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INVASIVE PLANT (Alliaria petiolata; garlic mustard) HOMOGENIZES FUNGAL COMMUNTIY COMPOSITION AND INCREASES FUNGAL RICHNESS

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ABSTRACT

INVASIVE PLANT (*Alliaria petiolata;* garlic mustard) HOMOGENIZES FUNGAL COMMUNTIY COMPOSITION AND INCREASES FUNGAL RICHNESS

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Non-native invasive plants can disrupt native plant communities and soil function (e.g., C and N cycling), but few studies have examined how soil microbial community structure differs in association with invasion. This work focused on *Alliaria petiolata* (garlic mustard), a non-mycorrhizal Brassicaceae that can displace native plants and reduce aboveground diversity. Garlic mustard produces toxic phytochemicals that can suppress mycorrhizal fungi, but we currently do not know if garlic mustard invasion affects the general fungal community, including specific mycorrhizal fungi, saprotrophic fungi, and plant pathogens and parasites. The objective of this work was to compare uninvaded and invaded soils from deciduous forest understories in the Northeastern U.S. in terms of fungal community structure, edaphic soil properties, and the correlation between these variables and garlic mustard abundances in the invaded plots. We show that garlic mustard invasion was associated with dramatic differences in fungal diversity, with a particular increase in saprotrophic fungal diversity. Saprotrophic diversity was positively correlated with the relative abundance of garlic mustard in invaded plots. In terms of edaphic soil properties, invaded soils also possessed reduced C:N ratio relative to uninvaded soils due to lower organic C concentrations in invaded soils. C:N ratio was negatively correlated with the fungal community through direct changes in saprotrophic fungal relative abundance and the ratio of saprotrophic fungi to ectomycorrhizal fungi. Invasion was also associated with higher relative

abundance and diversity of plant pathogens and parasites, including the occurrence of novel pathogens, such as *Olpidium brassicae*, a fungus that transmits necroviruses infectious to herbaceous plants. In summary, invasion was associated with fundamentally different soil fungal communities and this was correlated with altered edaphic soil properties and the abundance of garlic mustard across the invaded landscape.

Introduction

Diverse evolutionary histories and functional roles of soil microorganisms can shape aboveground plant dominance through plant-microbe feedbacks (Bever, 1997), however relatively little is known about relationships between soil microbial communities and non-native invasive plants (van der Putten *et al.* 2007, Ehrenfeld, 2004, 2010). In general, invasive plants can reduce native plant diversity and alter soil carbon (C) and nitrogen (N) cycling, and a significant portion of these impacts are thought to be mediated by soil microbes (Inderjit, 2015). In particular, it is critical to understand how soil fungi respond to invasion as they have been previously found to promote invasive plant growth (Klironomos, 2002) and establishment (Reinhart *et al.* 2003), and are critically involved in decomposition and nutrient cycling (Talbot *et al.* 2015). While the entire fungal community may solicit a meta-response to invasion, discrete functional groups may be differentially impacted by invasion. Functional groups may respond to specific plant traits, including interactions between litter quality and quantity and saprotrophic fungi, the mycorrhizal affinity of an invasive and mycorrhizal fungi (Lekberg *et al.* 2013), and the lack of evolutionary antecedent between an invasive plant and native pathogens and parasites (Flory and Clay, 2013). Disentangling which fungi become more or less abundant and diverse in invaded soils relative to uninvaded soils is an important step towards realizing how fungal communities shape aboveground plant communities.

Our work focused on an invasive Brassicaceae, *Alliaria petiolata* (garlic mustard) (Nuzzo, 1993), which was originally introduced into the U.S. and Canada from Europe in the late 1800s (Durka *et al.* 2005). Previous work has already shown that garlic mustard effects soil fungal communities because it is non-mycorrhizal and allelopathic, producing a suite of toxic phytochemicals regarded for their ability to suppress mycorrhizal fungi (Stinson *et al.* 2006,

Cantor *et al.* 2011). Dismantled mycorrhizae due to garlic mustard invasion have been linked to the inhibition of highly mycorrhizal dependent plants and lower native plant diversity (Stinson *et al.* 2007) in deciduous forest understories. Garlic mustard is able to suppress the two dominant mycorrhizal fungal types in deciduous forests, AMF and ectomycorrhizal fungi (EcM fungi) (Barto *et al.* 2011, Lankau and Norduft, 2013, Castellano and Gorchov, 2011), but the effects of invasion on specific mycorrhizal fungal taxa in soils remain largely unknown. Our study is the first to compare the general fungal community structure across uninvaded and invaded soils using fungal metabarcoding. This sequencing resolution can yield high resolution diversity estimates and annotation of fungi by taxonomy and functional strategy could reveal the comprehensive effects of invasion on soil fungi.

The specific objectives of this study were to compare fungal structure between uninvaded and invaded soils and differences across taxa and functional groups (e.g., AM, EcM, saprotrophic, and pathogenic). In addition to fungal community structure, we also quantified fungal biomass and compared edaphic soil properties between uninvaded and invaded soils. This work was conducted at six deciduous forests in a region of the Northeastern U.S., spanning a gradient of garlic mustard invasion severity (Table 1). We compared uninvaded and invaded plots in terms of edaphic (texture, pH, organic C content, total N, amino acid abundances, and inorganic N availability) and microbial characteristics (microbial biomass, microbial community composition, and fungal community structure). Since plant-soil feedbacks are both edaphic and microbial (Ke *et al.* 2015), which may be more parsimonious than we currently realize, a structural equation model was constructed to describe relationships between the fungal community and the soil properties.

Materials and methods

I. Sites and study design

This work was conducted at six temperate, deciduous forest sites in New York and Massachusetts, U.S. (Figure 1). The overstory at all sites is of mixed composition, with dominant canopy trees being maple (*Acer saccharum, A. rubrum),* oak *(Quercus rubra*), ash (*Fraxinus Americana*), and white pine (*Pinus strobus*), while Canadian mayflower (*Mianthemum canadense*) and jack-in-the-pulpit (*Arisaema triphyllum*) are the dominant understory plants. Soil type and texture varies across sites, as well as garlic mustard abundance in the invaded forest patches (Table 1). We established three replicate 3 m^{-2} plots in adjacent uninvaded and invaded forest patches at each site. Each invaded plot was paired with an uninvaded reference plot based on similar understory vegetation composition, earthworms presence, slope, and aspect. All invaded plots contained a minimum of 20 garlic mustard plants m^{-2} and were separated by at least 10 m.

II. Sample collection

Soil sampling at all sites was performed in the first two weeks of June, 2013. We collected three soil cores from each plot using a tulip bulb corer (5 cm wide x 10 cm deep). Each core were separated into the organic horizon (~3-5 cm depending on site) and mineral soil (top ~5-7 cm), and replicate samples from each plot were pooled by depth and manually homogenized. There were a total of 72 samples (6 sites x 2 invasion status x 3 replicates x 2 depths). A subsample (~2 g) from each plot and depth increment was flash frozen in liquid N immediately in the field and stored at -80˚C for subsequent molecular analysis. The remaining soil was kept on blue ice until being stored at 4˚C in the lab within 12 hours of sampling. Samples for edaphic characterization and nutrient analysis were processed and analyzed within 48 hours. The organic horizon samples were not sieved, but all visible roots, rocks and coarse woody debris were manually removed.

Mineral soil was passed through a 4 mm sieve, and roots, rocks and organic debris >4 mm were removed.

III. Soil analyses

Soil samples were analyzed for pH, total organic C and N, microbial biomass, and, in the mineral soil, inorganic N and amino acid concentrations. Soil pH was measured in distilled water (1:10 wt/vol). Total soil organic C and N were analyzed on air dried, finely ground samples using dry combustion in a Perkin Elmer 2400 Series II CHN elemental analyzer (Waltham, MA). Total inorganic N (NO_3^- and NH_4^+) was extracted from mineral soil using 2M KCl (10 g/ 40 mL) and analyzed using a vanadium (III) reduction for NO_3^- and a modified Berthelot reaction for NH_4^+ (Braman and Hendrix, 1989). Amino acid concentrations were quantified in mineral soil using 0.5M sodium acetate soil extracts and the fluorometric *o*-phthaldialdehyde and β-mercaptoethanol (OPAME) method with a leucine standard curve (Jones *et al.* 2002).

Microbial biomass was estimated using phospholipid fatty acid (PLFA) analysis on samples that were flash frozen in liquid N within 24 hours of sampling and subsequently freeze-dried (Freezone 6, Labconco, Kansas City, MO). Soil lipids were extracted from homogenized, root-free, freeze-dried soil (1 g) using phosphate buffer, chloroform, and methanol (0.8:1:2; v: v). The polar lipids were isolated and purified using silicic acid chromatography and collected using a methanol wash. Lipids were then methylated by adding 0.2M methanolic potassium hydroxide (1 mL) and incubating the reaction at 60°C for 30 min to form fatty acid methyl esters (FAMES). The FAMES were dried down under inert N_2 gas and reconstituted in hexane for quantification on a Varian CP-3800 gas chromatograph equipped with a flame ionization detector (front FID). We compared FAME peaks against a standard library of FAMES specific to bacteria (i15:0, a15:0, c15:0, i16:0, 16:1ω7t, 16:1ω7c, i17:0, a17:0, 18:1ω7c and cy19), actinomycetes (10Me16:0), fungi

(18:2ω6, 9c, 18:1ω9c) and AMF (16:1ω5c) (Matreya, LLC, Pleasant Gap, PA). A standard control biomarker (c19:0) was used to convert peak area concentrations into nmol PLFA g^{-1} dry soil.

III. Fungal diversity and community composition

DNA was extracted from organic and mineral soil (0.25 g) using the PowerSoil® DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA). The ITS2 region was amplified using the fungal specific primer pair fITS9 and ITS4 (fITS9: Ihrmark *et al.* 2012, ITS4: White *et al.* 1990). PCR primers contained the Illumina adaptor sequence, an 8 bp pad sequence, a 2 bp linker sequence, and one of 36 unique 8 bp index sequences (see custom PCR primer constructs, Supplementary Table 1). PCR reactions were performed in triplicate for each sample in 25 µL reactions with the following reagents: PCR Grade H_2O (13 µL), Five Prime Hot Master Mix (10 µL; 5 PRIME, Inc., Gaithersburg, MD), 10 µM fITS9 (0.5 µL), 10 µM ITS4 (0.5 µL), and template DNA (1 µL). Thermocycler conditions followed that of Caporaso *et al.* (2010). PCR products were cleaned using the AxyPrep MAG PCR Clean-up kit (Corning, Tewksbury, MA). Final PCR products were inspected on an agarose gel and DNA concentration was measured by fluorometry on a Qubit® 3.0 Fluorometer (Life Technologies, Grand Island, NY). Equimolar libraries of the 72 samples (36 organic horizon and 36 mineral soil) were split by soil depth on separate Illumina MiSeq runs (2 x 250 bp chemistry) at the Center for Genomics and Bioinformatics at Indiana State University, Bloomington, IN.

