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ORIGINAL PAPER

Long-term legacies and partial recovery of mycorrhizal communities after invasive plant removal

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Abstract Invasive plants can have strong impacts on native communities, which have prompted intense efforts at invasive removal. However, relatively little is known about how native communities will reassemble after a dominant invader has been removed from the system. Legacy effects of invasive plants on soil microbial communities may alter native plant community reassembly long after the invader is gone. Here we found that arbuscular mycorrhizal fungal (AMF) communities have shown some recovery in experimental plots following 6 years of removal of the invasive *Alliaria petiolata* (garlic mustard, a species known to degrade AMF communities) in terms of taxonomic richness and community composition. However, despite this recovery, the density of *A. petiolata* at the beginning of the experiment (in 2004) still correlated with lower AMF richness and altered community composition after 6 years of annual weeding, suggesting long-term legacies of dense *A. petiolata* infestations. Because native plant and mycorrhizal fungal communities may show interdependence, reassembly of one community may be limited by the reassembly of the other. Restoration may be more effective if practices address both communities simultaneously.

Keywords Arbuscular mycorrhizal fungi · *Alliaria petiolata* · Restoration · T-RFLP · Soil microbial community

Introduction

Invasive plants can have strong impacts on native plant communities as well as soil microbial communities and soil function. These impacts can occur as a consequence of the high dominance achieved by many problematic invaders, but can also feedback to promote and maintain this high dominance if the invader changes soil properties in a way that benefits its own growth relative to native species (Wolfe and Klironomos 2005; Klironomos 2002; van der Putten et al. 2007). Exotic species may have altered interactions with diverse soil communities relative to native species due to their lack of evolutionary history in their new range; for instance, they may be released from specialized pathogens or their chemical defenses may

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be especially effective against naïve soil organisms (Callaway et al. 2008; Callaway and Ridenour 2004).

Over the last 50 years, we have gained an increasingly sophisticated understanding of how and why exotic species can become invasive in their new range. Exotic species may gain ecological advantages through a plethora of mechanisms, including enhanced responses to disturbance, release from natural enemies, production of novel weapons, etc. (Catford et al. 2009; Hallett 2006). As a consequence of their high abundance and negative ecological impacts, considerable effort has gone toward extirpating or greatly reducing invasive populations from natural areas (Simberloff 2009). However, we still know relatively little about how native communities will respond to the removal of dominant invaders. Very few studies have investigated the long-term legacies of invasions through rigorous experimental means. Once invaders are removed, do native communities return to a pre-invasion state (in terms of diversity and composition)? Do they remain in the typical low diversity state created by the invader? Or do new communities assemble that differ from the original and invaded communities?

Predicting the response of native plant communities to invader removal may be complicated if the invader leaves behind long-lived legacy effects on soil properties. Legacy effects occur when the presence of a species alters ecosystem functions or community composition in ways that persist even when the species is removed from the system (Kardol et al. 2007; Kulmatiski et al. 2006). These legacies can occur through changes to soil structure, nutrient cycling, or microbial communities. While many studies have found that microbial communities can shift in response to the presence of certain plant species (Batten et al. 2008; Callaway et al. 2004; Klironomos 2002; Pringle et al. 2009; van Grunsven et al. 2010), we know relatively little about how persistent these shifts will be in natural settings once the invasive plants have been removed. If microbial communities quickly revert to a pre-invasion stage following invasive removal, then these effects might have little impact on restoration and community reassembly, even if they are important in the original establishment and persistence of the invader. On the other hand, if microbial communities (or certain subsets of the microbial community) are slow to revert, or develop in unexpected ways following invasive removal, this could inhibit successful establishment of the desired native plant community in restoration projects.

Here, we investigated the arbuscular mycorrhizal fungal (AMF) community structure in soils in plots naturally invaded by *Alliaria petiolata* and in plots where the invader had been experimentally removed for 6 years. *A. petiolata* is a biennial mustard native to Europe that has become a wide spread invader of understories in the eastern deciduous forests of North America. This species likely benefits from multiple ecological advantages in its introduced range (Rodgers et al. 2008), including the production of secondary compounds shown to reduce the abundance and composition of mycorrhizal fungal communities (Callaway et al. 2008; Cantor et al. 2011; Roberts and Anderson 2001; Barto et al. 2011; Stinson et al. 2006). Since *A. petiolata*, like all mustards, is non-mycorrhizal, this can provide a competitive advantage to the invader over mycorrhizal native plants, which depend on this mutualism for the acquisition of soil nutrients (Smith and Read 2008).

To determine how *A. petiolata* invasions affected native plant communities, we established permanent plots in a central Illinois woodland invaded by *A. petiolata* (garlic mustard), and experimentally weeded the invader from two-thirds of these plots (either in early or late spring), between 2005 and 2012. Sampling of the vegetation in the permanent plots occurred in mid to late May every year between 2004 and 2012, as well as sampling in April starting in 2008 and continuing through 2011. Data from an early spring sample (April 19 and 20, 2008) showed that cover of native spring ephemeral species increased in removal versus control sites for one of two experimental sites (upland vs. lowland forests), but there were no changes in the cover of summer dominant species in this early sample (Herold et al. 2011). However, this recovery of native spring species may be driven largely by non- or facultative mycorrhizal species. The percent cover of the three dominant mycorrhizal plant species at this site did not differ among treatments in 2008, while the percent cover of the three dominant non-mycorrhizal plant species nearly doubled in removal plots (Anderson et al. 2010). Mycorrhizal inoculum potential (MIP) also increased in removal treatments relative to controls by 2009 (25.72 vs. 18.29 %) (Anderson et al. 2010). Similarly, vegetation data collected in a late sample

(May) of 2011 indicated total cover of native species was significantly higher in removal plots than controls, but the differences in cover among treatments was relatively small, and treatments had no significant effects on species composition of summer dominant species after 7 years (2005–2011) of weeding. Nearly all of these species are mycorrhizal and collectively had only a small increase in cover (Anderson et al. 2012). These data suggest that native communities have begun to respond to the removal of *A. petiolata*, but this recovery is relatively slow. The speed of native response may be reduced by legacy effects of *A. petiolata* on mycorrhizal fungal communities. Here, we investigated the mycorrhizal fungal community richness and composition in soils taken from these experimental plots in fall of 2010 after 6 years of *A. petiolata* removal. In particular, we asked:

1. After 6 years of *A. petiolata* removal, does the richness or composition of AMF communities differ between weeded and unweeded plots (i.e. are mycorrhizal fungal communities recovering from this invasion)?
2. After 6 years of *A. petiolata* removal, do mycorrhizal fungal communities reflect the initial density of *A. petiolata* in these plots (i.e. is there a long-term legacy of *A. petiolata* invasion)?

