tips colonized by ECM was compared between seedlings from Reinhart and Tanglewood with fixed

effects three-way analysis of variance (ANOVA) ($\alpha = 0.05$) using the GLM procedure in SAS 9.1, with site, harvest date, and seedling type (field-germinated or bareroot) as fixed effects, and proportional colonization as the response variable. The UNIVARIATE procedure was used to ensure the assumption of normally distributed sample variance was met, and proportional data were arcsine square root transformed to meet this condition.

For naturally occurring seedlings, proportional colonization was compared between sites with a weighted one-way ANOVA ($\alpha = 0.05$), with colonization weighted by the number of root tips examined.

Fungal identification

Mycorrhizal fungi were identified using direct genetic sequencing of the internal transcribed spacer (ITS) region of nuclear rDNA (Gardes and Bruns 1993; Walker et al. 2005, 2008; White et al. 1990). DNA from frozen samples was extracted using the DNeasy Plant Mini Kit protocol, and the ITS region was amplified with polymerase chain reaction (PCR) using the fungal specific primer pair ITS1-F and ITS4 (Gardes and Bruns 1993, White et al. 1990). Thermocycle parameters followed Gardes and Bruns (1993). Negative controls (no DNA) were run with every PCR to test for DNA contamination of reagent mixtures and buffers. PCR product was cleaned using Promega PCR cleanup protocol and stored in nuclease-free water at 4 °C until sequencing. Multiple fragment bands resulting from individual samples were manually excised from the gel, purified using the cleanup protocol above, and re-amplified.

Clean PCR product was prepared for sequencing using BigDye Terminator v3.1Cycle Sequencing Kit using the ITS-1F and ITS4 primer pair; sequencing was carried out on an ABI Prism sequencer. Sequences were hand edited using the software application Squencher 4.8 and compared to known ITS sequences using the UNITE database (Kõljalg et al. 2005). Sequence searches were performed using the galaxie BLAST search option, which finds the best BLAST matches and performs web-based multiple phylogenetic alignments using a maximum parsimony model, and is recommended for the identification of unknown ITS sequences (Nilsson et al. 2004). Species identity was determined by the best match, i.e., the species with lowest *E*-value, resulting from alignments. The *E*-value, or expected value, represents the number of sequence matches expected by random chance. When equally close matches to different species of the same genus were obtained for either one individual morphotype or two different morphotypes, taxon identification was described only by generic name. Any morphotype that was not successfully sequenced or that lacked BLAST matches was left as unidentified. The ITS sequences are deposited at GenBank, and identifications can be re-evaluated as additional sequences become available.

Comparison of fungal communities

To compare fungal community composition among sites, we quantified diversity of ECM morphotypes for each seedling using richness (*S*, the total number of taxa) and the Shannon-Weiner index ($H' = -\sum p_i[\ln p_i]$, where p_i = proportion

of total seedling ECM belonging to morphotype *i*) (Brower et al. 1998, Moser et al. 2005). Differences in values of each parameter between sites were tested by treating each seedling as a replicate and using a two sample *t*-test, or, when the parameters did not conform to assumptions of normality, a nonparametric two-sample Wilcoxon test. Separate tests were carried out for the two harvests of out-planted seeds/seedlings.

To compare morphotype community composition among sites for each approach, we carried out non-metric multidimensional scaling (NMDS) ordination, using the metaMDS function in the R package vegan (Oksanen 2008, R Development Core Team 2004). Abundance data was square-root transformed and standardized using a double Wisconsin standardization, which preserves the relevance of morphotype abundance while diminishing the effect of highly dominant species and increasing the importance of more rare morphotypes (Oksanen 2008). The Bray-Curtis index was used as the dissimilarity measure to calculate the distance matrix of the standardized data. To test the null hypothesis of no difference in fungal morphotype composition between sites, we performed multiple response permutation procedure (MRPP) on Wisconsin double standardized abundance of morphotypes for each seedling with non-zero richness. This non-parametric, multivariate test calculates the fraction of permuted pair-wise dissimilarities that are less than observed dissimilarities between sites (Oksanen 2008, Walker et al. 2008). MRPP was performed using the mrpp function in the package vegan in R (Oksanen 2008, R Development Core Team 2004); group size (*n*) was used as a weighting factor, and a total of 10,000 permutations were run.

Results

Ectomycorrhizal colonization of seedlings

For out-planted seedlings, ECM colonization was significantly lower at Tanglewood, the high Garlic Mustard site, than at Reinhart, the no Garlic Mustard site (Table 2). Seedlings grown from field-germinated seed also had a higher proportional ECM colonization than the bare-root planted seedlings, but harvest date and all interactions were not significant (Table 2). In the first harvest (fall 2007), a total of 4066 living root tips were observed from 17 seedlings harvested from Reinhart; of these, 1050 showed evidence of ECM

Table 2. Three-way ANOVA of the proportion of colonized root tips for Northern Red Oak seedlings planted in Tanglewood (high density of Garlic Mustard) and Reinhart (no Garlic Mustard). Seedlings were harvested in Aug 2007 and May 2008. Seedling type refers to field-germinated and bareroot-derived seedlings. Data were arcsine square root transformed to meet assumptions of variance homogeneity; two- and three-way interactions were not significant and were dropped from the ANOVA model.

Source term	df	MS	*F*	*P*
Site	1	1.3505	45.72	<0.0001
Harvest date	1	0.0009	0.03	0.86
Seedling type	1	0.1324	4.48	0.04
Error	52	0.0295		
Corrected total	55			

colonization. The mean proportion of root tips colonized per seedling was 0.26. In sharp contrast, only 248 of 3250 tips analyzed from 13 seedlings harvested from Tanglewood had ECM; mean proportion colonized was 0.076 for these seedlings (Fig. 1A). In the second harvest (spring 2008), a total of 26 living seedlings were harvested: 17 from Reinhart and 9 from Tanglewood. From Reinhart, 1085 of 4144 root tips scored showed evidence of ECM colonization while only 133 of 2046 root tips did from Tanglewood; mean proportion colonized for these seedlings was 0.26 and 0.06, respectively (Fig. 1B).