IV. Sequence processing and bioinformatics

Illumina MiSeq sequencing resulted in 11,920,894 and 18,039,010 sequences for the organic horizion and mineral soil runs, respectively. Sequences were quality checked and demultiplexed by removing Illumina adapters, sequences < 100 bp, and bases with Phred scores < 2 using Trimmomatic (Bolger *et al.* 2014). The remaining forward and reverse reads were then

merged using fastq-join (Aronesty, 2013) at a 50 bp overlap and allowing 5% mismatch. After these steps, we retained 3,757,458 and 7,340,112 paired-end reads for organic and mineral samples, respectively. Chimeric sequences were removed and the ITS2 region was extracted using ITSx (Bengtsson-Palme *et al.* 2013). After ITS2 extraction, we retained 3,665,773 (organic horizon) and 5,870,260 (mineral soil) ITS2 sequences. Quality filtering and ITS2 excision resulted in retention of 65% (organic) and 62% (mineral) of the initial paired end sequences.

The USEARCH (v8) pipeline was used to create operational taxonomic unit (OTU) tables (Edgar, 2010). We removed singletons and chimeric sequences not detected by ITSx and clustered OTUs at 97% sequence similarity using the cluster_otus algorithm. Taxonomy was assigned using the UCLUST consensus taxonomy assigner in QIIME. Sequences that were not assigned a taxonomy at the phylum level were parsed from the OTU table and subjected to blastn inquiry against the NCBI nucleotide database. We then used MEGAN (v5) (Huson *et al.* 2011) to assign sequences a taxonomy from the blastn output and removed all non-fungal sequences (< 0.05%). Lastly, we assigned functional annotation to genera as saprotrophic, plant pathogenic, and parasitic using curation from Tedersoo *et al*. (2014), as EcM fungi using the UNITE EcM database (Kõljalg *et al,* 2005), and as AMF if part of the Glomeromycetes.

IV. Statistical Analyses

All statistical analyses were conducted in R 3.0.2 (R Development Core Team, 2008). Significance across all tests was set at $P \leq 0.05$. Statistical analyses were run after rarifying the OTU table to the lowest sequencing depth of 40,311 sequences per sampling unit using the rarefy function. We calculated the Shannon index, Simpson's Index, species richness, and performed rarefaction using the diversity, simp, specnumber, and rarefy functions within the vegan package (Oksanen *et al.* 2015). Multivariate analyses of microbial community composition were

characterized using resemblance based permutation methods including permutation ANOVA (PERMANOVA; Anderson, 2005) and heterogeneity of multivariate dispersion (PERMDISP; Anderson and Walsh, 2013) using the functions adonis and betadisper within the vegan package, respectively. All distance based analyses were performed on the Bray-Curtis dissimilarity matrices calculated from the relative abundance of OTUs. Significance of permutation methods was determined after 1,000 permutations. We used non-metric multidimensional scaling (NMDS) to visually display fungal community composition using the metaMDS function (vegan).

We used linear mixed effects models to assess soil and microbial parameters associated with invasion, site, and invasion x site interactions using the lme function within the nlme package (Pinheiro *et al.* 2007). Consistent with Contosta *et al*. (2011), we created beyond optimal models that parameterized for autocorrelation and unequal variance across predictor variables. We used partial least squares regression (PLSR) to resolve the covariables related to fungal community diversity and trophic group abundances using the plsr function within the pls package. We fit models to the kernel algorithm and used leave-one out cross-validation. Predictor variables included the abundance of garlic mustard (# plants m^{-2} and relative abundance of garlic mustard) and all soil measurements (Table 2). We refined models to the most important predictor variables based on the variable importance for the projection statistic (VIP), which is the weighted sum of squares of the PLS-weight (< 0.8 is considered significant; Wold *et al.* 2001).

We paired our PLSR output with structural equation modeling (SEM) to build models to test specific pathways linking the fungal community and edaphic soil properties (Grace, 2006). This analysis was performed after all other statistical analyses and specifically examined covariance among multiple variables and the pathways connecting these variables (Colman and Schimel, 2013). Our model focused on the relationship between fungal community structure

(diversity and composition), soil pH, and soil C:N ratio. This focus was due to significant differences in soil pH and C:N ratio between uninvaded and invaded soils and clear univariate correlations between these edaphic properties and the soil fungal community (see results *I. Soil characteristics*). We first created a conceptual model of how the fungal community and soil edaphic properties related to one another and soil C:N ratio. This was accomplished using the most significant predictor variables of C:N ratio from the PLSR output. The model included fungal richness, the relative abundance of saprotrophic fungi, and the ratio of saprotrophic fungi to EcM fungi as a composite variable, soil pH, and soil C:N ratio. All variables were log-transformed and the model was tested using the sem function. Since we had *a priori* knowledge of the significant correlations between each predictor variable and soil C:N ratio, no significant path was left out of the model, an important component of SEM (Grace, 2006). Our metric for model fit was based on the *P*-value and R^2. We inspected all parametric models based on QQnorm plots and Shapiro-Wilk tests of normality on model residuals.

Results

I. Soil characteristics

Invaded plots contained varying densities of garlic mustard, ranging from 44-238 plants m^{-2} across the sites (Table 1). Soil pH was elevated in association with invasion (Table 2) and was negatively correlated with soil C:N ratio ($R^2 = 0.21$, $P = 0.03$). The soil C:N ratio was also reduced by 14% (organic horizon) and 13% (mineral soil) in association with invasion, which was due to reduced organic C as opposed to higher total N. Total inorganic N (ammonium + nitrate), ammonium, and amino acid concentrations were not affected by invasion, but nitrate concentrations specifically were 147% higher in invaded compared to uninvaded soil. On average, there was no difference in microbial biomass between uninvaded and invaded soils (Table 2), but there was considerable variation in the biomass of different microbial groups between uninvaded and invaded soils across the sites, resulting in a significant site x invasion interaction (Supplementary Figure 1). Lastly, there was a small, but significant effect of invasion on the PLFA community composition in the organic horizon (PERMANOVA: $F = 3.15$, $P = 0.05$), but not the mineral soil ($F = 2.08$, $P = 0.11$).

II. Fungal diversity and community composition

Fungal communities in invaded soils were compositionally distinct from uninvaded communities (Figure 2; PERMANOVA: organic horizon: $F = 1.89$, $P = 0.002$; mineral soil: $F = 0.63$, $P = 0.001$), due primarily to reduced spatial variation in invaded relative to uninvaded plots (β-diversity; Supplementary Figure 2; PERMDISP: organic horizon: $F = 12.98$, $P = 0.001$; mineral soil: $F = 19.18$, $P = 0.0001$). On average, invaded soils contained 39% (organic horizon) and 75% (mineral soil) more fungal OTUs, fewer dominant fungal taxa, and greater fungal evenness than

uninvaded soils, as observed by higher richness estimates and Simpson's and Shannon's indices of diversity, respectively (Table 2). Invasion was also associated with shifts in community composition of EcM fungi, pathogens, and saprotrophs (Supplementary Table 2), with the largest effect of invasion on the pathogenic (PERMANOVA: organic horizon: $F = 3.28$, $P < 0.001$; mineral soil: $F = 4.33$, $P < 0.001$) and saprotrophic fungal communities (organic horizon: $F = 2.53$, $P < 0.001$; mineral soil: $F = 2.31$, $P < 0.001$).

The Basidiomycota were the most prevalent group in both soil horizons (organic: 40%, mineral: 55%), but the proportion of Ascomycetes was comparable to the Basidiomycetes in the invaded organic horizon. At the phylum level, there was a reduced relative abundance of Basidiomycetes and greater relative abundance of Ascomycetes, Mucoromycotina, and Chytridiomycetes in association with invasion (Figure 2). Because the ITS region is not highly informative for Glomeromycetes (AMF) (Krüger *et al.* 2012), the AMF represented less than 1% of the total sequences and at the phylum level were not affected by invasion. At the class level, the Agaricomycetes dominated the total proportion of sequences in all plots, but this group was comprised of significantly fewer Russulales and Polyporales in association with invasion (Supplementary Tables 4 & 5). Russulales were the most abundant EcM fungi (17%, organic horizon; 14%, mineral soil) and were less common in both soil horizons in association with invasion (Supplementary Table 6, 7). In contrast, there were significantly greater relative abundances of Mortierellomycetes and Sordariomycetes in invaded soil compared to uninvaded soil (Supplementary Table 3). While invasion was associated with higher relative abundance of saprotrophic Mortierellomycetes, the Sordariomycetes contained greater relative abundance of plant pathogens in the organic horizon (Supplementary Table 8) and saprotrophs in the mineral soil (Supplementary Table 9).

In uninvaded organic horizon and mineral soil, EcM and saprotrophic fungi each comprised 20-30% of the total sequences, while the invaded soils contained reduced relative abundance of EcM fungi and greater relative abundance of saprotrophic fungi (Figure 4). Invasion was associated with a more diverse assemblage of saprotrophic fungi across all sites, with average richness estimates increasing from 164 (organic horizon) and 125 (mineral soil) OTUs in uninvaded soil to 216 and 194 OTUs in invaded soil, respectively (Table 2). EcM fungal richness was less influenced by invasion; however, it did significantly increase in invaded mineral soil compared to uninvaded soil. Additionally, all invaded soils contained greater relative abundances and more diverse communities of pathogenic and parasitic (hereafter pathogen) fungi compared to the uninvaded soils (Table 2). Around 50% of the sequences were assigned an unknown functional strategy either because sequences could not be assigned a genus level identification, which was used to annotate sequences with a functional strategy, or because the fungal genus was not present in the reference database (Figure 4). Within the unassigned sequences, there was no difference in relative abundance between invasion statuses.

III. Relationship between garlic mustard abundance, soil parameters, and the fungal community

Garlic mustard abundance was positively correlated with soil nitrate concentration (R^2 = 0.51, P = 0.03) and mineral soil silt content (R^2 = 0.31, P = 0.008), but not other soil variables (Supplementary Figure 3). In turn, fungal richness was significantly correlated with the relative abundance of garlic mustard, though the degree of correlation varied across fungal functional groups and soil horizons (Supplementary Figure 4). Of the three functional groups (saprotrophs, EcM fungi, pathogens), organic horizon saprotrophic fungal richness was distinguished as being most strongly and positively correlated with the relative abundance of garlic mustard (Figure 5).

There was no relationship between garlic mustard abundance and EcM fungal richness, but there was a weak positive correlation with pathogen richness in the mineral soil ($R^2 = 0.21$, $P < 0.05$).

Soil C:N ratio was significantly correlated with the ratio of saprotrophic to EcM fungi ($R^2 = 0.33$, $P < 0.0001$), the relative abundance of saprotrophic fungi ($R^2 = 0.31$, $P < 0.0001$), fungal richness ($R^2 = 0.16$, $P = 0.003$), and soil pH ($R^2 = 0.34$, $P < 0.001$), and the fungal variables were all correlated to varying degrees with soil pH. We focused on soil C:N ratio because it was reduced in association with invasion (Table 1) and significantly correlated with univariate fungal community metrics and soil pH, whereas the other soil variables were not. These relationships were modelled using structural equation modeling (SEM), resulting in both direct and indirect effects of the fungal community on the soil C:N ratio (Figure 6). Thick green arrows connecting the three fungal variables indicate that they are all positively correlated and that fungal richness is most strongly correlated to soil pH while the relative abundance of saprobes and the saprobe to EMF ratio were most indicative of the fungal community composite variable. The fungal community composite variable was directly negatively correlated with the C:N ratio and fungal richness was indirectly correlated with C:N ratio through the positive correlation it had with soil pH and the negative effect of soil pH on C:N ratio. In total, the final SEM model described 43% of the variation in C:N ratio ($P = 0.05$).