Methods

Experimental design

Our study area is located 30 km northeast of Normal, Illinois, USA in a second growth hardwood forest in the ParkLands Foundation's Merwin Nature Preserve. There are well established populations of *A. petiolata* on the study plots, which are located on an upland site and a lowland site located in a low-lying area near a creek. The upland site has a well-developed herbaceous spring ground layer, whereas the lowland site does not. Both sites have a well-developed herbaceous summer ground layer.

We used a randomized complete block design with blocks nested within sites. More complete details of the sampling design are provided in (Bauer et al. 2010) and are summarized here. Each of our two study sites has two blocks ($\sim$23 $\times$ 30 m) each containing 60 plots for a total of 120 study plots per site. Each plot has a treatment area (2.5 $\times$ 2.5 m) and a central sampling area (50 $\times$ 50 cm). Parallel transects were located 5 m apart in each block and plots were placed at 2.5-m intervals along each transect.

In each block, one-third of the plots (20) were randomly assigned to a control or one of the two treatments in which second-year *A. petiolata* plants were hand removed either early (March 4–9) or late (May 15–18). In early-treatment plots when all second-year *A. petiolata* plants were being removed, *A. petiolata* seeds had germinated, second-year plants were still in the rosette stage, and nearly all native species were dormant. In late-treatment plots, all second-year plants were removed after second-year *A. petiolata* plants had bolted, flowering was occurring, and native species were actively growing. Thus, seed input from second-year plants growing directly on the plots was little to none. Treatments have been applied annually 2005–2013 following a pre-treatment sample of vegetation in plots in 2004.

Plant and soil sampling

Percent aerial cover of all plants rooted within the sampling areas was estimated by species or by genus for Sedges (*Carex)* and Violets (*Viola*) in May (2005–2013) and in April 2008–2011 to sample spring ephemeral species. Cover of first- and second-year *A. petiolata* was also estimated and second-year plants were counted. Counts of first-year *A. petiolata* were made in decimeter quadrats nested in the NE and SE corners of the sample plots. For pretreatment data there were no significant differences in the cover of *A. petiolata* or native vegetation between treatment or control plots.

Two soil cores ($\sim$5 g soil each) were taken from a total of 192 plots (evenly distributed among the two sites, the two blocks at each site, and the three treatments within each block, for a total of 16 per site $\times$ block $\times$ treatment combination) in November, 2010. Soil was kept on ice until transported back to the laboratory and frozen at -80 °C. Genomic DNA was extracted from 1 g of each sample using an E.Z.N.A. Soil DNA kit (Omega Bio-tek, Atlanta, GA), and diluted 1:10 with water for PCR amplification.

Molecular characterization of AMF communities

AMF communities in soil samples were characterized with a database terminal restriction fragment length

polymorphism (TRFLP) approach (Dickie and Fitz-John 2007). In brief, this approach involved amplifying mixed community AMF DNA with PCR, digesting the products with restriction enzymes to convert sequence variation to length variation, and sizing the terminal fragments using capillary electrophoresis. Clone libraries were created from a subsample of the PCR products, sequenced, and the forward and reverse terminal fragment sizes of each unique sequence type for two enzymes were determined. We then used the TRAMP package in R to predict which sequence types could be present in a given sample based on the pattern of terminal fragment sizes (Fitzjohn and Dickie 2007).

DNA was amplified by the AML1–AML2 primer pair (Lee et al. 2008) using the following program: initial denaturation at 94 °C for 15 min, followed by 30 cycles of 94 °C for 30 s, 58 °C for 40 s, 72 °C for 55 s and an additional extension of 72 °C for 5 min. PCR was carried out using 20 ng of diluted template DNA, 1.2 μg T4 protein and 1.25 U Taq polymerase (Promega Go Taq Flexi) in 1× accompanying buffer including a concentration of 1.5 mM MgCl, 20 μM of each dNTP, and 0.4 μM of both AML1-FAM and AML2-NED, in a final volume of 20 μl. PCR products were digested with MboI (Promega) and submitted for capillary electrophoresis.