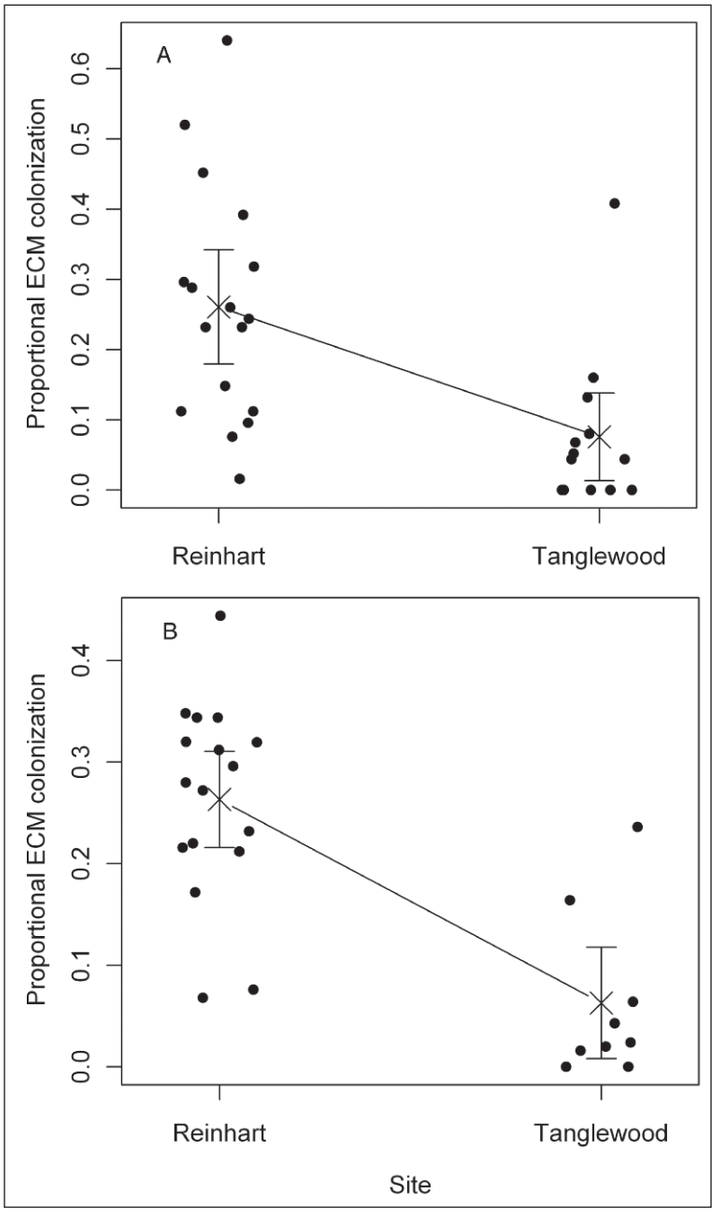

Figure 1. Proportional colonization by ectomycorrhizal fungi on root tips of Northern Red Oak planted in forest sites with high (Tanglewood Preserve) and no (Reinhart Preserve) Garlic Mustard and harvested in A) fall 2007 and B) spring 2008. Crosses represent the mean of the distribution and the error bars are extended to 2x SEM.

For the naturally occurring seedlings, a total of 5466 living root tips were observed from 19 seedlings collected from "Garlic Mustard present" sites at Kramer, of which 1344 showed signs of ECM colonization, an average proportional colonization of 0.25 (Fig. 2). A total of 3626 root tips were examined from 20 "Garlic Mustard absent" seedlings from Reinhart; of these, 1184 were colonized, for a proportional colonization rate of 0.33 (Fig. 2). There was a trend toward more colonization on the "Garlic Mustard absent" samples, but this difference was not significant (Table 3) due to higher variability.

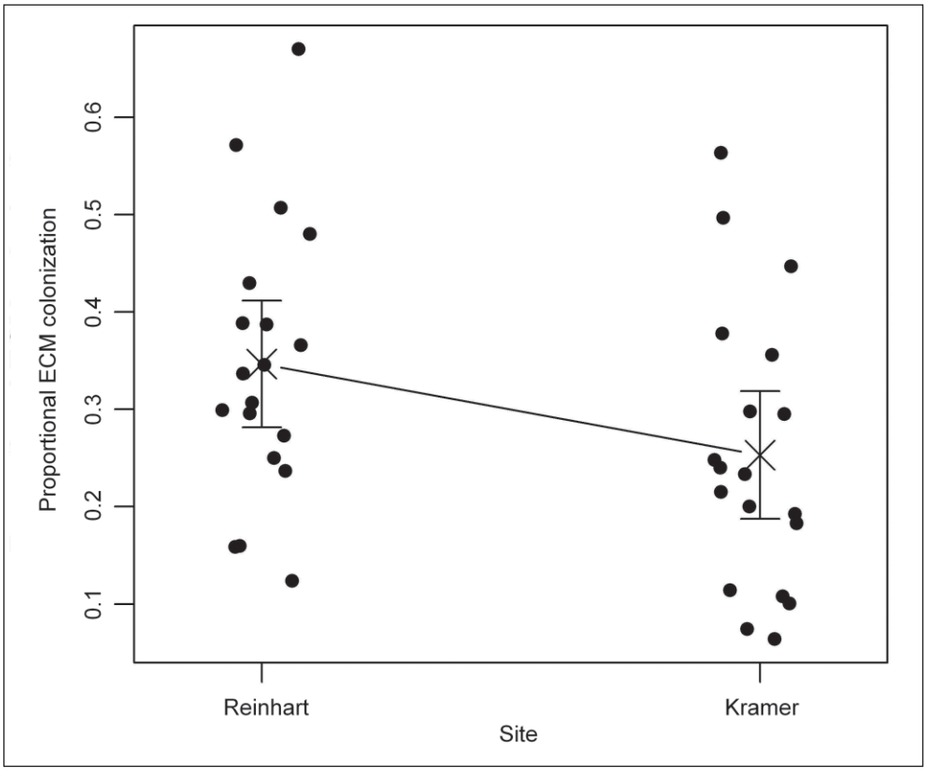

Figure 2. Proportional colonization by ectomycorrhizal fungi on root tips of Northern Red Oak naturally growing in forest sites with moderate (Kramer Woods) and with no (Reinhart Preserve) Garlic Mustard. Crosses represent the mean of the distribution and the error bars are extended to 2x SEM.

Table 3. Weighted one-way ANOVA comparison of the proportion of colonized root tips for Northern Red Oak seedlings naturally growing in Kramer Woods (near Garlic Mustard) and Reinhart (no-Garlic Mustard).

Source term	df	MS	*F*	*P*
Site	1	14.18	3.19	0.082
Error	37	4.45		
Corrected total	38			

Fungal morphotype community

In all, 17 morphotypes were distinguished (Fig. 3). ITS sequences of 13 of these types were successfully amplified and sequenced (Table 4). DNA from