Discussion

Previous studies have documented shifts in AMF community composition and diversity in association with garlic mustard invasion (Lankau, 2011, Barto *et al.* 2011, Lankau and Norduft, 2013), however our study is the first to report a difference in the general fungal community structure between uninvaded and invaded soils and to correlate differences in fungal community structure with garlic mustard abundances and soil properties. We also found that garlic mustard invasion was associated with higher soil nitrate concentrations, elevated soil pH, and lower soil C:N ratio, due to reduced organic C content as opposed to higher total N (Table 1). While garlic mustard is problematic because it can invade relatively resistant forest understories (Nuzzo, 1993, Rodgers *et al.* 2008), it is an important invasive plant to manage because it has a high competitive ability over native plants (Meekins and McCarthy, 1999) and can negatively affect aboveground plant diversity (Stinson *et al.* 2007). Shifts in belowground fungal communities associated with invasion may feedback to influence the negative effect of invasion on aboveground plant communities due to reduced relative abundance of EcM fungi, higher relative abundance of saprotrophic fungi, and the accumulation of fungal pathogens that may inhibit indigenous plants.

I. Fungal diversity and community composition and their relationship to soil properties

Our study was the first to find a shift in fungal community structure in association with garlic mustard invasion (Table 1, Figure 2) even though previous studies have tested for differences in the general fungal community using molecular finger printing techniques (e.g. T-RFLP or LH-PCR; Lankau, 2011, Rodgers *et al.* 2008b, Burke *et al.* 2011). Previous work has shown that the degree of change in AMF richness is related to the duration of invasion with garlic mustard, where long coexistence with garlic mustard may reduce the impacts of invasion on AMF diversity (Lankau, 2011, Lankau and Norduft, 2013). Since there are few herbarium or natural

history records of garlic mustard invasion at our sites, we do not know the duration of invasion at five of the six sites. We do know that the most western site (BR) has been invaded for more than 65 years (Lankau, 2011), and we found higher fungal diversity in association with invasion at this site (e.g. 1,062 OTUs in the uninvaded soil and 1,361 OTUs in the invaded soil; $P = 0.04$). Higher α-diversity was consistent cross all sites, but β-diversity or the variation in fungal community composition across all invaded plots was significantly restricted relative to the uninvaded plots (Figure 2). Although the AMF community has not been sequenced using metabarcoding in garlic mustard invaded soils, our results suggest that the general fungal community responds differently from the AMF community to garlic mustard invasion.

From taxonomic to functional levels, there were many fungal groups that shifted in terms of relative abundance between invasion statuses. There were fewer EcM fungi in association with invasion (Figure 4), and this was consistent across all sites. Previous work on EcM fungal sensitivity to garlic mustard has demonstrated that EcM fungi on roots of *Quercus rubra* (red oak) seedlings were less diverse and less abundant when grown in an invaded compared to an uninvaded forest (Castellano and Gorchov, 2011). Our work shows that reduced relative abundance of EcM fungi in soil is driven by loss of the most prevalent EcM fungal genus, *Russula* (Supplementary Table 6, & 7). This EcM genus was the most prevalent in the uninvaded soils (14-17% relative abundance) and was dramatically and significantly lower in invaded soils (<1-3%). EcM fungi can exhibit high specificity with host plants (Tedersoo *et al.* 2009). For example, *Russula* in a temperate forest in Japan exhibited strong host affinity for two dominant oak trees and did not commonly colonize neighboring pine and deciduous trees (Toju *et al.* 2013). Although it not entirely clear how the loss of specific EcM fungi effects tree fitness, it is likely that reduced EcM

fungal abundance in garlic mustard invaded soil contributes to the invasion sensitivity of EcM fungal associated tree seedlings (Meekins and McCarthy, 1999).

Saprotrophic fungal response to invasive species is likely different from most mycorrhizal fungi because the plant-fungus relationship is much less specific (Wardle *et al.* 2004). Instead, plant traits such as litter biomass and nutrient content, have been proposed to more strongly influence saprotrophic fungal communities (van der Putten *et al.* 2007). We found that garlic mustard invasion was associated with increased relative abundance of saprotrophic Ascomycetes (Supplementary Table 8) and Mucoromycotina (Figure 3), resulting in overall greater relative abundance of saprotrophic fungi (Figure 4) and altered saprotrophic fungal community composition (Supplementary Table 2). From a methodological perspective, it is possible that a loss of EcM fungi in invaded soil permitted greater sequencing detection of non-EcM fungi, including saprotrophs. This argument would be supported if fungal biomass were reduced in invaded soil relative to uninvaded soil (due to lower EcM fungal biomass); however, fungal biomass was not significantly different between uninvaded and invaded soils (Table 1), suggesting that garlic mustard invasion is associated with real changes in the saprotrophic fungal community.

Saprotrophic fungi can strongly influence nutrient cycling through their function as decomposers (Baldrian *et al.* 2011), and saprotrophic fungi may play important roles in mediating the impacts of invasion on C and N cycling (Ashton *et al.* 2005). For example, Japanese knotweed (*Fallopia japonica*) produces immense biomass that is chemically recalcitrant (Tamura and Tharyil, 2014). Japanese knotweed litter decomposes 3-4 times slower than native litter and this is correspondent with higher fungal biomass, altered fungal community composition (Mincheva *et al.* 2014), and organic carbon accumulation (Tamura and Tharyil, 2014). In contrast to Japanese knotweed, garlic mustard litter decomposes very quickly and invasion has been previously

associated with accelerated decomposition of native litter (Rodgers *et al.* 2008b), though the impacts of garlic mustard invasion on C and N cycling remain untested. That said, we found that invasion was associated with reduced soil C:N ratio due to lower organic C concentration shifts in the relative abundance of saprotrophic fungi and EcM fungi, both of which produce enzymes that decay soil organic matter (Talbot *et al.* 2015).

EcM fungi and saprotrophic fungi can compete with one another for soil nutrients, and this has recently attracted attention because this competition can affect organic C cycling (see review by Fernandez and Kennedy, 2015). EcM fungi receive a fairly constant C supply from host photosynthate translocation and in return, they decompose soil organic matter largely to obtain organically bound nutrients, particularly N and P (Smith and Read, 1997). In contrast, saprotrophs decompose soil organic matter as a C source in addition to mineral nutrients since soil C availability is often limiting to soil heterotrophs, saprotrophic fungi are sensitive to competition against EcM fungi for mineral nutrients (Bending, 2003). Here we show that garlic mustard invasion is associated with reduced EcM fungal relative abundance and increased saprotrophic fungal relative abundance, which could favor saprotrophic metabolism, thereby enhancing soil organic matter decay and reducing organic C concentrations in invaded soils.

Structural equation modeling indicated that the fungal community was both directly and indirectly correlated with C:N ratio. The fungal community had a direct negative relationship with soil C:N ratio, and this effect was driven by increased relative abundance of saprotrophic fungi and greater ratios of saprotrophic fungi to EcM fungi. There was also an indirect relationship between the fungal community on C:N ratio through a positive correlation between fungal richness and soil pH, which was elevated in association with invasion and had a direct negative relationship with C:N ratio. A paired PLSR-SEM approach suggests that soil fungi can directly (saprotrophic

fungal dynamics) and indirectly (fungal richness ~ pH) mediate the impacts of invasive plants on soil C:N ratio, and that saprotrophic fungal dominance was associated with lower organic C concentration.

Garlic mustard abundances and soil-fungus feedbacks

The abundance of garlic mustard in the invaded plots was positively correlated with edaphic and microbial soil properties. This relationship was fairly straightforward, and there were clear predictors of both the absolute and relative abundances of garlic mustard. Soil nitrate concentrations were elevated in association with invasion, and nitrate concentration was positively correlated with the absolute abundance of garlic mustard. Since nitrate is a negative compound, plants need to release a base in order to take nitrate up, which may contribute to the alkalization of invaded soils (Smiley and Cook, 1973). Garlic mustard is also a non-mycorrhizal Brassicaceae, and these plants are generally restricted to nutrient rich environments because they do not possess root adaptations for low soil fertility growth (Lambers and Teste, 2013). Whether garlic mustard invasion increased soil nutrient availability or was more successful in fertile soil patches is unclear, but our results suggest that nitrate rich soils may support more severe garlic mustard invasion.

In contrast, the relative abundance of garlic mustard was positively correlated with saprotrophic fungal richness. This was a unique correlation and there was no strong relationship between the relative abundance of garlic mustard and the entire fungal community, EcM fungi alone, and weakly with pathogenic fungal richness (Supplementary Figure 3). As saprotrophic richness was correlated with the proportion of garlic mustard relative to the entire plant community, saprotrophic fungal richness was positively correlated with garlic mustard at the expense of native plants. Of additional importance is the weak positive relationship between garlic mustard relative abundance and pathogenic fungal richness (Supplementary Figure 3).

Pathogenic fungal community

The ability of invasive plants to outcompete indigenous plants may be encouraged by the accumulation of local pathogens that can suppress native plants (Eppinga *et al.* 2006). Invasion by *Chromolaena odorata* (Siam weed) has been shown to increase the abundance of Fusarium, a general plant pathogen that can inhibit indigenous plants in the invaded range of the Western Ghats of India (Mangla *et al.* 2008). Our study is the first to suggest that garlic mustard invasion may also accumulate pathogenic and parasitic fungi, both in terms of relative abundance (Figure 4) and species richness (Table 1). Invaded soils contained higher relative abundance of Hypocreales, including Fusarium and more specialized plant pathogens, such as Cylindrocarpon and Phyllosticta (Supplementary Tables 10 & 11). Invasion was also associated with greater relative abundance of mycoparasites and animal parasites, including the Orbiliaceae, which can actually capture soil nematodes through specialized mycelium traps (Yang *et al.* 2007). Both general and specialized pathogens increased in relative abundance in association with invasion (Supplementary Table 10 & 11), but further work is required to understand how pathogen shifts associated with invasion may inhibit native plants already suffering from suppressed mycorrhizae.

Of final consideration are pathogens that were entirely unique to invaded soil, some of which may be able to infect garlic mustard. Although our study is one of the only examining pathogen accumulation in natural studies, invasive plants can become infected by pathogens with increasing invasion duration (Flory and Clay, 2013). There was novel occurrence of Erysiphales (powdery mildews) in association with invasion (Supplementary Table 10), which have been previously shown to reduce the competitive ability of garlic mustard when introduced in the greenhouse (Cipollini and Enright, 2009). There was also novel occurrence of Leptosphaeria, which causes black leg disease in Rutabega (Supplementary Table 11). Of particular interest

however were the Olpidiales, which were present in just one of the thirty six uninvaded soil samples and uniquely common in all of the invaded soils (Supplementary Table 10 & 11). There was only a single species in the Olpidiales, *Olpidium brassicae*, and it is one of the only vectors that transmits necroviruses capable of infecting herbaceous plants (Hartwright *et al.* 2010). While *O. brassicae* itself can infect a suite of Brassicaceae, the virus it carries can cause a variety of diseases in herbaceous plants (Lot *et al.* 2002). The resting spores of *O. brassicae* can also remain viable in soil for more than 20 years, making eradication extremely difficult (Campbell, 1985). Our results suggest that garlic mustard may not only promote native pathogen accumulation, but novel pathogen accumulation.