Based on the MboI TRFLP patterns, we selected a subset of ten samples from each site (upland and lowland) to use to make clone libraries. These ten samples per site were chosen to include each unique fragment length observed in the larger sample set for that site, to help ensure that all AMF taxa present at a site would be present in the mixed sample. We also made sure that each set of ten samples included several from each *A. petiolata* removal treatment and was equally balanced between the two blocks per site. The ten samples from each site were separately re-amplified with untagged AML1–AML2 primers, and then combined into two cloning reactions (one for each site). Details of the cloning protocols can be found in (Lankau and Nodurft 2013). In brief, the combined samples were gel purified, then ligated into pGEM-T Easy Vector and cloned into JM109 High Efficiency Competent Cells using the pGEM-T Easy Vector System (Promega). Transformed cells were grown over night at 37 °C on LB/Amp plates. Fifty clones per site were picked, regrown overnight on new plates, and then transferred to liquid media and frozen. The clones were then re-amplified with the M13F–M13R primer pair, and the products digested with MboI and visualized on a 1.5 % agarose gel. Of the 100 clones examined for restriction fragment length patterns, we detected six unique RFLPs across the two sites. We then chose 40 of these clones to sequence, ensuring that we sequenced multiple versions of each RFLP from each site. M13 PCR product from clones selected for sequencing were purified using EZNA (96) Omega PCR purification kits (Omega Biotek, Atlanta, GA). Purified product was then submitted to Georgia Genomics Facility for sequencing from the AML2 primer. Returned sequences were corrected, trimmed, and run through BLASTN. Sequences matching to glomeromycotan sequences in the NCBI database were then compared to the MaarjAM database (Öpik et al. 2010) using BLAST in order to assign each AMF clone to a virtual taxa (VT). Fifteen unique VTs were detected among our 40 sequences. Representative sequences from this and a parallel study (Lankau and Nodurft 2013) were submitted to GenBank with accession numbers KF386264–KF386347. To create a TRFLP database, we determined empirical terminal restriction fragment lengths for each VT by digesting several clones per VT with MboI and TfiI (New England Biolabs), previously shown to be the most discriminatory pair with this gene region (Lankau and Nodurft 2013). The resulting fragment patterns were then added to a larger TRFLP database from a previous project using soils and roots from six other *A. petiolata* invaded forests (Lankau and Nodurft 2013), which in total included 93 empirical TRFLs from 35 unique VTs. The TRFLP database is available as Supplemental Information. See Lankau and Nodurft (2013) for more methodological details.

Once the database had been made, all 192 samples were amplified again and digested with TfiI (New England Biolabs), and the two digest patterns combined to allow matching to the TRFLP database using the TRAMPR package in R (Fitzjohn and Dickie 2007). This algorithm searches for all possible matches between the fragment sizes present in a sample and the fragment size patterns of the taxa in the database.

Statistical analysis

Question 1: After 6 years of A. petiolata removal, does the richness or composition of AMF communities differ between weeded and unweeded plots?

The TRAMPR package produces a community matrix of the presence or absence of each VT in each sample.

We calculated the richness of a sample as the number of VTs detected. We used a generalized linear model with a negative binomial error distribution to test whether VT richness varied according to the site (upland vs. lowland), *A. petiolata* removal treatment (control, early, or late removal), and their interaction. To compare composition among samples, we used non-parametric MANOVA with the adonis function in the vegan package in R, using the same dependent variables (Oksanen et al. 2005). Additionally, we compared the beta diversity within each treatment group and site combination using the betadisper function in the vegan package followed by a linear model comparing distance to centroid for each group (Oksanen et al. 2005). Because previous research on these experimental plots has found strong differences between the upland and lowland woods, we also performed all analyses separately for each site.

The observed richness of a sample may depend on the sampling effort (more species are expected when more individuals are collected). The efficiency of the PCR may act in an analogous way, with more taxa detected in more efficient reactions (due both to differences in starting copy number of the gene of interest, and methodological variation). To control for this, we included the total fluorescence of each sample (minus that due to primer and primer dimer) as a covariate in all of our models.

Question 2: After 6 years of A. petiolata removal, do AMF communities reflect the initial density of A. petiolata in these plots?

To test whether the presence of *A. petiolata* creates long-term legacies in AMF community richness or composition, we repeated the above analysis with addition of the pre-treatment percent cover of first and second year *A. petiolata* in each plot, measured in 2004. We also included all interactions with both the site (upland vs. lowland) and removal treatments. As before, we also performed both analyses separately in each site.

Finally, since a plot of VT richness in 2010 versus pre-treatment percent cover of *A. petiolata* in 2004 showed a strong pattern of heteroscedasticity, with greater variance at low initial *A. petiolata* cover, we also performed a quantile regression for each site separately. We used five quantiles (tau = 0.1, 0.25, 0.5, 0.75, 0.9) to determine how the slope of this relationship varied across this range. Quantile regression is useful in ecological studies when many unmeasured factors can affect the variable of interest (in this case, AMF richness), and so relationships with a particular predictor (e.g. pre-treatment *A. petiolata* cover) are apparent only in a subset of samples where other factors are not limiting (Cade and Noon 2003). Since our interest is to test whether variation in *A. petiolata* density creates legacies that act long after the invader has been removed, we only used plots from the two removal treatments in this analysis.

Results

Question 1: After 6 years of *A. petiolata* removal, does the richness or composition of AMF communities differ between weeded and unweeded plots?

AMF communities were richer in the upland versus lowland site, but this difference was driven primarily by the early removal treatment at the upland site, resulting in a significant interaction between site and *A. petiolata* removal treatment (Table 1A; Fig. 1). In the upland site, AMF richness was significantly higher in the early removal treatment relative to either the control or late removal treatments (Table 1A; Fig. 1). In the lowland site, there were no differences in AMF richness among the treatments (Table 1A; Fig. 1).

Similarly, AMF community composition was most divergent between sites (Table 1B), with a marginal interaction between site and removal treatment. When sites were analyzed separately, there was a significant effect of removal treatment in the upland, but not lowland site. This difference came primarily from divergence in the early versus late removal treatments (Table 1B). Composition in the control treatments was more variable, encompassing the variation present within both removal treatments (Fig. 2). This increased variability was confirmed by a significant difference in the multivariate dispersion of control versus early or late removal samples in the upland site (mean distance to group centroid: control, 0.415; early, 0.320; late, 0.304; $F_{2,83} = 4.734$, $P = 0.011$) but not the lowland site (mean distance to group centroid: control, 0.363; early, 0.342; late, 0.368; $F_{2,83} = 0.158$, $P = 0.854$). The multivariate dispersion is a measure of beta diversity among communities

Table 1 Comparison of AMF richness and composition among experimental sites and treatments