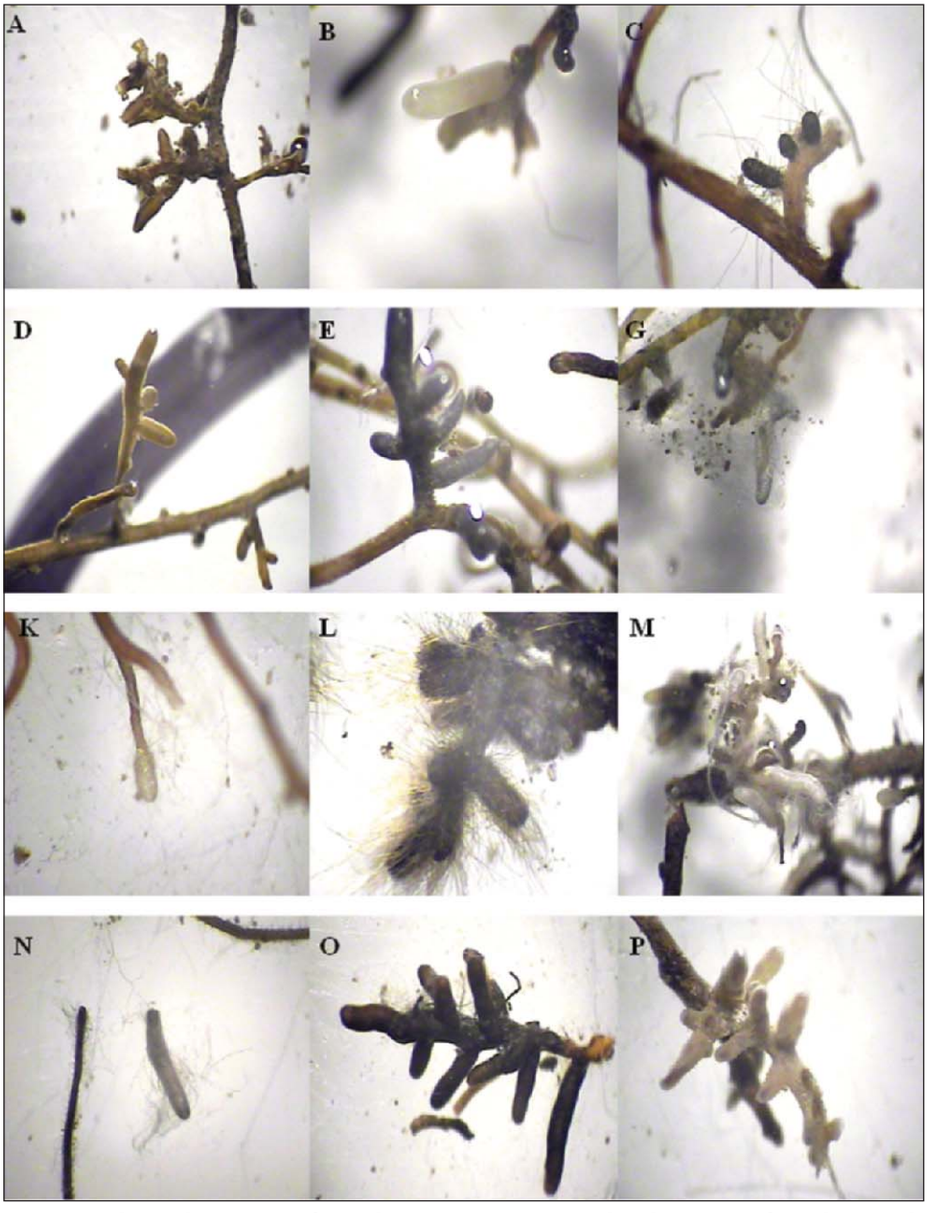

Figure 3. Select color images of morphotypes on ectomycorrhizal root tips of Northern Red Oak. See Table 4 for descriptions and identification statistics of these and other morphotypes: A) *Cystoderma lilacipes*, B) *Russula* sp., C) *Cenoccocum geophilum*, D) *Lactarius* sp., E) *Russula odorata*, G) *Byssocorticium atrovirens*, K) Unidentified, L) Unidentified, M) *Tomentella ellisii*, N) *Russula subrubens*, O) *Sebacina* sp., and P) *Inocybe* sp.

Table 4. Ectomycorrhizal (ECM) morphotypes found on Northern Red Oak seedlings from three forest stands. Names represent the closest match to ITS sequences catalogued in the UNITE database as determined by phylogenetic alignments using maximum parsimony method. We also show which genera have been recorded on *Quercus* species and corresponding references. It should be noted, as in the cases of types A and H, that the closest matches are saprophytic fungi; in these cases contaminants may have been amplified in place of the intended ECM or the fungus may have been decomposing the root tip. For these two taxa, no relationship to *Quercus* was found in the literature. In addition, these types also showed significant alignments, with only slightly lower *E* values, to a variety of ECM species. *Cenoccocum geophilum* was not successfully sequenced, but distinctive morphology allowed identification of this morphotype.

ID	Closest galaxieBLAST match	E	GenBank accession #	Morphotype description	Documented on/near *Quercus*
A	*Cystoderma lilacipes* Harmaja	5.00E-94	FJ389444	Gold/brown with soil debris	
B	*Russula* sp.	0	FJ389445	White/cream smooth, monopodal	Walker et al. 2005, Gebhart et al. 2007
C	*Cenoccocum geophilum* Fr.			Black monopodal w/ black emanating hyphae	Walker et al. 2005, Gebhart et al. 2007
D	*Lactarius* sp.	0	FJ389446	Gold, smooth, some branching	Walker et al. 2005, Gebhart et al. 2007
E	*Russula odorata* Romagn.	0	FJ389447	Grey smooth, monopodal pinnate	Walker et al. 2005, Ishida et al. 2007
F	*Inocybe* sp.1	8.00E-93	FJ389448	Gray/Black, some branching	Mosca et al. 2007
G	*Byssocorticium atrovirens* (Fr.) Bondartsev & Singer ex Singer	0	FJ389449	Silver/blue metallic shiny w/some hyphae	Ishida et al. 2007
H	*Mycena purpureofusca* (Peck) Sacc.	0	FJ389450	White/gray fluff, cottony hyphae	
I	Unidentified 1			Glassy, translucent, monopodal	
J	*Sebacina* sp.1	0	FJ389451	Yellow/Gray, some hyphae	Ishida et al. 2007
K	Unidentified 2			White/Gray, fluffy, monopodal	
L	Unidentified 3			Black/gray-gold hyphae, furry, some branching	
M	*Tomentella ellisii* (Sacc.) Jülich & Stalpers	0	FJ389452	White-cream w/ fluffy hyphae/some branching	Mosca et al. 2007
N	*Russula subrubens* (J.E. Lange) Bon	0	FJ389453	Gray, hairy, emanating hyphae	Walker et al. 2005, Gebhart et al. 2007
O	*Sebacina* sp. 2	0	FJ389454	Black, no hyphae	Ishida et al. 2007
P	*Inocybe* sp. 2	8.00E-93	FJ389455	Milky cream w/ hyphae, smooth, branching	Mosca et al. 2007
Q	*Tomentella stuposa* (Link) Stalpers	0	FJ389456	Brown, wooly, formed in clusters	Ishida et al. 2007

one morphotype, C, was not successfully sequenced, but it was identified as *Cenococcum geophilum* based on its distinct morphology (LoBuglio 1999). It should be noted that although several samples of each morphotype were collected (Table 4), many samples were not successfully extracted and/or amplified. Thus, some species identities are based solely on one ITS sequence. Most of the genera identified have been recorded in the literature as growing on oak species (Gebhart et al. 2007, Ishida et al. 2007, Mosca et al. 2007, Walker et al. 2005; Table 4).

For both harvest dates of out-planted seedlings, Reinhart (no Garlic Mustard) had significantly greater ECM morphotype richness than Tanglewood (high Garlic Mustard) (4.24 and 4.82 vs. 1.46 and 1.44, respectively; $P < 0.001$); Shannon-Weiner diversity was also greater at Reinhart (1.00 and 1.11 vs. 0.31 and 0.30, respectively; $P = 0.0010$ and $P = 0.0009$; Table 5). For the naturally occurring seedlings, richness and diversity tended to be slightly higher at Kramer (moderate Garlic Mustard) than at Reinhart, but differences were not significant ($P = 0.562$ and $P = 0.574$, respectively).

NMDS ordinations for out-planted seedlings revealed differences in ECM community composition of planted seedlings between sites (Fig. 4). MRPP

Table 5. Fungal morphotypes growing on Northern Red Oak root tips from three forested stands with no (Reinhart), low (Kramer), and high (Tanglewood) Garlic Mustard density. Proportional data is provided and represents the proportion of each morphotype out of all ECM root tips for each site. For Reinhart (out) and Tanglewood, proportion is derived from pooling both harvests; data in parentheses are from Harvest 1 (fall 2007) and data in brackets are from Harvest 2 (spring 2008) of the out-planted seedlings only. Kramer and Reinhart (nat) are from the naturally occurring seedlings only. Total number of ECM counted, richness, and diversity are also shown for each site.