Conclusion

Although garlic mustard can reduce aboveground plant diversity, we actually found the opposite trend of garlic mustard invasion on soil fungal richness and evenness. This may be due to unique traits of garlic mustard that cultivate a different assemblage of fungal functional groups, including reduced relative abundance of EcM fungi and greater relative abundance of saprotrophic and pathogenic fungi, both of which are taxonomically more diverse than EcM fungi. Greater fungal richness was also concomitant with reduced variation in fungal community composition, suggesting omnipresent meta-community response to a novel plant. Understanding which traits of garlic mustard drive changes in fungal community structure is important, as shifts in the fungal community were also correlated with greater relative abundances of garlic mustard and differences in edaphic soil properties, including higher soil pH and reduced organic C content. Future work should look at how eradication or naturalization of garlic mustard impacts the general fungal community, as many novel fungi were observed in invaded soils that were not detected in uninvaded soils, including potentially harmful plant pathogens and even a novel genus of AMF, the Paraglomus (AMF), which were entirely absent from the uninvaded soils but were the most abundant AMF genus in the invaded soils (Supplementary Figure 5). Our results suggest that invasive plants can fundamentally transform soil fungal communities and that fungi can influence the success and impacts of garlic mustard across the invaded deciduous forest landscape.

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Table 1. Site characteristics for the six temperate forests sampled in this study. Sites are listed from west to east (refer to map in Figure 1).

Site name (ID)	Location	Soil texture[1] (soil order)	Garlic mustard abundance[2] (plants m^{-2})
Black Rock (BR)	Cornwall, NY	Sandy clay loam (Inceptisol)	87 (68)
West Point (WP)	West Point, NY	Clay loam (Inceptisol)	68 (22)
Pittsfield Forest (PF)	Pittsfield, MA	Silty clay loam (Spodosol)	131 (78)
Questing Reserve (Q)	New Marlborough, MA	Sandy clay loam (Spodosol)	87 (8)
Harvard Forest (HF)	Petersham, MA	Clay loam (Inceptisol)	44 (10)
Drumlin Farm (DF)	Lincoln, MA	Clay loam (Entisol)	238 (115)

[1]Soil textural class was assigned from the average proportion of sand, silt and clay measured in the uninvaded plots at each forest.

[2]Garlic mustard densities represent the mean of three replicate plots ± one standard error (in parentheses).

Table 2. Soil chemical properties, microbial biomass, and fungal diversity for the organic horizon and mineral soil at uninvaded and invaded plots, averaged across six northeastern forests. Values represent the mean ± one standard error (n = 18). Values within a soil horizon followed by different lowercase letters are significantly different ($P \leq 0.05$). Dashes indicate where data were not collected.

	Organic horizon		**Mineral soil**	
	Uninvaded	Invaded	Uninvaded	Invaded
Soil chemical properties				
Ammonium (μg g^{-1} soil)	-	-	17.3 (9.70)a	17.0 (9.84)a
Nitrate (μg g^{-1} soil)	-	-	1.95 (0.72)a	4.82 (1.56)b
Amino acids (μg g^{-1} soil)	-	-	65.4 (13.04)a	66.4 (13.77)a
pH	4.8 (0.3)a	5.4 (0.2)b	4.7 (0.2)a	5.2 (0.2)b
Organic C (%)	13.2 (2.8)a	8.8 (0.9)b	6.0 (1.0)a	4.72 (0.5)a
Total N (%)	0.74 (0.13)a	0.59 (0.05)a	0.36 (0.05)a	0.33 (0.03)a
Soil C:N	17.4 (1.4)a	14.9 (0.4)b	16.3 (1.2)a	14.1 (0.5)b
Microbial biomass (nmol PLFA g^{-1} soil**)**				
Bacteria	211 (38)a	200 (39)a	103 (12)a	105 (13)a
Actinomycetes	15.6 (2.4)a	11.1 (2.80)a	7.7 (1.9)a	8.43 (1.3)a
Fungi	39.5 (9.2)a	34.0 (8.5)a	17.1 (2.6)a	19.3 (3.7)a
AM fungi	19.4 (4.9)a	21.2 (5.0)a	8.1 (1.9)a	7.6 (1.9)a
F:B ratio	0.17 (0.02)a	0.15 (0.02)a	0.16 (0.01)a	0.18 (0.02)a
Fungal diversity				
Shannon Index	3.40 (0.33)a	4.21 (0.25)b	3.00 (0.28)a	3.96 (0.17)b
Simpson's Index	0.84 (0.06)a	0.93 (0.03)b	0.81 (0.06)a	0.93 (0.01)b
Richness (*S*)	888 (117)a	1,229 (102)b	570 (63)a	979 (65)b
Saprotrophic *S*	165 (14)a	216 (10)b	125 (10)a	194 (6)b
Ectomycorrhizal *S*	34 (3)a	33 (3)a	24 (2)a	33 (3)b
Pathogenic *S*	33 (3)a	51 (3)b	20 (2)a	35 (2)b

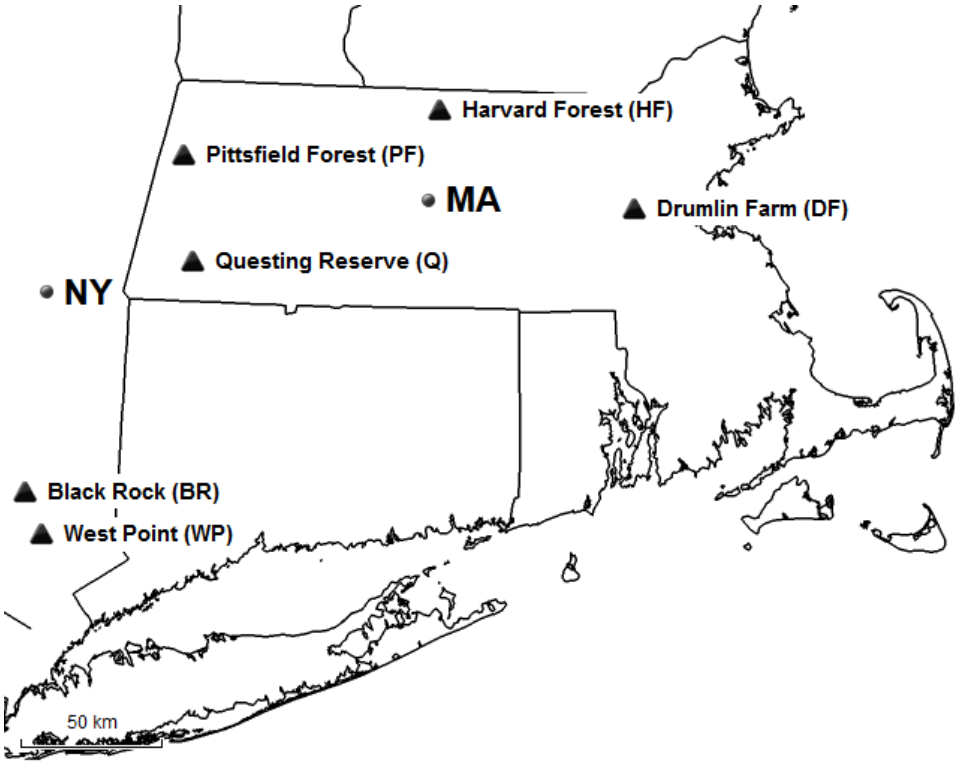


Figure 1. Map of study sites, all of which are located in New York (NY) and Massachusetts (MA), U.S. Site names and the site ID (in parentheses) are plotted beside each location.

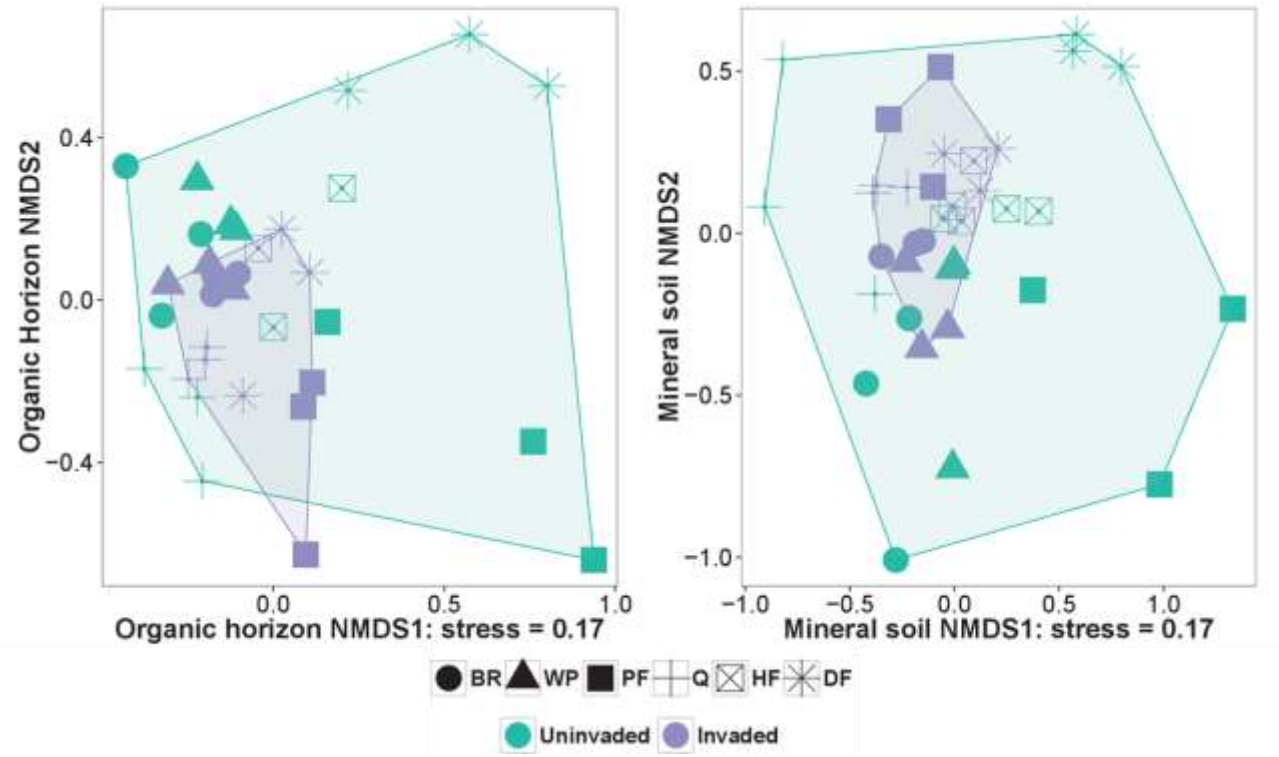


Figure 2. Non-metric multidimensional scaling (NMDS) ordination of fungal community composition in uninvaded and invaded plots at six northeastern deciduous forests. The relative abundance of fungal OTUs were converted to Bray-Curtis distances and collapsed into two NMDS axes. Convex hulls represent the range of uninvaded and invaded plots. Symbols for each site are displayed from west (BR) to east (DF).