Source	A. GLM of AMF richness		B. npMANOVA of AMF composition		
All samples	LR	*P*	Pseudo F	R^2	*P*
Site	**10.61**	**0.001**	**4.83**	**0.03**	**0.004**
Removal treatment	4.92	0.085	1.87	0.02	0.119
Site × removal treatment	**6.14**	**0.046**	1.87	0.02	0.101
Total fluorescence	3.65	0.056	0.40	0.00	0.689
Site 1: Upland					
Removal treatment	**8.71**	**0.013**	**2.79**	**0.06**	**0.027**
Total fluorescence	**4.69**	**0.030**	1.05	0.01	0.378
Control versus *early*	**2.59**	**0.011**	1.51	0.03	0.243
Control versus *late*	0.40	0.691	1.45	0.03	0.273
Early versus *late*	**−2.31**	**0.023**	**6.05**	**0.10**	**0.006**
Site 2: Lowland					
Removal treatment	2.68	0.262	0.31	0.00	0.746
Total fluorescence	0.62	0.431	1.05	0.03	0.410
Control versus *early*	−0.80	0.426	1.87	0.04	0.162
Control versus *late*	−1.56	0.124	0.65	0.01	0.586
Early versus *late*	−0.83	0.408	0.60	0.01	0.570

A. Results of a generalized linear model with a negative binomial error distribution. *LR* = likelihood ratio

B. Results of a non-parametric MANOVA of AMF community composition

For analyses within a site, non-orthogonal contrasts were performed between each treatment comparisons. Bold values are significant at $P < 0.05$. For non-orthogonal contrasts, all bold values remain significant following a sequential Bonferroni correction

in a group, and reflects a lower consistency of taxa composition in the control plots in the upland site than in the early or late removal samples.

By comparing the frequency of occurrence of each VT in samples from each site and removal treatment, it is apparent that the higher richness of the early removal treatment in the upland site is not due to one or a few taxa occurring uniquely in those samples (Fig. 3). Rather, the majority of VTs detected in the upland site were found in early removal plots at a higher frequency than in control or late removal plots (10 of 12, Fig. 3). Consistent with the lack of statistical differences in richness or composition among treatments in the lowland site, there was no clear pattern in the frequency of occurrence among the 15 VTs detected in that site (Fig. 3).

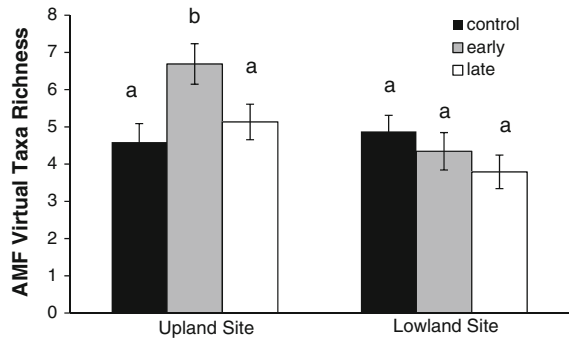

Fig. 1 Mean AMF richness (# of virtual taxa detected per plot) for the control (*black*), early removal (*gray*), and late removal (*white*) treatments, separately for the upland and lowland sites. *Error bars* are standard errors. *Bars* that share the *same letter* within a site are not significantly different, according to non-orthogonal contrasts

Question 2: After 6 years of *A. petiolata* removal, do mycorrhizal fungal communities reflect the initial density of *A. petiolata* in these plots?

When considering all plots, the pre-treatment density of *A. petiolata* in 2004 did not have a significant correlation with AMF richness in 2010 (Table 2). However, it was significantly correlated with AMF community composition, as evidenced by the npMANOVA (Table 2). Again, this effect was only evident in the upland site.

While pre-treatment *A. petiolata* cover was not a significant predictor in the overall GLM, this analysis may have been affected by the heterogeneity of variance in AMF richness across the gradient of pre-treatment *A. petiolata* density (Fig. 4). The "triangle"-shaped pattern of points in Fig. 4a suggests a threshold relationship, in which at low *A. petiolata* densities AMF richness can vary from low to high, but at high *A. petiolata* densities AMF richness tends to be low. This figure contains only those plots from *A. petiolata* removal treatments, to focus on the potential legacy effect for plots that have had no or very low *A. petiolata* densities for the last 6 years. Quantile regression confirmed that the relationship between pre-treatment *A. petiolata* cover and AMF richness varied across quantiles, with no relationship at low quantiles but an increasingly negative relationship at higher quantiles (Table 3). Again, this relationship is only evident in the upland site.