		Reinhart			
ID	Species ID	Out	Nat	Kramer	Tanglewood
A	*Cystoderma lilacipes*	0.061 (0.026) [0.078]	0.08	0.077	0.042 (0.000) [0.120]
B	*Russula* sp.	0.210 (0.247) [0.195]	0.19	0.376	0.026 (0.040) [0.000]
C	*Cenoccocum geophilum*	0.331 (0.356) [0.160]	0.47	0.245	0.092 (0.105) [0.068]
D	*Lactarius* sp.	0.084 (0.036) [0.131]	0.08	0.034	0.092 (0.040) [0.188]
E	*Russula odorata*	0.077 (0.114) [0.042]	0.07	0.142	0.105 (0.113) [0.090]
F	*Inocybe* sp.1	0.016 (0.030) [0.018]	0.00		
G	*Byssocorticium atrovirens*	0.017 (0.008) [0.001]	0.04	0.008	0.003 (0.004) [0.000]
H	*Mycena purpureofusca*	0.017 (0.000) [0.000]	0.05	0.047	
I	Unidentified 1	0.001 (0.000) [0.000]	0.00		
J	*Sebacina* sp.1		0.00	0.001	
K	Unidentified 2	0.028 (0.000) [0.068]	0.02	0.068	
L	Unidentified 3	0.011 (0.029) [0.006]			0.239 (0.239) [0.241]
M	*Tomentella ellisii*	0.104 (0.047) [0.273]			0.284 (0.381) [0.105]
N	*Russula subrubens*	0.022 (0.056) [0.014]			0.026 (0.040) [0.000]
O	*Sebacina* sp. 2	0.005 (0.000) [0.014]			0.092 (0.040) [0.188]
P	*Inocybe* sp. 2	0.015 (0.047) [0.000]			
Q	*Tomentella stuposa*	0.002 (0.006) [0.000]			
Total number of ECM counted:		2135 (1050) [1085]	1184	1344	381 (248) [133]
ECM morphotype richness (*S*):		(4.24) [4.82]	3.40	3.68	(1.46) [1.44]
Shannon-Weiner diversity (H′):		(1.00) [1.11]	0.80	0.87	(0.31) [0.30]

confirmed that the ECM morphotype community compositions of Reinhart and Tanglewood were marginally different at the first harvest (A = 0.0212, *P* = 0.076), and differed significantly at the second harvest (A = 0.0530, *P* = 0.002). A combined total (both harvests) of 10 out of 14 morphotypes were shared between Reinhart and Tanglewood. Four morphotypes were unique to Reinhart, while no morphotypes were unique to Tanglewood. The only morphotype dominant at both sites was *Tomentella ellisii*.

NMDS ordination from the naturally occurring seedlings shows considerable overlap of community composition at Reinhart and Kramer (Castellano 2008, data not shown); MRPP also failed to reveal differences in the composition of ECM between these sites (A = 0.0116, *P* = 0.105). Of 11 morphotypes, 8 were shared between sites, 1 was unique to Kramer, and 2 unique to Reinhart. Both sites had *Russula* sp. and *Cenoccocum geophilum* as dominant taxa.

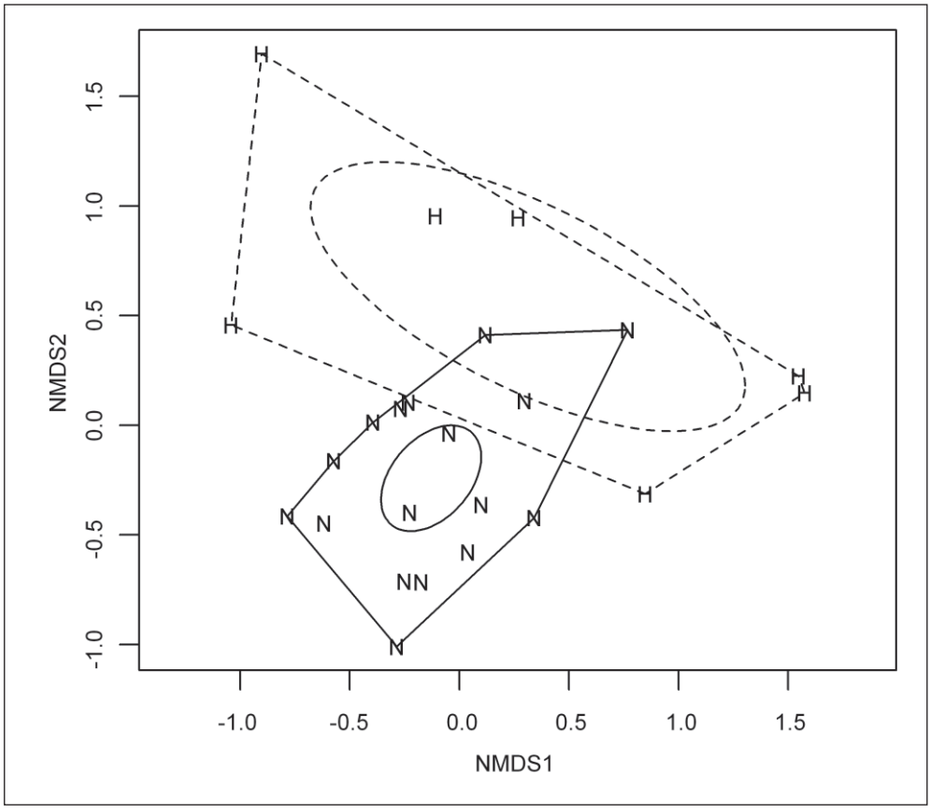

Figure 4. Non-metric multidimensional scaling (NMDS) ordination of ectomycorrhizal (ECM) abundance at high-density Garlic Mustard (dashed lines) and no Garlic Mustard (solid lines) sites for the spring 2008 harvest. Polygons represent range of data, and ellipses represent 95% confidence intervals drawn around the data centroids. For this harvest, the data range had some similarities, but lack of centroid overlap indicates differences in ECM composition.

Soil analysis

Tanglewood had a higher pH and higher organic fraction than Reinhart and was much higher in all nutrients measured except magnesium (Mg). Most striking were the much higher levels of nitrate (NO_3) and phosphorus (P) at Tanglewood than at Reinhart (17 vs. 2 ppm and 141 vs. 5 ppm, for NO_3 and P, respectively. Ammonium (NH_4^+) levels for these sites were not different (Castellano 2008).

Discussion

Our finding that Northern Red Oak seedlings grown from seeds and bareroot seedlings planted at Tanglewood Preserve, a site with dense Garlic Mustard, had significantly lower ECM root tip colonization than those at Reinhart Preserve, a site with almost no Garlic Mustard, is consistent with the hypothesis that Garlic Mustard reduces ectomycorrhizal colonization. Results were similar in both fall and spring harvests of Northern Red Oak seedlings; at each date, only about 7% of root tips at Tanglewood were infected with ECM compared to about 26% at Reinhart.