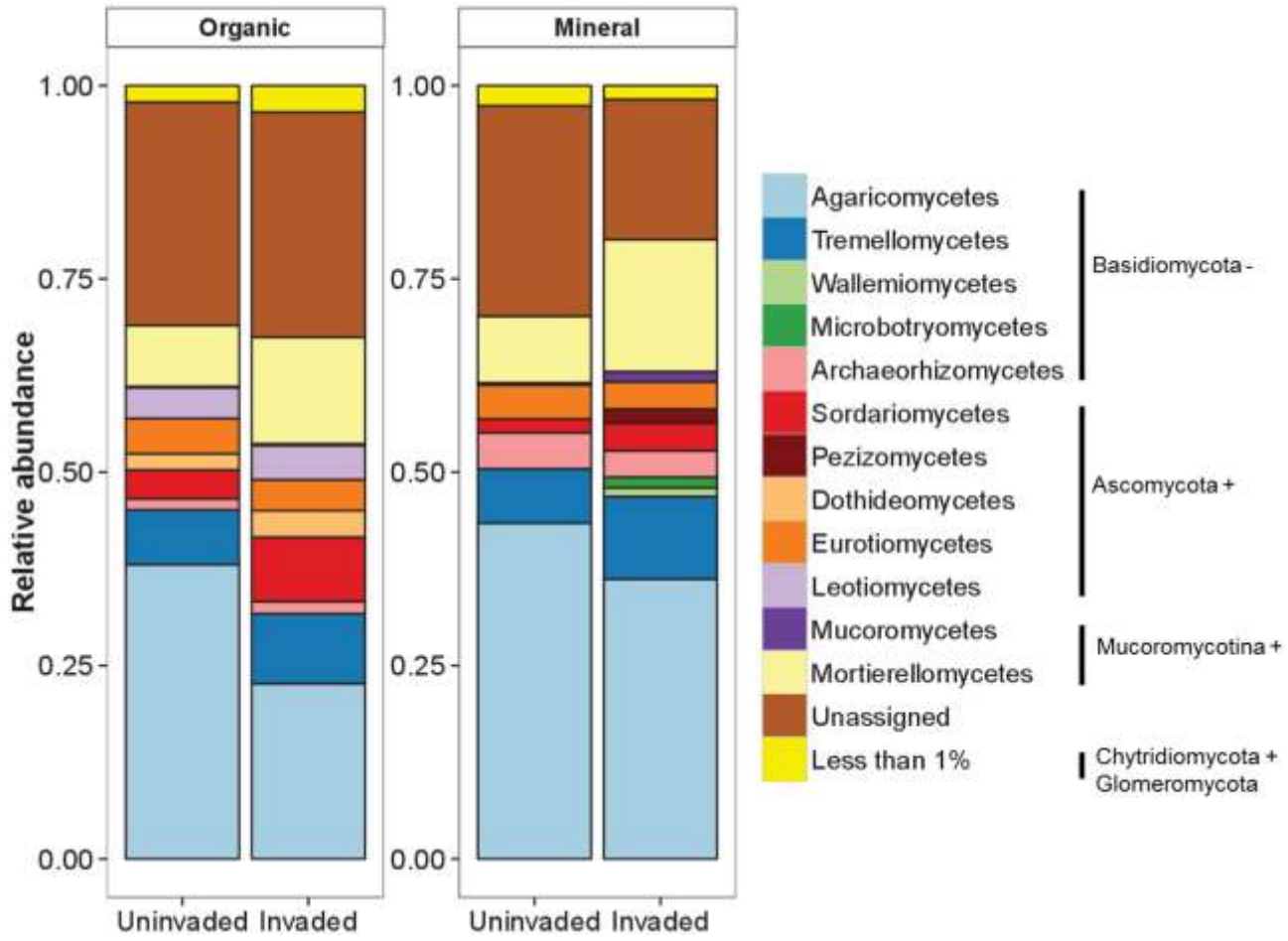


Figure 3. The relative abundance of fungal classes for uninvaded and invaded soil. Bars are stacked in order to show the classes associated with each phyla. Bars represent the mean relative abundance of three replicate plots at six northeastern forests (n = 18). Significant increases (+) and decreases (-) at the phyla level are indicated, but all other statistical results and fungal classes representing less than 1% of the total sequences are provided in Supplementary Table 3.

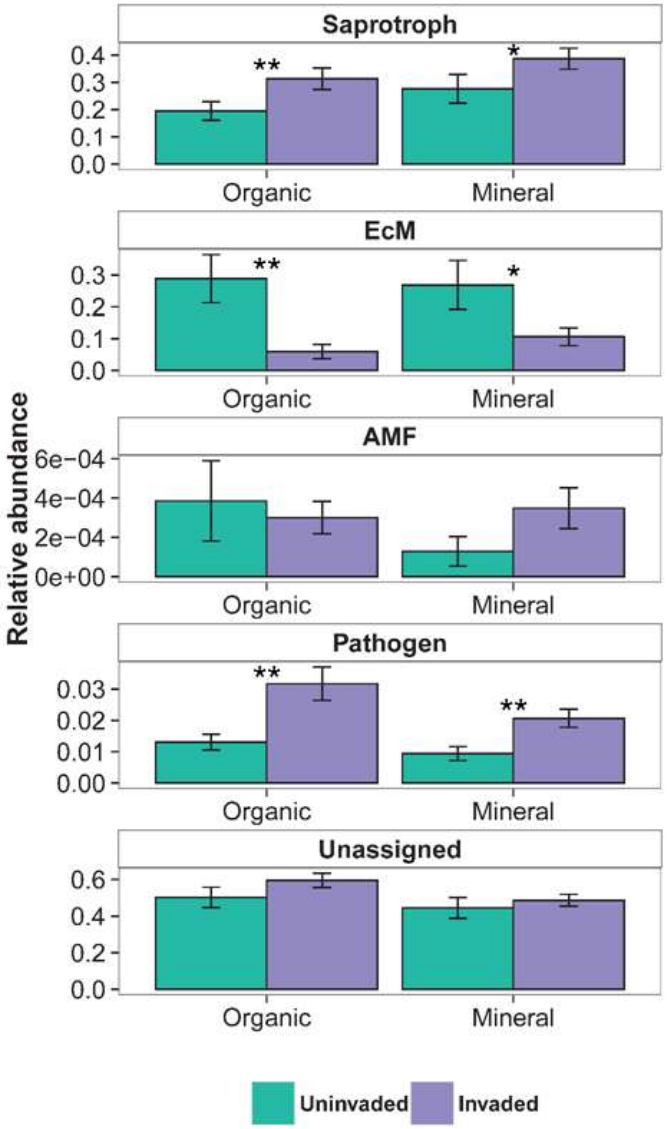


Figure 4. Relative abundance of different fungal functional groups in uninvaded and invaded plots in organic horizon and mineral soil. Bars represent the mean abundance and error bars are one standard error. Asterisks indicate statistically significant differences:* = $P < 0.001$ and ** = $P < 0.05$.

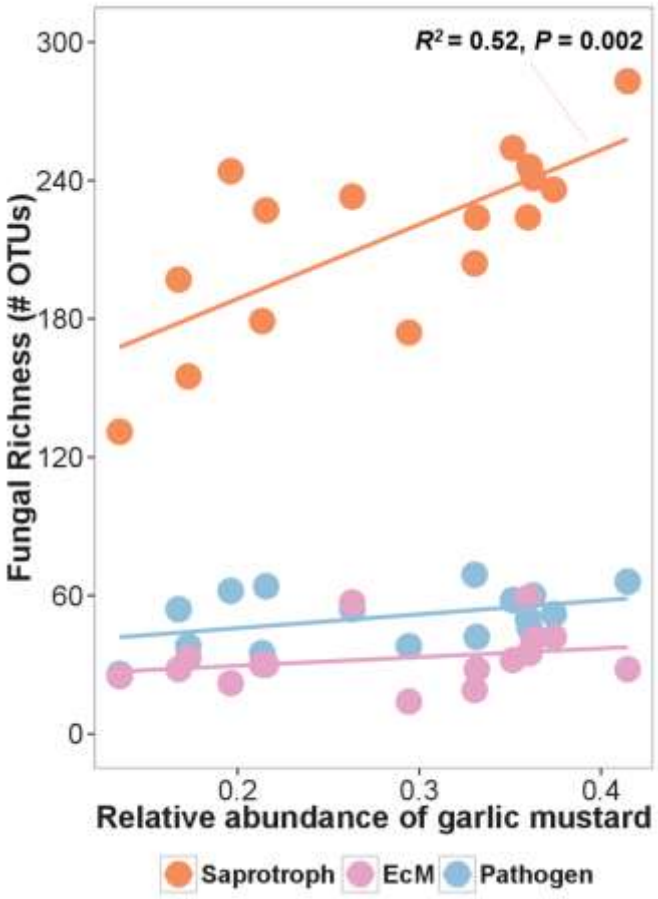


Figure 5. Relationship between garlic mustard relative abundance and saprotrophic fungal richness, ectomycorrhizal (EcM) fungal richness, and pathogenic richness in organic horizon soils.

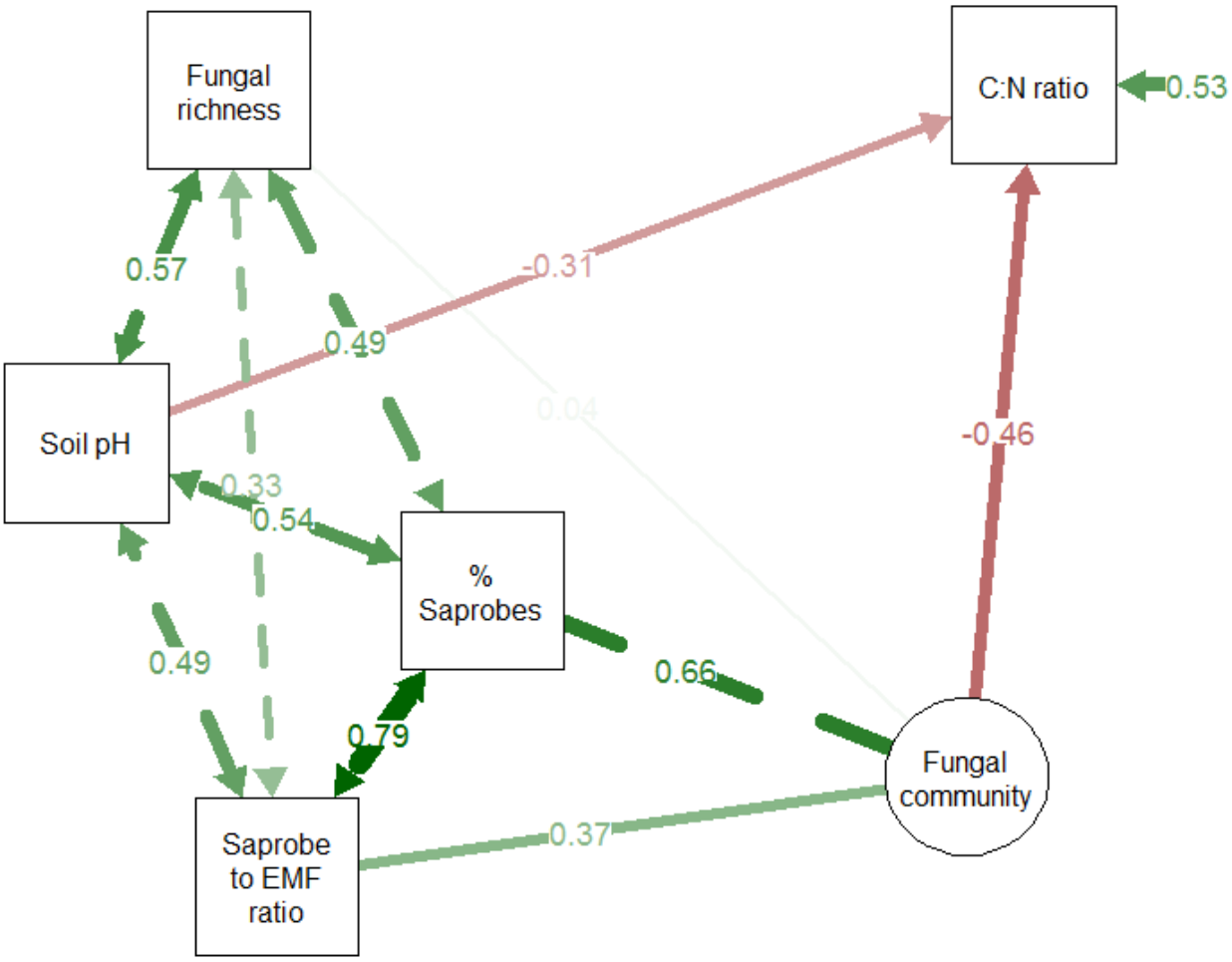


Figure 6. A SEM for soil C:N ratio. The arrow width and chroma indicate the degree of effects, arrow color shows negative (red) and positive (green) effects, line type shows regression (solid) versus correlation (dashed) formula types, and box shape shows composite variables (circle) and individual variables (squares). Values in the lines are the standardized path coefficients, and the arrow pointing to C:N ratio states the unexplained model variance. The total model output explained 47% of the variation in C:N ratio (P = 0.05). All variables were log transformed with the exception of soil pH and C:N ratio.