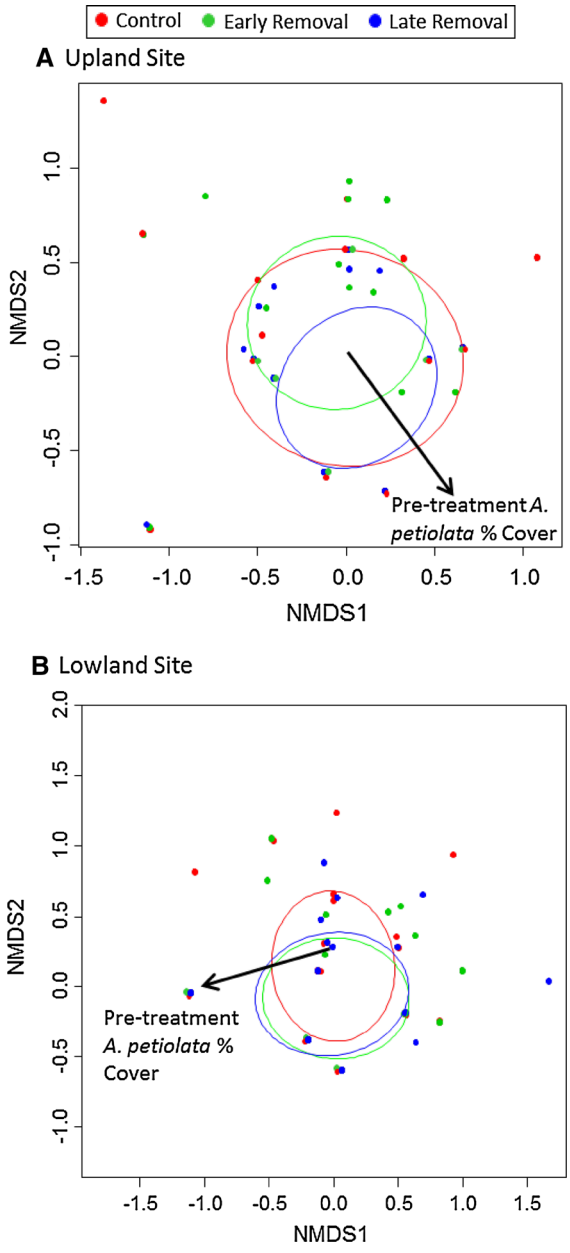

Fig. 2 Non-metric multidimensional scaling ordinations of AMF communities, separately for the upland (**a**) and lowland sites (**b**). *Ellipses* show 95 % confidence intervals around the group centroid. *Arrows* depict an environmental fit of pretreatment *A. petiolata* percent cover (significant for the upland but not lowland site). Due to identical VT composition, some samples overlap in the ordination plot

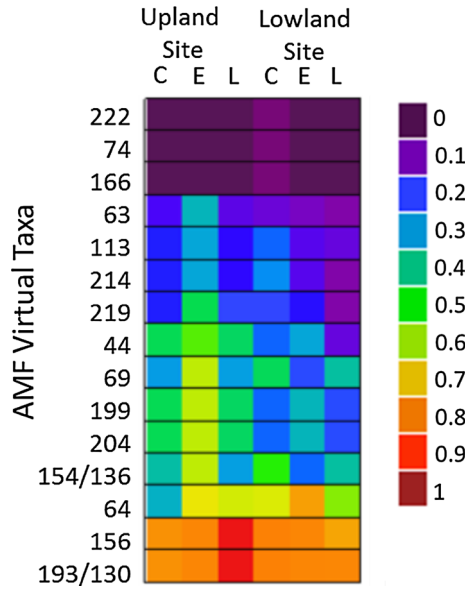

Fig. 3 Heat map of frequency of occurrence for each AMF virtual taxa in each site by treatment combination. Frequency of occurrence is calculated as the number of samples containing the taxa in each site by treatment combination, divided by the total number of samples in that site by treatment combination

Discussion

Considerable research has investigated the impacts of invasive species on native communities, and the strongly negative effects of some invaders on native diversity have prompted control efforts to reduce or eliminate invaders from select areas. However, little is known about how the native community will reassemble once a dominant invader has been removed. In particular, invasive plants that alter ecosystem functions, for instance by altering abiotic and biotic properties of soils, may create legacy effects that alter or inhibit the establishment of native communities long after the invasive population is gone. While such legacy effects have been documented in some cases (Grove et al. 2012; Kulmatiski and Beard 2011; Marchante et al. 2009), long-term experimental data are still rare. Here, we investigated the reassembly of AMF communities in soils previously invaded by the anti-mycorrhizal *A. petiolata* in a 6-year invader removal experiment. We found that in one of the two sites studied, AMF communities increased in richness and diverged in composition in the weeded plots, suggesting that mycorrhizal fungal communities can reassemble once the invader is removed. However, even after 6 years of invader removal, the original density of *A. petiolata* in a plot remained a strong predictor of AMF richness and composition, suggesting that this invader does leave a legacy of altered

Table 2 Comparison of AMF richness and composition versus pre-treatment *A. petiolata* density

Source	A. GLM of AMF richness		B. npMANOVA of AMFcomposition		
All samples	LR	*P*	Pseudo F	R^2	*P*
Site	**8.76**	**0.003**	**4.85**	**0.03**	**0.007**
Removal treatment	5.38	0.068	1.88	0.02	0.117
Site × treatment	**6.39**	**0.041**	1.53	0.02	0.202
Pre-treatment % cover A. pet	1.01	0.314	**3.07**	**0.02**	**0.045**
% cov × site	1.95	0.163	2.07	0.01	0.124
% cov × treatment	0.59	0.743	0.15	0.00	0.937
% cov × site × treatment	1.03	0.597	1.05	0.01	0.396
Total fluorescence	3.00	0.083	0.40	0.00	0.688
Site 1: Upland					
Removal Treatment	**8.19**	**0.017**	**2.87**	**0.06**	**0.018**
Pre-treatment % cover A. pet	2.38	0.124	**4.45**	**0.05**	**0.022**
% cov × treatment	1.55	0.461	0.39	0.01	0.799
Total fluorescence	3.75	0.053	1.07	0.01	0.367
Site 2: Lowland					
Removal treatment	2.82	0.245	1.03	0.03	0.415
Pre-treatment % cover A. pet	0.00	0.956	0.06	0.00	0.876
% cov × treatment	0.15	0.928	0.78	0.02	0.556
Total fluorescence	0.50	0.478	0.31	0.00	0.736

A. Results of a generalized linear model with a negative binomial error distribution. *LR* = likelihood ratio

B. Results of a non-parametric MANOVA of AMF community composition

Bold values are significant at $P < 0.05$

AMF communities for years after it has been successfully removed.

We consistently found different effects of *A. petiolata* removal between the two experimental sites. This is consistent with previous studies of this experiment, which also found a stronger effect of *A. petiolata* removal on plant communities in the upland site (Anderson et al. 2010, Herold et al. 2011). When we did find an effect of *A. petiolata* removal, it was generally much stronger in the early versus late removal treatments. This is not surprising, since the early removal treatments likely prevented *A. petiolata* soil impacts to a greater degree by removing second-year plants at the beginning of spring, before their rapid growth and flowering, while the late removal treatment weeded plants after several additional months in which the adult plants were growing rapidly and potentially producing high volumes of secondary compounds.