These findings complement those of Wolfe et al. (2008) on inhibition of ECM by Garlic Mustard. They found lower ECM root tip biomass in soils invaded by this biennial than in soils of nearby uninvaded plots, but did not distinguish whether this pattern was due to reduced infection rate of ECM tree species, or simply to reduced biomass of roots of these species. Wolfe et al. (2008) also found that Garlic Mustard reduced ECM colonization of Eastern White Pine in pots in a glasshouse experiment. Our findings indicate similar patterns on hardwood seedlings growing under natural conditions. These results also parallel reports that Garlic Mustard interferes with endomycorrhizal associations (Callaway et al. 2008, Roberts and Anderson 2001, Stinson et al. 2006).

We also found ECM infection tended to be lower on seedlings growing naturally near Garlic Mustard in an area of moderate Garlic Mustard density (Kramer) than in seedlings growing at the Garlic Mustard-absent site (Reinhart), but this trend was only marginally significant. We think this effect was weak because of the low density of Garlic Mustard at Kramer.

The lower diversity and richness of fungal morphotypes forming ECM associations at the high Garlic Mustard density site are also consistent with our hypothesis that Garlic Mustard negatively impacts ECM fungi. The community composition between sites was also different, albeit only marginally for the fall harvest. We found that while all the morphotypes documented at Tanglewood, the high-density site, were also documented at Reinhart, the latter had unique types. This pattern suggests that fungal species may differ in their resistance to Garlic Mustard effects; less tolerant fungi may be restricted from growing at high-density sites, where species more tolerant of Garlic Mustard would be expected to dominate. However, due to limitations in study design, we cannot make this claim with confidence; variation in fungal community may result from differences in site condition or other variables. While our results do not explicitly attribute a shift in dominant ECM species to Garlic Mustard presence, we did find dominant species differed among sites. At the high Garlic Mustard density site, dominant species included *Tomentella ellisii* and type L, whereas

the dominant species at the low and moderate density sites were the unresolved *Russula* sp. and *Cenoccocum geophilum*.

There were some minor shifts in dominant types on each site from one harvest to the next, but these may be seasonal trends; other studies have found relative abundance and frequencies of species varying with season (Koide et al. 2007, Walker et al. 2008). *Cenoccocum geophilum* was more common in samples we collected in the fall than in spring, consistent with Walker et al.'s (2008) finding that this species was more abundant in fall samples than in summer. However, in another study, *C. geophilum* showed little seasonal variation (Koide et al. 2007). We also found a reduction in abundance of *Russula* spp. and an increase in *Lactarius* sp. and *Sebacina* sp. from fall to spring at both sites. *Tomentella ellisii* increased in Reinhart Preserve and decreased at Tanglewood Preserve between harvests. These findings indicate that community dynamics may be site specific and illustrates the importance of multi-seasonal ECM collection when describing the ECM community of a site.

While our predictions of reduced ECM colonization, reduced morphotype diversity and richness, and differences in community composition were mostly supported by our data from the out-planted seedlings, we interpret these results cautiously. Differences between the two sites in these ECM parameters are not necessarily due to differences in Garlic Mustard density. Other differences between these sites may have influenced ECM; these potentially confounding environmental effects were not controlled in this study.

Lower ECM infection at Tanglewood might have been due to higher soil nutrients, at least during the spring when sampling took place. Nitrate nitrogen (NO_3) was 8x greater than at Reinhart, and phosphorus (P) was 28x greater. As plants have greater access to soil nutrients, they are expected to form fewer associations with ECM as carbon allocation is adjusted, and fungi become C limited. In contrast, as nutrients become more limiting, a greater C investment to mycorrhizal fungi is expected as associated fungi are beneficial in nutrient acquisition (Kiers and van der Heijden 2006). In a meta-analysis comparing mycorrhizal response to these nutrients across field studies done in a variety of biomes, Treseder (2004) reports that nitrogen fertilization tended to reduce mycorrhizae by about 14% and phosphorus reduced mycorrhizae by 32%, with no difference in response between ECM and arbuscular mycorrhizae (AM). However, this percent reduction in N comes from studies using different quantification methods, including hyphal length and percent colonization. Among studies only considering percent colonization, the method used in the current study, the reduction in colonization was somewhat lower (5.8%; Treseder 2004). Other studies found nitrogen fertilization caused no ECM reduction and actually increased arbuscular mycorrhizae (AM) (Garcia et al. 2008), but AM increase occurred only when P was limited, with AM decreasing in P-rich sites (Egerton-Warburton et al. 2007, Eom et al. 1999, Johnson et al. 2003). High nitrogen possibly reduced ECM on our P-rich site.

The higher N and P of Tanglewood, rather than its high density of Garlic Mustard, may have also been responsible for its lower ECM species richness and different community composition. Edgerton-Warburton and Allen (2000) observed that increasing nitrogen resulted in lower species richness and a shift in

AM community composition. These nutrient conditions could have existed prior to Garlic Mustard invasion and could be responsible for a long history of low ECM, even in the absence of the invader.

The high nitrogen levels at Tanglewood might have been due to an outbreak of *Malacosoma disstria* Hübner (Forest Tent Caterpillar) that occurred in spring 2007 and, to lesser extent, in the spring of 2008. Frost and Hunter (2004) found that *Malacosoma americanum* Fab. (Eastern Tent Caterpillar) frass deposition increased total soil N, the NH_4^+ soil pool, and soluble NO_3 in Northern Red Oak mesocosms. Our results from Tanglewood show a much higher soil NO_3, while the NH_4^+ level was comparable to Reinhart.

It is possible that the high N and P at Tanglewood is itself the cause of, or a consequence of, the invasion of Garlic Mustard. Ehrenfeld (2003) found that most introduced, invasive plants were associated with higher levels of inorganic soil N, increased N mineralization, and increased nitrification. Rodgers et al. (2008b) found that plots invaded by Garlic Mustard had higher N, P, Ca, and Mg availability, and higher pH, than uninvaded plots in five forest stands, and experimentally showed that Garlic Mustard leaves accelerate the decomposition of tree leaves, providing a mechanism for such increases. Our data are consistent with this pattern, but we cannot distinguish if Garlic Mustard caused the higher nutrients or if Garlic Mustard was more successful invading this nutrient-rich site. Despite the greater nutrient levels on sites with Garlic Mustard, native plants typically show lower growth and survival, indicating any positive effects on nutrient availability may be outweighed by negative effects of Garlic Mustard (Rodgers et al. 2008a).

Thus, in addition to the potential direct suppression of ECM via allelochemicals, Garlic Mustard may indirectly reduce ECM by creating eutrophic soil conditions unfavorable to infection or which cause the host tree to suppress infection. Potential interactions among N, P, ECM, and Garlic Mustard should be explored in greater depth using long-term manipulative experiments to distinguish between environmental variables and Garlic Mustard as potential stressors to ECM colonization, and how cumulative effects impact this important association and the diversity of the fungal community in general.

The greater herbivory at Tanglewood may have contributed to the low abundance and distinct community composition of ECM at this site. While herbivory from *Odocoileus virginianus* (Zimmermann) (White-tailed Deer) and insects was evident at both sites, the seedlings at the Tanglewood suffered greater deer-related damage, and Tanglewood experienced the outbreak of Forest Tent Caterpillars, which defoliated some seedlings and portions of the canopy. Rossow et al. (1997) found browsing mammals reduced the ECM abundance on willow, and Mueller et al. (2005) report that *Quercus turbinella* Greene (Sonoran Scrub Oak) had reduced ECM with increased insect herbivory. Other studies, however, report that herbivory did not affect ECM abundance and species richness, but did affect community composition (Cullings et al. 2001, Saikkonen et al. 1999).