Supplementary Tables and Figures (Appendix)

Supplementary Table 1. The primer constructs used for Illumina MiSeq sequencing.

Name	Reverse compliment 3' Illumina adapter	Barcode	Reverse primer pad	linker	Reverse ITS4
reverse 1	CAAGCAGAAGACGGCATACGAGAT	TCCCTTGTCTCC	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 2	CAAGCAGAAGACGGCATACGAGAT	ACGAGACTGATT	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 3	CAAGCAGAAGACGGCATACGAGAT	GCTGTACGGATT	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 4	CAAGCAGAAGACGGCATACGAGAT	ATCACCAGGTGT	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 5	CAAGCAGAAGACGGCATACGAGAT	TGGTCAACGATA	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 6	CAAGCAGAAGACGGCATACGAGAT	ATCGCACAGTAA	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 7	CAAGCAGAAGACGGCATACGAGAT	GTCGTGTAGCCT	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 8	CAAGCAGAAGACGGCATACGAGAT	AGCGGAGGTTAG	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 9	CAAGCAGAAGACGGCATACGAGAT	ATCCTTTGGTTC	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 10	CAAGCAGAAGACGGCATACGAGAT	TACAGCGCATAC	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 11	CAAGCAGAAGACGGCATACGAGAT	ACCGGTATGTAC	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 12	CAAGCAGAAGACGGCATACGAGAT	AATTGTGTCGGA	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 13	CAAGCAGAAGACGGCATACGAGAT	TGCATACACTGG	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 14	CAAGCAGAAGACGGCATACGAGAT	AGTCGAACGAGG	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 15	CAAGCAGAAGACGGCATACGAGAT	ACCAGTGACTCA	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 16	CAAGCAGAAGACGGCATACGAGAT	GAATACCAAGTC	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 17	CAAGCAGAAGACGGCATACGAGAT	GTAGATCGTGTA	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 18	CAAGCAGAAGACGGCATACGAGAT	TAACGTGTGTGC	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 19	CAAGCAGAAGACGGCATACGAGAT	CATTATGGCGTG	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 20	CAAGCAGAAGACGGCATACGAGAT	CCAATACGCCTG	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 21	CAAGCAGAAGACGGCATACGAGAT	GATCTGCGATCC	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 22	CAAGCAGAAGACGGCATACGAGAT	CAGCTCATCAGC	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 23	CAAGCAGAAGACGGCATACGAGAT	CAAACAACAGCT	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 24	CAAGCAGAAGACGGCATACGAGAT	GCAACACCATCC	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 25	CAAGCAGAAGACGGCATACGAGAT	GCGATATATCGC	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 26	CAAGCAGAAGACGGCATACGAGAT	CGAGCAATCCTA	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 27	CAAGCAGAAGACGGCATACGAGAT	AGTCGTGCACAT	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 28	CAAGCAGAAGACGGCATACGAGAT	GTATCTGCGCGT	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 29	CAAGCAGAAGACGGCATACGAGAT	CGAGGGAAAGTC	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 30	CAAGCAGAAGACGGCATACGAGAT	CAAATTCGGGAT	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 31	CAAGCAGAAGACGGCATACGAGAT	AGATTGACCAAC	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 32	CAAGCAGAAGACGGCATACGAGAT	AGTTACGAGCTA	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 33	CAAGCAGAAGACGGCATACGAGAT	GCATATGCACTG	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 34	CAAGCAGAAGACGGCATACGAGAT	CAACTCCCGTGA	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 35	CAAGCAGAAGACGGCATACGAGAT	TTGCGTTAGCAG	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
reverse 36	CAAGCAGAAGACGGCATACGAGAT	TACGAGCCCTAA	AGTCAGTCAG	CC	TCCTCCGCTTATTGATATGC
Index Sequencing Primer	CGGCGACCACCGAGATCTACACTATGGTAATTGTGTGAATCATCGAATCTTTG				

Supplementary Table 2. Community composition of the entire fungal community and for different functional groups. The PERMANOVA statistical model output for the general fungal and functional group specific community Bray-Curtis distance matrices is shown in response to invasion, site, and invasion x site after 999 permutations. Significant values are bolded.

		Organic horizon				Mineral soil			
	DF	SS	*F*	R^2	*P*	SS	*F*	R^2	*P*
General fungal community									
Invasion	1	0.58	1.89	0.04	**0.002**	0.63	0.63	0.05	**0.001**
Site	5	3.75	2.47	0.28	**0.001**	3.49	0.70	0.26	**0.001**
Invasion:Site	5	1.77	1.17	0.13	**0.042**	2.04	0.41	0.15	**0.002**
Saprotrophs									
Invasion	1	0.55	2.53	0.06	**0.001**	0.54	2.31	0.05	**0.001**
Site	5	2.97	2.71	0.31	**0.001**	3.41	2.94	0.31	**0.001**
Invasion:Site	5	1.51	1.38	0.16	**0.017**	1.58	1.36	0.14	**0.004**
EMF									
Invasion	1	0.54	1.34	0.04	**0.034**	0.64	1.47	0.04	**0.005**
Site	5	3.54	1.73	0.23	**0.001**	3.28	1.51	0.19	**0.001**
Invasion:Site	5	2.49	1.22	0.16	**0.009**	2.53	1.16	0.15	**0.014**
Pathogens									
Invasion	1	0.69	3.28	0.07	**0.001**	0.95	4.33	0.09	**0.001**
Site	5	2.93	2.81	0.32	**0.001**	2.93	2.66	0.26	**0.001**
Invasion:Site	5	1.24	1.19	0.13	0.130	2.01	1.82	0.18	**0.001**

Supplementary Table 3. The relative abundance of fungal classes from uninvaded and invaded plots in six northeastern forests. Values represent the mean and standard error (in parentheses). Values followed by different lowercase letters within a fungal class and soil horizon are significantly different at $P < 0.05$.

	Organic horizon		Mineral soil	
	Uninvaded	Invaded	Uninvaded	Invaded
Agaricomycetes	0.38 (0.1)a	0.23 (0.07)a	0.43 (0.09)a	0.36 (0.06)a
Agaricostilbomycetes	6.2^{-6} (5.1^{-6})a	0a	0a	6.9 (6.2^{-6})a
Archaeorhizomycetes	0.01 (0.004)a	0.02 (0.005)a	0.05 (0.02)a	0.03 (0.02)a
Chytridiomycetes	0.001 (0.0007)a	0.001 (0.0006)a	0.0002 (0.9^{-4})a	0.001 (0.0005)b
Dothideomycetes	0.02 (0.009)a	0.03 (0.01)a	0.002 (0.0007)a	0.005 (0.002)b
Eurotiomycetes	0.05 (0.02)a	0.04 (0.007)a	0.04 (0.01)a	0.04 (0.005)a
Exobasidiomycetes	3.8^{-5} (3.9^{-5})a	9.3^{-6} (9.5^{-6})a	1.38^{-5} (1.1^{-5})a	1.9^{-5} (1.9^{-5})a
Incertae sedis	0.005 (0.003)a	0.007 (0.002)a	2.8^{-5} (1.7^{-5})a	7.8^{-4} (3.9^{-4})b
Lecanoromycetes	0.0003 (2.1^{-4})a	0.0001 (7.6^{-5})a	1.9^{-5} (1.4^{-5})a	2.8 (2.7^{-6})a
Leotiomycetes	0.04 (0.01)a	0.04 (0.01)a	0.0009 (0.0006)a	0.002 (0.001)a
Microbotryomycetes	0.004 (0.001)a	0.009 (0.005)b	0.008 (0.005)a	0.01 (0.004)a
Mortierellomycetes	0.08 (0.02)a	0.14 (0.03)b	0.09 (0.02)a	0.18 (0.03)b
Orbiliomycetes	9.8^{-5} (6.8^{-5}-)a	2.4^{-4} (1.4^{-4})a	1.4^{-5} (9.1^{-6})a	2.4^{-4} (1.5^{-4})b
Pezizomycetes	0.003 (0.003)a	0.007 (0.005)a	0.008 (0.007)a	0.02 (0.01)a
Pucciniomycetes	1.46^{-6} (2.1^{-6})a	9.9^{-5} (1.4^{-4})a	5.5^{-6} (3.5^{-6})a	4.1 (5.3^{-6})a
Saccharomycetes	0.0003 (1.2^{-4})a	0.0002 (8.0^{-5})a	0.0005 (0.0003)a	0.0009 (0.0005)a
Sordariomycetes	0.04 (0.008)a	0.08 (0.02)b	0.02 (0.005)a	0.04 (0.006)b
Taphrinomycetes	5.8^{-6} (4.8^{-6})a	2.2^{-5} (1.1^{-5})a	6.9^{-6} (6.8^{-6})a	1.5^{-5} (9.03^{-6})a
Tremellomycetes	0.07 (0.03)a	0.09 (0.02)a	0.07 (0.03)a	0.11 (0.03)a
Unassigned	0.29 (0.08)a	0.29 (0.05)a	0.27 (0.08)a	0.18 (0.03)a
Ustilaginomycetes	0.0001 (0.0001)a	0.002 (0.002)a	1.4^{-5} (1.03^{-5})a	1.07^{-3} (1.1^{-3})a
Wallemiomycetes	0.005 (0.007)a	0.004 (0.002)a	0.003 (0.001)a	0.01 (0.007)a

Supplementary Table 4. The relative abundance of organic horizon fungal orders from uninvaded and invaded plots at six northeastern forests. Significantly different abundances are bolded ($P < 0.05$).