Interestingly, the increase in AMF richness in the early removal treatment in the upland site did not appear to come from the reestablishment of particular taxa that had been extirpated by the presence of *A. petiolata*. Rather, the majority of taxa detected at that site (10 out of 12) occurred more frequently in early removal plots compared to control or late removal ones. This can also be seen in the non-metric dimensional scaling ordination, where the AMF communities in control plots are more dispersed, but still largely overlap those in the early removal plots. It appears that *A. petiolata* invaded plots had overall less rich but also less consistent AMF communities, while the early removal plots were more consistent in their communities because each plot contained a higher proportion of the available taxa pool. One interpretation of this result is that the AMF taxa present in this site do not differ drastically in their resistance to *A. petiolata*, but that overall lower population sizes in the presence of the invader leads to stochastic loss of taxa from particular locations. Similarly, the pattern of higher richness in removal plots may reflect generally higher population sizes across all of the taxa, resulting in a higher probability of detection by our molecular methods. Unfortunately, we do not have data on the AMF community composition of this site prior to the establishment of *A. petiolata*. Therefore, we cannot determine whether the increase in richness or change in composition in early removal plots represents a

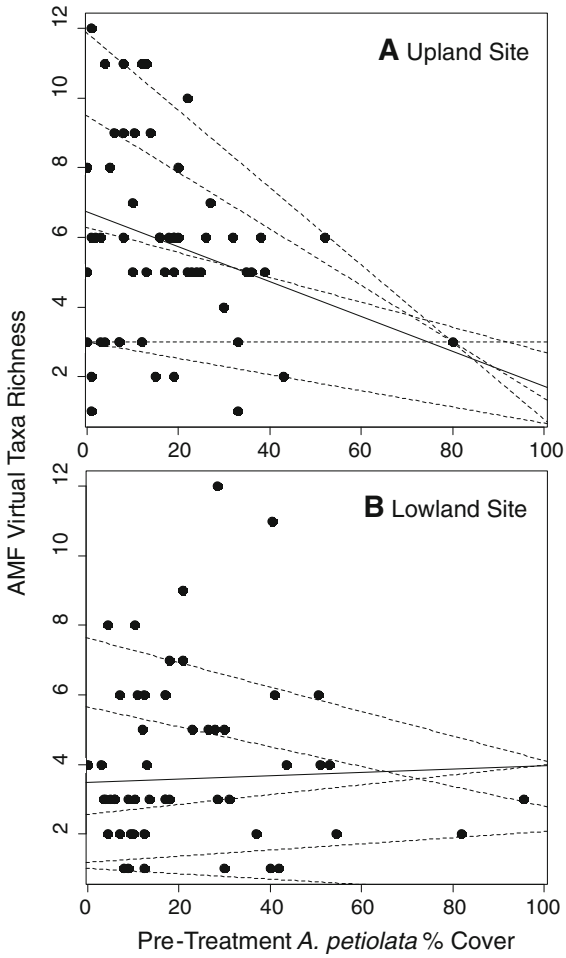

Fig. 4 Scatter plot of AMF richness versus pretreatment *A. petiolata* percent cover (measured in 2004), separately for the upland (**a**) and lowland (**b**) sites. *Trend lines* represent slopes for the five quantiles used in the quantile regression analysis. The ordinary least squares regression is represented with a *solid line*, while the quantiles (tau = 0.1, 0.25, 0.5, 0.75, 0.9) are represented with *broken lines*. Quantiles can be identified by their y-intercepts (increasing with increasing tau). Intercepts and slopes differ slightly from Table 3 due to the absence of the "total fluorescence" covariate

return to a pre-invaded state, or the development of a novel AMF community.

Removing *A. petiolata* by hand-pulling causes some soil disturbance, and such disturbances can negatively affect both AMF abundance and diversity. However, we are fairly confident that this effect was minor compared to the direct effects of *A. petiolata*, for several reasons. First, we only removed second-year garlic mustard plants, and the abundance of second year plants in our weeded plots declined dramatically over the course of the experiment, with a consequent decline in the yearly soil disturbance. Secondly, pulling second-year plants disturbs a relatively small volume of soil. The root mass is only 15.7 % of the total biomass of the plant (Anderson et al. 1996), and *A. petiolata* has a rather narrow tap root so the area of soil being disturbed by removal of second-year plants is less than the area of plant cover. Finally, and perhaps most importantly, our data show patterns opposite to what one would expect were soil disturbance the primary force in the experiment. We have shown in a previous study that there is an increase in soil MIP in removal plots compared to the control plots (Anderson et al. 2010). Here we find a higher diversity of AMF taxa in removal plots. Soil disturbance tends to reduce both AMF abundance and diversity. Thus, to the extent soil disturbance was important in this experiment, it likely made our findings conservative (i.e. if there were a way to remove *A. petiolata* root systems without disturbing the soil, we may have seen stronger increases in diversity in our removal plots).

Although we detected patterns consistent with some recovery of AMF communities in the early removal plots, we also found evidence of long-term legacy effects of *A. petiolata* on AMF community structure even after 6 years of experimental weeding. In the two removal treatments, both richness and composition correlated with the initial density of *A. petiolata* measured before the start of the experiment. For richness, this was reflected in a "triangle" shaped distribution of community richness versus initial *A. petiolata* density, in which AMF richness can vary from low to high when initial *A. petiolata* density was low, but is restricted to be low when initial *A. petiolata* density was high. Such patterns are common in ecological data when the metric of interest (AMF richness) can be limited by multiple factors, such that the effect of any one factor is only evident in the subset of samples in which other factors are not limiting (Cade and Noon 2003). There are likely many reasons why AMF richness may be low, with *A. petiolata* density being one of them. However, when initial *A. petiolata* density was high, AMF richness appeared to be restricted to staying low even after years of weeding had essentially eliminated *A. petiolata* from these plots.