Our results may have implications for restoration of native plants following control or eradication of Garlic Mustard. Long-term control of Garlic Mustard may be possible with biological control (e.g., Gerber et al. 2008). Nuzzo (2000)

suggests that high quality communities, containing greater diversity of native species and community structure, should recover on their own, provided that removal occurs before high Garlic Mustard densities are reached. Such communities likely have abundant and diverse assemblages of mycorrhizal fungi, much like Reinhart Preserve and Kramer Woods, with which naturally occurring as well as replanted species can associate. At high-density Garlic Mustard sites, however, natural regeneration is less likely as these sites tend to be devoid of abundant native species, and replanting vegetation following Garlic Mustard removal may be required (Nuzzo 2000). We argue that replanting is unlikely to be successful where soils are depauperate in mycorrhizae. In such sites, inoculation of fungi common to the particular area, or to the species planted, would greatly enhance restoration of the plant community. However, we do not know how long a negative effect of Garlic Mustard on mycorrhizal fungi would persist following control or eradication of this invasive. Tree seedlings planted in Garlic Mustard-invaded soils had reduced fungal colonization and seedling growth than those planted in non-invaded soils, even in the absence of Garlic Mustard individuals (Stinson et al. 2006). Long-term studies involving removal treatments would reveal the persistence of such residual effects. Future studies should focus on replication across multiple sites and removal of Garlic Mustard to further test this hypothesis. Loss of ECM and other beneficial soil organisms due to invasive plants may alter ecosystem function and has the potential to cause lasting changes in plant and animal communities.

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Literature Cited

Abrams, M.D. 1992. Fire and the development of oak forests. BioScience 42:346–353.

Agerer, R. 1993. Some checklists for anatomical studies on ectomycorrhizae: Comments on check-list “A”, Morphological characteristics. Pp. 7i–16i, *In* R. Agerer (Ed.). Colour Atlas of Ectomycorrhizae with Glossary. Einhorn-Verlag, Munich, Germany.

Anderson, R.C., S.S. Dhillion., and T.M. Kelley. 1996. Aspects of the ecology of an invasive plant, Garlic Mustard (*Alliaria petiolata*), in central Illinois. Restoration Ecology 4:181–191.

Bartuszevige, A.M., R.L. Hrenko, and D.L. Gorchov. 2007. Effects of leaf litter on establishment, growth, and survival of invasive plant seedlings in a deciduous forest. American Midland Naturalist 158:472–477.

Baskin, J.M., and C.C. Baskin. 1992. Seed-germination biology of the weedy biennial *Alliaria petiolata*. Natural Areas Journal 12:191–197.

Bever, J.D. 2003. Soil community feedback and the coexistence of competitors: Conceptual framework and empirical tests. New Phytologist 157:465–473.

Brower, J.E., J.H. Zar, and C.N. von Ende. 1998. Field and Laboratory Methods for General Ecology. McGraw Hill, Boston, MA. Pp. 177–187.

Burke, D.J. 2008. Effects of *Alliaria petiolata* (Garlic Mustard; Brassicaceae) on mycorrhizal colonization and community structure in three herbaceous plants in a mixed deciduous forest. American Journal of Botany 95(11):1416–1425.

Byers, D.L., and J.A. Quinn. 1998. Demographic variation in *Alliaria petiolata* (Brassicaceae) in four contrasting habitats. Journal of the Torrey Botanical Society 125:138–149.

Callaway, R.M., D. Cipollini, K. Barto, G.C. Thelen, S.G. Hallet, D. Prati, K. Stinson, and J. Kilronomos. 2008. Novel weapons: Invasive plant suppresses fungal mutualists in America, but not in its native Europe. Ecology 89(4):1043–1055.

Carlson, A.M., and D.L. Gorchov. 2004. Effects of herbicide on the invasive biennial *Alliaria petiolata* (Garlic Mustard) and initial responses of native plants in a southwestern Ohio forest. Restoration Ecology 12(4):559–567.

Castellano, S.M. 2008. Effect of *Alliaria petiolata* invasion on ectomycorrhizal colonization of *Quercus rubra*. M.Sc. Thesis. Miami University, Oxford, OH. 59 pp.

Cavers, P.B., M.I. Heagy, and R.F. Kokron. 1979. The biology of Canadian weeds, 35: *Alliaria petiolata* (M. Bieb.) Cavara and Grande. Canadian Journal of Plant Science 59:217–229.

Chapin, F.S., B.H. Walker, R.J. Hobbs, D.U. Hooper, J.H. Lawton, O.E. Sala, and D. Tilman. 1997. Biotic control over the functioning of ecosystems. Science 277:500–504.

Cincinnati Park Board. 2008. Park Board web site. Available online at http://www.cincinnati-oh.gov/cityparks/pages/-3036-/. Accessed 3 July 2008.

Cipollini, D., and B. Gruner. 2007. Cyanide in the chemical arsenal of Garlic Mustard *Alliaria petiolata*. Journal of Chemical Ecology 33:85–94.

Cipollini, D., R. Stevenson, and K. Cipollni. 2008. Contrasting effects of allelochemicals from two invasive plants on the performance of a non-mycorrhizal plant. International Journal of Plant Science 169(3):371–375.

Cruden, R.W., A.M. McClain, and G. Shrivastava. 1996. Pollination biology and breeding system of *Alliaria petiolata* (Brassicaceae). Bulletin of the Torrey Botanical Club 123:273–280.

Cullings, K.W., D.R. Vogler, V.T. Parker, and S. Makhija. 2001. Defoliation effects on the ectomycorrhizal community of a mixed *Pinus contorta*/*Picea engelmannii* stand in Yellowstone Park. Oecologia 127:433–539.

Dickie, I.A., and P.B. Reich. 2005. Ectomycorrhizal fungal communities at forest edges. Journal of Ecology 93:244–255.

Dickie, I.A., R.T. Koide, and K.C. Steiner. 2002. Influences of established trees on mycorrhizas, nutrition, and growth of *Quercus rubra* seedlings. Ecological Monographs 72:505–521.

Dickie, I.A., S.A. Schnizter, P.B. Reich, and S.E. Hobbie. 2005. Spatially disjunct effects of co-occurring competition and facilitation. Ecology Letters 8:1191–1200.

Egerton-Warburton, L.M., and E.B. Allen. 2000. Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. Ecological Applications 10(2):484–496.

Egerton-Warburton, L.M., N.C. Johnson, and E.B. Allen. 2007. Mycorrhizal community dynamics following nitrogen fertilization: A cross-site test in five grasslands. Ecological Monographs 77:527–544.

Ehrenfeld, J.G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6:503–523.

Eom, A.H., D.C. Hartnett, G.W.T. Wilson, and D.A.H. Figge. 1999. The effect of fire, mowing and fertilizer amendment on arbuscular mycorrhizas in tallgrass prairie. American Midland Naturalist 142:55–70.

Fahey, J.W., A.T. Zalcmann, and P. Talalay. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56:5–51.

Freeland, W.J., and D.H. Janzen. 1974. Strategies in herbivory by mammals: The role of plant secondary compounds. American Naturalist 108:269–289.

Frost, C.J., and M.D. Hunter. 2004. Insect canopy herbivory and frass deposition affect soil nutrient dynamics and export in oak mescocosms. Ecology 85(12):3335–3347.