	Invaded	Uninvaded	*P*-value
Unassigned	0.465852	0.389298	0.282271
Agaricales	0.0885	0.090003	0.974454
Tremellales	0.058156	0.054351	0.849332
Hypocreales	**0.039575**	**0.02347**	**0.01844**
Cantharellales	0.035118	0.016165	0.438824
Helotiales	0.034452	0.02885	0.426386
Trechisporales	0.033522	0.019777	0.405812
Russulales	**0.026308**	**0.183567**	**0.021178**
Eurotiales	0.025136	0.033718	0.475175
Pleosporales	0.022489	0.010162	0.190637
Filobasidiales	0.02226	0.01136	0.121494
Boletales	0.019278	0.003725	0.110974
Sordariales	0.016802	0.002501	0.059675
Archaeorhizomycetales	0.015968	0.014206	0.711065
Chaetothyriales	0.014358	0.011424	0.497364
Polyporales	**0.009219**	**0.003365**	**0.010252**
Incertae	0.008748	0.003807	0.070902
Sporidiobolales	0.008462	0.003931	0.20132
Trichosporonales	0.007962	0.003723	0.075828
Pezizales	0.00714	0.002841	0.312421
Venturiales	0.006847	0.001335	0.353811
Thelephorales	0.005439	0.012204	0.086736
Sebacinales	0.003899	0.003423	0.885591
Geminibasidiales	0.003688	0.005031	0.78802
Atheliales	0.002479	0.045724	0.116957
Glomerales	0.002198	0.002142	0.947652
Urocystidales	0.002076	0.000114	0.151229
Xylariales	0.001792	0.000881	0.098802
Cystofilobasidiales	0.001592	0.000784	0.110604
Capnodiales	0.001343	0.000887	0.255013
Rhizophydiales	0.00105	0.000845	0.70588
Geoglossales	0.000809	0.000467	0.663803
Archaeosporales	0.000764	0.000428	0.141384
Hysteriales	**0.000699**	**0.006803**	**0.028431**
Diaporthales	0.000668	0.000222	0.085895
Chaetosphaeriales	0.000597	0.000544	0.821898
Auriculariales	0.000564	0.000312	0.50754
Microascales	**0.0004**	**3.36E-05**	**0.047985**

Onygenales	**0.000375**	**0.000131**	**0.050913**
Thelebolales	0.000327	3.5E-05	0.362614
Diversisporales	0.00029	0.000268	0.884935
Leucosporidiales	0.000267	9.19E-05	0.08887
Coniochaetales	**0.000264**	**4.09E-05**	**0.026331**
Saccharomycetales	0.000242	0.000283	0.702886
Spizellomycetales	0.000211	0.000182	0.820073
Hymenochaetales	0.000181	0.000588	0.402105
Olpidiales	0.000175	0	0.105462
Botryosphaeriales	0.000169	0.000289	0.623459
Dothideales	**0.000155**	**3.79E-05**	**0.007335**
Rhytismatales	0.000152	3.94E-05	0.301373
Orbiliales	0.000147	6.57E-05	0.256014
Paraglomerales	**0.000141**	**1.61E-05**	**0.025995**
Ophiostomatales	9.92E-05	8.9E-05	0.895695
Platygloeales	9.61E-05	0	0.33317
Gomphales	5.27E-05	2.92E-05	0.448182
Pyrenulales	2.79E-05	0	0.251767
Phallales	2.48E-05	2.48E-05	0.999866
Taphrinales	2.17E-05	5.84E-06	0.086421
Ustilaginales	1.55E-05	1.46E-05	0.912134
Geastrales	9.3E-06	0	0.33317
Exobasidiales	9.3E-06	3.79E-05	0.329258
Agaricostilbales	6.2E-06	0	0.103771
Lecanorales	6.2E-06	0.000188	0.220973
Leotiales	4.65E-06	0.004994	0.330446
Pucciniales	3.1E-06	1.46E-06	0.636783
Erysiphales	1.55E-06	0	0.33317
Microbotryales	1.55E-06	2.92E-06	0.682542
Ostropales	1.55E-06	1.17E-05	0.122923
Calosphaeriales	0	5.84E-06	0.215616
Corticiales	0	1.46E-06	0.332195
Myriangiales	0	1.46E-06	0.332195

Supplementary Table 5. The relative abundance of mineral soil fungal orders from uninvaded and invaded plots at six northeastern forests. Significantly different abundances are bolded ($P < 0.05$).

	Invaded	Uninvaded	*P*-value
Unassigned	0.381609	0.37465	0.917207
Agaricales	0.197612	0.125871	0.18837
Tremellales	0.075378	0.05347	0.325091
Russulales	0.051637	0.145284	0.1483
Cantharellales	0.041205	0.009523	0.154134
Archaeorhizomycetales	0.033904	0.046619	0.480569
Filobasidiales	0.025815	0.01382	0.094217
Sebacinales	0.023609	0.004752	0.159451
Hypocreales	0.020583	0.011771	**0.043481**
Pezizales	0.018501	0.007905	0.323412
Chaetothyriales	0.017716	0.020022	0.67393
Eurotiales	0.015622	0.022267	0.421575
Sporidiobolales	0.013277	0.007716	0.227383
Boletales	0.012902	0.053188	0.373884
Geminibasidiales	0.011468	0.002585	0.078289
Polyporales	0.010022	0.002947	**0.003718**
Trechisporales	0.008869	0.0179	0.222979
Atheliales	0.007566	0.05957	0.167094
Thelephorales	0.006651	0.008694	0.641721
Glomerales	0.003975	0.002949	0.482108
Trichosporonales	0.003284	0.001998	0.131436
Xylariales	0.002321	0.000292	**0.001904**
Pleosporales	0.001805	0.0012	0.340001
Cystofilobasidiales	0.001684	0.000358	**0.05515**
Geoglossales	0.00164	0.000679	0.317734
Archaeosporales	0.001294	0.000863	0.300054
Incertae sedis	0.00126	0.000411	**0.005751**
Urocystidales	0.001058	1.1E-05	0.196758
Rhizophydiales	0.001041	0.000205	**0.014649**
Saccharomycetales	0.000907	0.000484	0.317818
Capnodiales	0.000835	0.00026	0.305091
Helotiales	0.00062	4.55E-05	0.105239
Onygenales	0.000547	4.13E-05	0.142399
Geastrales	0.00044	0	0.325058
Botryosphaeriales	0.000437	2.89E-05	0.177786
Diversisporales	0.000418	0.00032	0.545421
Leucosporidiales	0.000398	0.000127	0.200999
Paraglomerales	0.000365	1.38E-06	**0.**056603

Microascales	0.000249	1.1E-05	0.140313
Auriculariales	0.000248	9.23E-05	0.188206
Olpidiales	0.000218	1.38E-06	**0.041508**
Hymenochaetales	0.000194	0.000147	0.492173
Diaporthales	0.000183	0.000525	0.43898
Gomphales	0.000152	3.17E-05	0.316674
Orbiliales	0.000113	1.24E-05	0.032616
Dothideales	8.54E-05	3.86E-05	0.221298
Coniochaetales	6.48E-05	0	**0.04904**
Sordariales	5.65E-05	2.34E-05	0.159583
Spizellomycetales	4.41E-05	3.03E-05	0.595926
Ophiostomatales	2.62E-05	1.52E-05	0.589644
Taphrinales	1.52E-05	6.89E-06	0.309628
Phallales	1.38E-05	5.51E-06	0.217912
Tilletiales	1.38E-05	0	0.331333
Ustilaginales	9.65E-06	2.76E-06	0.175183
Agaricostilbales	6.89E-06	0	0.135453
Boliniales	6.89E-06	1.1E-05	0.700448
Exobasidiales	5.51E-06	1.38E-05	0.337502
Verrucariales	5.51E-06	0	0.331333
Pucciniales	4.13E-06	5.51E-06	0.726889
Pyrenulales	2.76E-06	2.76E-06	1
Arachnomycetales	1.38E-06	1.38E-06	1
Erythrobasidiales	1.38E-06	0	0.331333
Lecanorales	1.38E-06	1.52E-05	0.109045
Teloschistales	1.38E-06	0	0.331333
Cystobasidiales	0	1.38E-06	0.331333
Leotiales	0	0.000165	0.331333
Mycocaliciales	0	1.38E-06	0.331333
Ostropales	0	4.13E-06	0.187176
Rhizophlyctidales	0	2.76E-06	0.331333

Supplementary Table 6. The relative abundance of EcM fungal orders from uninvaded and invaded mineral soil collected at six northeastern forests. Values represent the mean and standard error (in parentheses). Values in bold are significantly different at $P < 0.05$.

	Invaded	**Uninvaded**	**P-value**
Russulales	**0.007113**	**0.165157**	**0.015228**
Atheliales	0.002468	0.04561	0.117911
Agaricales	0.0082	0.030409	0.435192
Cantharellales	0.026812	0.015704	0.6352
Eurotiales	0.003397	0.014705	0.336485
Hysteriales	**0.000673**	**0.006759**	**0.029175**
Thelephorales	**0.001343**	**0.006549**	**0.035213**
Pezizales	0.005437	0.002152	0.439567
Sebacinales	0.000707	0.00094	0.773136
Boletales	0.003706	0.000865	0.294188
Gomphales	4.81E-05	7.29E-06	0.079001

Supplementary Table 7. The relative abundance of EcM fungal orders from uninvaded and invaded organic horizon samples collected at six northeastern forests. Values represent the mean and standard error (in parentheses).

	Invaded	**Uninvaded**	***P*-value**
Russulales	0.025256	0.137132	0.083963
Atheliales	0.007561	0.05957	0.167052
Agaricales	0.037308	0.04482	0.829179
Eurotiales	0.00141	0.011462	0.195796
Cantharellales	0.018308	0.007977	0.423214
Thelephorales	0.002015	0.003494	0.446087
Pezizales	0.004212	0.001844	0.263828
Sebacinales	0.005941	0.001805	0.496683
Boletales	0.004033	0.000915	0.117294
Gomphales	0.000149	1.52E-05	0.259981

Supplementary Table 8. The relative abundance of organic horizon Sordariomycetes lifestyle groups from uninvaded and invaded plots at six northeastern forests. Significantly different abundances are bolded ($P < 0.05$).

	Invaded	**Uninvaded**	***P*-value**
Animal parasites	0.002135	0.002087	0.957746
Mycoparasites	0.006498	0.00354	0.223168
Plant pathogens	**0.004521**	**0.001319**	**8.8E-06**
Saprotrophs	0.008577	0.005652	0.092029
Unassigned	**0.06144**	**0.024832**	**0.015951**

Supplementary Table 9. The relative abundance of mineral soil Sordariomycetes lifestyle groups from uninvaded and invaded plots at six northeastern forests. Significantly different abundances are bolded ($P < 0.05$).