The legacy pattern could be explained in multiple ways. Since the secondary compounds of *A. petiolata*

Table 3 Quantile regression parameters of AMF richness versus pretreatment *A. petiolata* cover for the *A. petiolata* removal plots for five quantiles (tau levels)

	tau				
	0.100	0.250	0.500	0.750	0.900
Site 1: Upland					
Intercept	**3.232**	**2.982**	**5.176**	**6.726**	**11.738**
Pre-treatment % cover A. pet	−0.021	−0.011	−0.023	**−0.075**	**−0.112**
Total fluorescence	0.000	0.000	0.000	0.000	0.000
Site 2: Lowland					
Intercept	**1.019**	**1.184**	**2.565**	**5.659**	**7.641**
Pre-treatment % cover A. pet	−0.008	0.009	0.014	−0.029	−0.035
Total fluorescence	0.000	0.000	0.000	0.000	0.000

Bold values are significant at $P < 0.05$ based on bootstrapped standard errors

have a short half-life in soils (hours to days), it is unlikely that the soil itself remained toxic to AMF after *A. petiolata* was weeded (Barto and Cipollini 2009). Instead, low dispersal ability of the fungi may have prevented them from reestablishing in the heavily invaded plots after weeding. Since AMF are obligate mutualists, their reestablishment would require the prior establishment of suitable host plants in these plots. If the host plants are themselves reliant on AMF for establishment, this could present a substantial barrier to community reassembly since neither partner could successfully establish without the other. Indeed, much of the increase in native cover in the weeded plots came from non-mycorrhizal plant species (Anderson et al. 2010), supporting the possibility of co-limitation of establishment for native AMF and mycorrhizal plants.

Another possibility is that *A. petiolata* preferentially invaded microsites that were already low in AMF richness for other reasons, creating a correlation between initial *A. petiolata* density and AMF richness for opposite causal reasons. Since *A. petiolata* is non-mycorrhizal, it is possible that it would be competitively favored in areas that had low AMF abundance and/or diversity for other reasons. The factors controlling AMF diversity in natural systems are not well understood, so it is not clear whether we should expect variation in AMF diversity due to fine-scale edaphic or microclimate/topography heterogeneity on the scale of this experiment (tens of meters). However, the observed increased in AMF richness following experimental weeding of *A. petiolata*, along with numerous studies in laboratory and field conditions (Stinson et al. 2006; Callaway et al. 2008; Cantor et al. 2011), points to a direct effect of *A. petiolata* on AMF abundance and diversity.

We are only beginning to understand the role of AMF diversity in natural systems. AMF taxa are considered broadly generalized because almost any AMF taxa will form connections with almost any mycorrhizal host plant (Smith and Read 2008). However, the functional consequences of these interactions for plant growth can be widely variable (Klironomos 2003). In surveys of natural communities, plant species were found to associate with non-random subsets of the available fungal pool, suggesting a level of ecological specificity not captured in one-to-one laboratory trials (Davison et al. 2011). Additionally, increased diversity of AMF communities on individual plants may increase plant growth and fitness if different AMF taxa provide complementary benefits (Maherali and Klironomos 2007).

While more research is needed to determine the impact of reduced AMF diversity per se (compared to reduced abundance overall) on native plant communities, our current understanding suggests that maintaining AMF diversity at naturally high levels may be a valuable management objective. Additionally, understanding the potential co-limitation of native plant and mycorrhizal fungal recovery in post-invasion landscapes could provide insight in restoration failure and suggest alternative restoration practices (e.g. soil restoration) to overcome this issue and help prevent reinvasion. For instance, previous studies in *A. petiolata* invaded forests found that oak seedlings inoculated with living, uninvaded forest soil communities established and grew better in the presence of *A. petiolata* compared to seedlings lacking this inoculum, in those populations where the invader produced high levels of allelochemicals (Lankau 2012). Other studies have found that inoculation with soil communities from reference sites can promote the establishment of

late successional native species in restorations (Middleton and Bever 2012). While natural recovery of soil communities following invasive removal may occur eventually, restoration success may be improved by direct interventions to accelerate this process.

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References

Anderson RC, Dhillion SS, Kelley TM (1996). Aspects of the ecology of an invasive plant, garlic mustard (Alliaria petiolata), in central Illinois. Restor Ecol 4:181–191

Anderson RC, Anderson MR, Bauer JT, Slater M, Herold J, Baumhardt P, Borowicz V (2010) Effect of removal of garlic mustard (*Alliaria petiolata*, Brassicaceae) on arbuscular mycorrhizal fungi inoculum potential in forest soils. Open Ecol J 3:41–47

Anderson RC, Anderson MR, Bauer JT et al (2012) Garlic mustard's (*Alliaria petiolata*)effectiveness as an invader of Eastern North American deciduous forest groundlayers. Abstracts, ESA 97th meeting, Portland Oregon. http://esameetings.allenpress.com/2012/webprogram/Paper38382.html

Barto EK, Cipollini D (2009) Half-lives and field soil concentrations of *Alliaria petiolata* secondary metabolites. Chemosphere 76(1):71–75

Barto EK, Antunes PM, Stinson K, Koch AM, Klironomos JN, Cipollini D (2011) Differences in arbuscular mycorrhizal fungal communities associated with sugar maple seedlings in and outside of invaded garlic mustard forest patches. Biol Invasions 13(12):2755–2762. doi:10.1007/s10530-011-9945-6

Batten KM, Scow KM, Espeland EK (2008) Soil microbial community associated with an invasive grass differentially impacts native plant performance. Microb Ecol 55(2):220–228. doi:10.1007/s00248-007-9269-3

Bauer JT, Anderson RC, Anderson MR (2010) Competitive interactions among first-year and second-year plants of the invasive, biennial garlic mustard (*Alliaria petiolata*) and native ground layer vegetation. Restor Ecol 18(5):720–728