Garcia, M.O., T. Ovasapyan, M. Greas, and K.K. Treseder. 2008. Mycorrhizal dynamics under elevated CO_2 and nitrogen fertilization in a warm temperate forest. Plant and Soil 303:301–310.

Gardes, M., and T.D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes: Application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113–118.

Gebhardt, S., K. Neubert, J. Wollecke, B. Munzenberger, and R.F. Huttl. 2007. Ectomycorrhiza communities of Red Oak (*Quercus rubra* L.) of different age in the Lusatian lignite mining district, East Germany. Mycorrhiza 17:279–290.

Gerber, E., J.L. Hinz, and B. Blossey. 2008. Pre-release impact assessment of two stemboring weevils proposed as biological control agents for *Alliaria petiolata*. Biological Control 45:360–367.

Goodman, R.M., and G.A. Mattson. 1980. Low field temperatures optimum for field germination of Northern Red Oak. Tree Planters' Notes 32–34.

Goodman, D.M., D.M. Durall, and J.A. Trofymow. 1998. Describing ectomycorrhizae. Pp. 3A1–3A5, *In* D.M. Goodman, D.M. Durall, J.A. Trofymow, and S.M. Berch, (Eds.). A Manual of Concise Descriptions of North American Ectomycorrhizae. Mycologue Publications, Sidney, BC, Canada.

Gordon, D.R. 1998. Effects of invasive, non-indigenous plant species on ecosystem processes: Lessons from Florida. Ecological Applications 8(4):975–989.

Gribko, L.S., T.M. Schuler, and W.M. Ford. 2002. Biotic and abiotic mechanisms in the establishment of Northern Red Oak seedlings: A review. General Technical Report NE-295. US Department of Agriculture, Forest Service, Northeastern Research Station, Newton Square, PA. 18 pp.

Gupta, V., T. Satyanarayana, and S. Garg. 2000. General aspects of mycorrhiza. Pp. 29–44, *In* K.G. Mukerji, B.P. Chamola, and J. Singh (Eds.) Mycorrhizal Biology. Kluwer Academic/Plenum Publishers, New York, NY. 336 pp.

Hochstedler, W.W., B.S. Slaughter, D.L. Gorchov, L.P. Saunders, and M.H.H. Stevens. 2007. Forest floor plant community response to experimental control of the invasive biennial, *Alliaria petiolata* (Garlic Mustard). Journal of the Torrey Botanical Society 134(2):155–165.

Ishida, T.A., K. Nara, and T. Hogetsu. 2007. Host effects on ectomycorrhizal communities: Insight from eight host species in mixed conifer-broadleaf forests. New Phytologist 174:430–440.

Johnson, N.C., D.L. Rowland, L. Corkidi, L.M. Egerton-Warburton, and E.B. Allen. 2003. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. Ecology 84:1895–1908.

Jones, J.B. 2001. Laboratory Guide for Conducting Soil Tests and Plant Analysis. CRC Press LLC, Boca Raton, FL. 363 pp.

Kiers, E.T., and M.G.A. van der Heijden. 2006. Mutualistic stability in the arbuscular mycorrhizal symbiosis: Exploring hypotheses of evolutionary cooperation. Ecology 87:1627–1636.

Koide, R.T., D.L. Shumway, B. Xu, and J.N. Sharda. 2007. On temporal partitioning of a community of ectomycorrhizal fungi. New Phytologist 174:420–429.

Kõljalg, U., K.-H. Larsson, K. Abarenkov, R.H. Nilsson, I.J. Alexander, U. Eberhardt, S. Erland, K. Hoiland, R. Kjoller, E. Larsson, T. Pennanen, R. Sen, A.F.S. Taylor, L. Tedersoo, T. Vralstad, and B.M. Ursing. 2005. UNITE: A database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytologist 166:1063–1068.

Lilleskov, E.A., T.D. Bruns, T.R. Horton, D.L. Taylor, and P. Grogan. 2004. Detection of forest stand-level spatial structure in ectomycorrhizal fungal communities. FEMS Microbiology Ecology 49:319–322.

LoBuglio, K.F. 1999. Cenoccum. Pp. 287–305, *In* J.W.G. Cairney and S.M. Chambers (Eds.). Ectomycorrhizal Fungi: Key Genera in Profile. Springer, Berlin, Germany.

Lorimer, C.G., J.W. Chapman, and W.D. Lambert. 1994. Tall understory vegetation as a factor in the poor development of oak seedlings beneath mature stands. Journal of Ecology 82:227–237.

Mack, R.N., D. Simberloff, W.M. Lonsdale, H. Evans, M. Clout, and F.A. Bazzaz. 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. Ecological Applications 10(3):689–710.

Mayton, H.S., C. Olivier, S.F. Vaughn, and R. Loria. 1996. Correlation of fungicidal activity of *Brassica* species with allyl isothiocyanate production in macerated leaf tissue. Phytopathology 86(3):267–271.

McCarthy, B.C. 1997. Response of a forest understory community to experimental removal of an invasive nonindigenous plant (*Alliaria petiolata*, Brassicaceae). Pp. 117–130, *In* J.O. Luken, and J.W. Thieret (Eds.). Assessment and Management of Plant Invasions. Springer, New York, NY. 324 pp.

Medley, K.E. 1996. Dieback in the native shrub, *Lindera benzoin*: A subtle effect of forest fragmentation. Ohio Journal of Science 96(4/5):76–80.

Medley, K.E., and B. Krisko. 2007. Physical site conditions and land-use history as factors influencing the conservation of regrowth in a southwestern Ohio Nature Reserve. Natural Areas Journal 27:31–40.

Meekins, J.F., and B.C. McCarthy. 1999. Competitive ability of *Alliaria petiolata* (Garlic Mustard, Brassicaceae), an invasive, nonindigenous forest herb. International Journal of Plant Science 160(4):743–752.

Meekins, J.F., and B.C. McCarthy. 2001. Effect of environmental variation on the invasive success of a non-indigenous forest herb. Ecological Applications 11:1336–1348.

Meekins, J.F., and B.C. McCarthy. 2002. Effect of population density on the demography of an invasive plant (*Alliaria petiolata*, Brassicaceae) population in a southeastern Ohio forest. American Midland Naturalist 147:256–278.

Mooney, H.A., and E.E. Cleland. 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Science 98(10):5446–5451.

Mosca, E., L. Montecchio, L. Sella, and J. Garbaye. 2007. Short-term effect of removing tree competition on the ectomycorrhizal status of a declining pedunculate oak forest (*Quercus robur* L.). Forest Ecology and Management 244:129–140.

Moser, M.A., C.A Peterson, J.A. D'Allura, and D. Southworth. 2005. Comparison of ectomycorrhizas of *Quercus garryana* (Fagaceae) on serpentine and non-serpentine soils in southwestern Oregon. American Journal of Botany 92(2):224–230.

Mueller, R.C., C.M. Sthultz, T. Martinez, C.A. Gehring, and T.G. Whitham. 2005. The relationship between stem-galling wasps and mycorrhizal colonization of *Quercus turbinella*. Canadian Journal of Botany 83(10):1349–1353.

Natural Resources Conservation Service (NRCS). 2007. NCSS Web Soil Survey. Natural Resources Conservation Service, US Department of Agriculture. Available online at http://websoilsurvey.nrcs.usda.gov/app/. Accessed 25 May 2008.

NRCS. 2010. The PLANTS Database: National Plant Data Center, Baton Rouge, LA 70874-4490 USA. Available online at http://plants.usda.gov. Accessed April 2010.

Nilsson, R.H., K-H. Larsson, and B.M. Ursing. 2004. Galaxie - CGI scripts for sequence identification through automated phylogenetic analysis. Bioinformatics 20:1447–1452.

Nuzzo, V.A. 1991. Experimental control of Garlic Mustard (*Alliaria petiolata* (Bieb.) Cavara & Grande) in northern Illinois using fire, herbicide, and cutting. Natural Areas Journal 11:158–167.

Nuzzo, V.A. 1993. Current and historic distribution of Garlic Mustard (*Alliaria petiolata*) in Illinois. Michigan Botanist 32:21–33.

Nuzzo, V.A. 1999. Invasion pattern of the herb Garlic Mustard (*Alliaria petiolata*) in high quality forests. Biological Invasions 1:169–179.

Nuzzo, V.A. 2000. Element stewardship abstract for *Alliaria petiolata* (*Alliaria officinalis*), Garlic Mustard. The Nature Conservancy, Arlington, VA. 19 pp.

O'Dell, T.E., H.B. Massicotte, and G. Kernaghan. 1998. How to store, clean, and photograph ectomycorrhizae and prepare voucher material. Pp. 2.1–2.3, *In* D.M. Goodman, D.M. Durall, J.A. Trofymow, and S.M. Berch (Eds.). A Manual of Concise Descriptions of North American Ectomycorrhizae. Mycologue Publications, Sidney, BC, Canada.

Oksanen, J. 2008. Multivariate analysis on ecological comminutes in R: Vegan tutorial. Vegan: R functions for vegetation ecologists. Available online at http://cc.oulu.fi/~jarioksa/softhelp/vegan.html. Accessed 24 June 2008.

Orr, S.P., J.A. Rudgers, and K. Clay. 2005. Invasive plants can inhibit native tree seedlings: Testing potential allelopathic mechanisms. Plant Ecology 181(2):153–165.

Peterson, R.L., H.B. Massicotte, and L.H. Melville. 2004. Mycorrhizas: Anatomy and Cell Biology. CABI Publishing, Ottawa, ON, Canada. 196 pp.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52(3):273–288.

Prati, D., and O. Bossdorf. 2004. Allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae). American Journal of Botany 91(2):285–288.

R Development Core Team. 2004. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-00-3, Available online at http://www.R-project.org. Accessed 3 March 2010.

Reinhart, K.O., and R.M. Callaway. 2006. Tansley review: Soil biota and invasive plants. New Phytologist 170:445–457.

Roberts, K.J., and R.C. Anderson. 2001. Effect of Garlic Mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. American Midland Naturalist 146:146–152.

Rodgers, V.L., K.A. Stinson, and A.C. Finzi. 2008a. Ready or not, Garlic Mustard is moving in: *Alliaria petiolata* as a member of eastern North American Forests. Bioscience 58(5):426–436.

Rodgers, V.L., B.E. Wolfe, L.K. Werden, and A.C. Finzi. 2008b. The invasive species *Alliaria petiolata* (Garlic Mustard) increases soil nutrient availability in northern hardwood-conifer forests. Oecologia 157:459–471.

Rossow, L.J., J.P. Bryant, and K. Kielland. 1997. Effects of above-ground browsing by mammals on mycorrhizal infection in an early successional taiga ecosystem. Oecologia 110:94–98.

Saikkonen, K., U. Ahonen-Jonnarth, A.M. Markkola, M. Helander, J. Tuomi, M. Roitto, and H. Ranta. 1999. Defoliation and mycorrhizal symbiosis: A functional balance between carbon sources and below-ground sinks. Ecology Letters 2:19–26.

Sander, I.L. 1990. *Quercus rubra*. Pp. 727–733, *In* R.M. Burns and B.H. Honkala (Eds.). Silvics of North America. v. 2. Hardwoods. Agriculture Handbook No. 654. US Department of Agriculture, Forest Service, Washington, DC. 877 pp.

Schreiner, R.P., and R.T. Koide. 1993. Mustards, mustard oils, and mycorrhizas. New Phytologist 123:99–105.

Slaughter, B.S., W.W. Hochstedler, D.L. Gorchov, and A.M. Carlson. 2007. Response of *Alliaria petiolata* (Garlic Mustard) to five years of fall herbicide application in a southern Ohio deciduous forest. Journal of the Torrey Botanical Society 134(1):18–26.

Smith, S.E., and D.J. Read**.** 1997. Mycorrhizal Symbiosis, 2nd Edition. Academic Press, New York, NY. 605 pp.

Stinson, K.A., S.A. Campbell, J.R. Powell, B.E. Wolfe, R.M. Callaway, G.C. Thelen, S.G. Hallett, D. Prati, and J.N. Kilronomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biology 4(5):727–731.

Stinson, K., S. Kaufman, L. Durbin, and F. Lowenstein. 2007. Impacts of Garlic Mustard invasion on a forest understory community. Northeastern Naturalist 14(1):73–88.

Taylor, A.S.F., and I. Alexander. 2005. The ectomycorrhizal symbiosis: Life in the real world. Mycologist 19(3):102–112.

The Nature Conservancy (TNC). 2008. Land Protection Statistics. Available online at http://www.nature.org/wherewework/northamerica/states/ohio/preserves/art11493.html. Accessed 3 July 2008.

Treseder, K**.**K. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytologist 164:347–355.

Vaughn, S.F., and M.A. Berhow. 1999. Allelochemicals isolated from tissues of the invasive weed Garlic Mustard (*Alliaria petiolata*). Journal of Chemical Ecology 25(11):2495–2504.

Visser, S. 1995. Ectomycorrhizal fungal succession in Jack Pine stands following wildfire. New Phytologist 129:389–401.

Vitousek, P.M., H.A. Mooney, J. Lubchenco, and J.M. Melilo. 1997. Human domination of earth's ecosystems. Science 277:494–499.

Walker, J.F., O.K. Miller, and J.L. Horton. 2005. Hyperdiversity of ectomycorrhizal fungus assemblages on oak seedlings in mixed forests in the southern Appalachian Mountains. Molecular Ecology 14:829–828.

Walker, J.F., O.K. Miller, and J.L. Horton. 2008. Seasonal dynamics of ectomycorrhizal fungus assemblages on oak seedlings in the southeastern Appalachian Mountains. Mycorrhiza 18:123–132.

Whigham, D.F. 2004**.** Ecology of woodland herbs in temperate deciduous forests. Annual Review of Ecology, Evolution, and Systematics 35:583–621.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322, *In* M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White (Eds.). PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA. 482 pp.

Wilcove, D.S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States**.** BioScience 48(8):607–615.

Wolfe, B.E., V.L. Rodgers, K.A. Stinson, A. Pringle. 2008. The invasive plant *Alliaria petiolata* (Garlic Mustard) inhibits ectomycorrhizal fungi in its introduced range. Journal of Ecology DOI: 10.1111/j.1365-2745.2008.01389.x.

Young, J.A., and C.G. Young. 1992. Seeds of Woody Plants in North America, Revised and Enlarged Edition. Dioscorides Press, Portland, OR. 407 pp.