Cade BS, Noon BR (2003) A gentle introduction to quantile regression for ecologists. Front Ecol Environ 1(8):412–420. doi:10.2307/3868138

Callaway RM, Ridenour WM (2004) Novel weapons: a biochemically based hypothesis for invasive success and the evolution of increased competitive ability. Front Ecol Environ 2:436–443

Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. Nature 427(6976):731–733. doi:10.1038/nature02322

Callaway RM, Cipollini D, Barto K, Thelen GC, Hallett SG, Prati D, Stinson K, Klironomos J (2008) Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology 89(4):1043–1055

Cantor A, Hale A, Aaron J, Traw MB, Kalisz S (2011) Low allelochemical concentrations detected in garlic mustard-invaded forest soils inhibit fungal growth and AMF spore germination. Biol Invasions 13(12):3015–3025. doi:10.1007/s10530-011-9986-x

Catford JA, Jansson R, Nilsson C (2009) Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Divers Distrib 15:22–40

Davison J, Opik M, Daniell TJ, Moora M, Zobel M (2011) Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. FEMS Microbiol Ecol 78(1):103–115. doi:10.1111/j.1574-6941.2011.01103.x

Dickie IA, FitzJohn RG (2007) Using terminal restriction fragment length polymorphism (T-RFLP) to identify mycorrhizal fungi: a methods review. Mycorrhiza 17(4):259–270. doi:10.1007/s00572-007-0129-2

Fitzjohn RG, Dickie IA (2007) TRAMPR: an R package for analysis and matching of terminal-restriction fragment length polymorphism (TRFLP) profiles. Mol Ecol Notes 7(4):583–587. doi:10.1111/j.1471-8286.2007.01744.x

Grove S, Haubensak KA, Parker IM (2012) Direct and indirect effects of allelopathy in the soil legacy of an exotic plant invasion. Plant Ecol 213(12):1869–1882. doi:10.1007/s11258-012-0079-4

Hallett SG (2006) Dislocation from coevolved relationships: a unifying theory for plant invasion and naturalization? Weed Sci 54(2):282–290

Herold J, Anderson MR, Bauer JT, Borowicz V, Anderson RC (2011) Comparison of the effect of early and late removal of second-year garlic mustard (*Alliaria petiolata*) on first-year plants and deciduous forest spring and summer dominant herbaceous groundlayer species in central Illinois, USA. Ecol Restor 29:225–233

Kardol P, Cornips NJ, van Kempen MML, Bakx-Schotman JMT, van der Putten WH (2007) Microbe-mediated plant–soil feedback causes historical contingency effects in plant community assembly. Ecol Monogr 77(2):147–162

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417(6884):67–70

Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84(9):2292–2301

Kulmatiski A, Beard KH (2011) Long-term plant growth legacies overwhelm short-term plant growth effects on soil microbial community structure. Soil Biol Biochem 43(4):823–830. doi:10.1016/j.soilbio.2010.12.018

Kulmatiski A, Beard KH, Stark JM (2006) Soil history as a primary control on plant invasion in abandoned agricultural fields. J Appl Ecol 43(5):868–876. doi:10.1111/j.1365-2664.2006.01192.x

Lankau RA (2012) Interpopulation variation in allelopathic traits informs restoration of invaded landscapes. Evol Appl 5(3):270–282

Lankau RA, Nodurft RN (2013) An exotic invader drives the evolution of plant traits that determine mycorrhizal fungal diversity in a native competitor. Mol Ecol 22(21):5472–5485. doi:10.1111/mec.12484

Lee J, Lee S, Young JPW (2008) Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 65(2):339–349. doi:10.1111/j.1574-6941.2008.00531.x

Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316(5832):1746–1748. doi:10.1126/science.1143082

Marchante E, Kjoller A, Struwe S, Freitas H (2009) Soil recovery after removal of the N-2-fixing invasive *Acacia longifolia*: consequences for ecosystem restoration. Biol Invasions 11(4):813–823. doi:10.1007/s10530-008-9295-1

Middleton EL, Bever JD (2012) Inoculation with a native soil community advances succession in a grassland restoration. Restor Ecol 20(2):218–226. doi:10.1111/j.1526-100X.2010.00752.x

Oksanen J, Kindt R, O'HAre RB (2005) vegan:Community Ecology Package version 1.6-10

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier U, Zobel M (2010) The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol 188(1):223–241. doi:10.1111/j.1469-8137.2010.03334.x

Pringle A, Bever JD, Gardes M, Parrent JL, Rillig MC, Klironomos JN (2009) Mycorrhizal symbioses and plant invasions. Annu Rev Ecol Evol Syst 40:699–715. doi:10.1146/annurev.ecolsys.39.110707.173454

Roberts KJ, Anderson RC (2001) Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. Am Midl Nat 146(1):146–152

Rodgers VL, Stinson KA, Finzi AC (2008) Ready or not, garlic mustard is moving in: *Alliaria petiolata* as a member of eastern North American forests. Bioscience 58(5):426–436. doi:10.1641/b580510

Simberloff D (2009) We can eliminate invasions or live with them. Successful management projects. Biol Invasions 11(1):149–157. doi:10.1007/s10530-008-9317-z

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic Press, London

Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM, Thelen GC, Hallett SG, Prati D, Klironomos JN (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biol 4(5):727–731. doi:e140

van der Putten WH, Klironomos JN, Wardle DA (2007) Microbial ecology of biological invasions. ISME J 1(1):28–37. doi:10.1038/ismej.2007.9

van Grunsven RHA, van der Putten WH, Bezemer TM, Berendse F, Veenendaal EM (2010) Plant–soil interactions in the expansion and native range of a poleward shifting plant species. Glob Chang Biol 16(1):380–385. doi:10.1111/j.1365-2486.2009.01996.x

Wolfe BE, Klironomos JN (2005) Breaking new ground: soil communities and exotic plant invasion. Bioscience 55(6):477–487