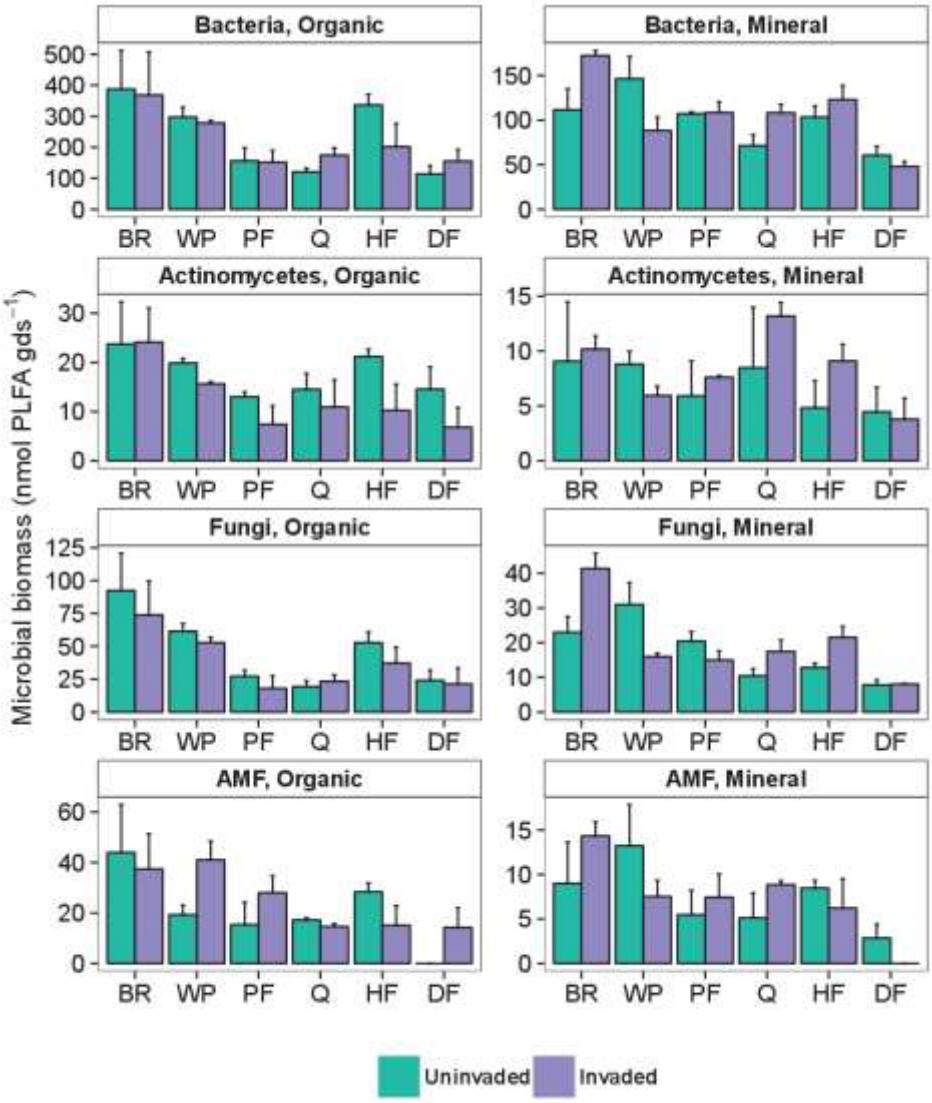
	Invaded	**Uninvaded**	**P-value**
Animal parasite	0.003474	0.002599	0.643089
Mycoparasite	0.00707	0.003069	0.134683
Plant pathogen	0.000717	0.000548	0.71714
Saprotroph	**0.006731**	**0.002488**	**0.005724**
Unassigned	**0.01757**	**0.009016**	**0.006363**

Supplementary Table 10. The relative abundance of pathogenic organic horizon soil fungal orders from uninvaded and invaded plots at six northeastern forests. Significantly different abundances are bolded ($P < 0.05$).

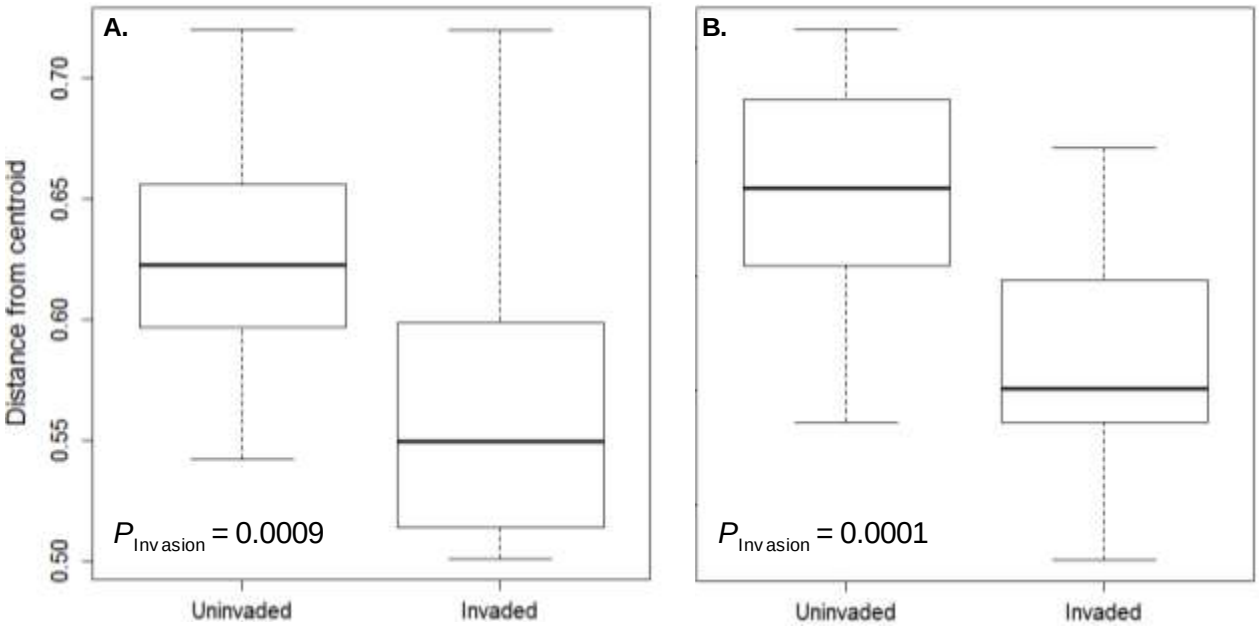
	Invaded	**Uninvaded**	***P*-value**
Hypocreales	0.012684	0.00667	0.**050539**
Trichosporonales	0.007958	0.003644	0.070766
Polyporales	**0.004329**	**0.001338**	**0.007323**
Pleosporales	**0.002076**	**0.000573**	**0.00747**
Spizellomycetales	0.000211	0.000182	0.820073
Xylariales	0.000274	0.000121	0.188389
Urocystidales	0.002076	0.000114	0.151229
Diaporthales	0.000174	9.34E-05	0.335093
Capnodiales	9.77E-05	7.15E-05	0.5963
Botryosphaeriales	0.000129	6.57E-05	0.198781
Ophiostomatales	2.17E-05	6.13E-05	0.491603
Exobasidiales	9.3E-06	3.79E-05	0.329258
Helotiales	0.001197	2.33E-05	0.338523
Rhytismatales	0.000149	2.04E-05	0.238767
Cantharellales	6.2E-06	1.75E-05	0.549473
Ustilaginales	1.4E-05	1.02E-05	0.615241
Hymenochaetales	1.55E-06	5.84E-06	0.261548
Taphrinales	2.17E-05	5.84E-06	0.086421
Microbotryales	1.55E-06	2.92E-06	0.682542
Pucciniales	3.1E-06	1.46E-06	0.636783
Erysiphales	1.55E-06	0	0.33317
Olpidiales	0.000175	0	0.105462
Platygloeales	9.61E-05	0	0.33317

Supplementary Table 11. The relative abundance of pathogenic mineral horizon soil fungal families from uninvaded and invaded plots at six northeastern forests. Significantly different abundances are bolded ($P < 0.05$).

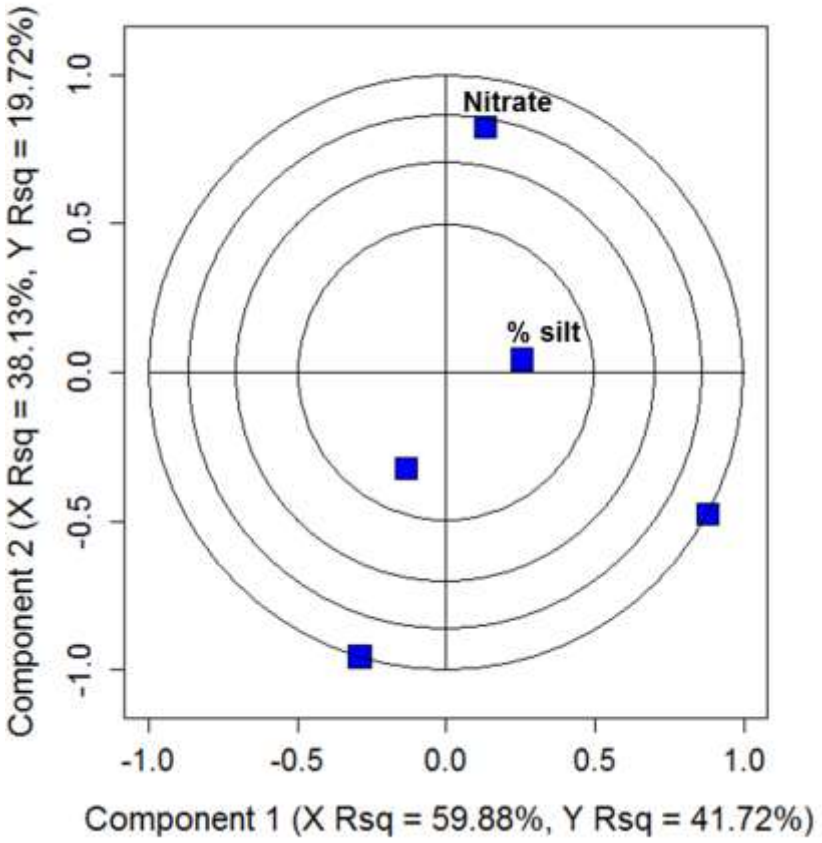
	Invaded	**Uninvaded**	***P*-value**
Clavicipitaceae	0.003404	0.002539	0.64644
Hypocreaceae	0.004967	0.002295	0.181696
Trichosporonaceae	0.003284	0.001993	0.129897
Ganodermataceae	**0.0043**	**0.001094**	**0.003504**
Ophiocordycipitaceae	0.002135	0.000812	0.135528
Schizoparmaceae	3.58E-05	0.000464	0.335051
Spizellomycetaceae	4.41E-05	3.03E-05	0.595889
Amphisphaeriaceae	0.00016	2.62E-05	0.045231
Nectriaceae	**0.000255**	**2.48E-05**	**0.023314**
Cordycipitaceae	3.86E-05	2.34E-05	0.281467
Meripilaceae	2.89E-05	1.93E-05	0.541624
Exobasidiaceae	5.51E-06	1.38E-05	0.337502
Ophiostomataceae	2.62E-05	1.24E-05	0.472485
Urocystidaceae	0.001054	1.1E-05	0.198528
Botryosphaeriaceae	**0.000127**	**1.1E-05**	**0.010331**
Pleosporaceae	**0.00024**	**1.1E-05**	**0.01265**
Taphrinaceae	1.38E-05	6.89E-06	0.397225
Valsaceae	3.45E-05	6.89E-06	0.253625
Diatrypaceae	**3.31E-05**	**5.51E-06**	**0.022475**
Togniniaceae	2.07E-05	4.13E-06	0.335601
Diaporthaceae	5.51E-06	2.76E-06	0.561497
Phragmidiaceae	1.38E-06	2.76E-06	0.560107
Pucciniastraceae	2.76E-06	2.76E-06	1
Ustilaginaceae	5.51E-06	2.76E-06	0.465243
Bionectriaceae	0.000146	1.38E-06	0.021432
Cystobasidiaceae	0	1.38E-06	0.331333
Olpidiaceae	**0.000218**	**1.38E-06**	**0.041512**
Ceratobasidiaceae	2.48E-05	0	0.250328
Hymenochaetaceae	4.13E-06	0	0.331333
Leptosphaeriaceae	6.89E-06	0	0.096161
Mycosphaerellaceae	1.1E-05	0	0.176866
Physalacriaceae	1.1E-05	0	0.331333
Tilletiaceae	1.38E-05	0	0.331333



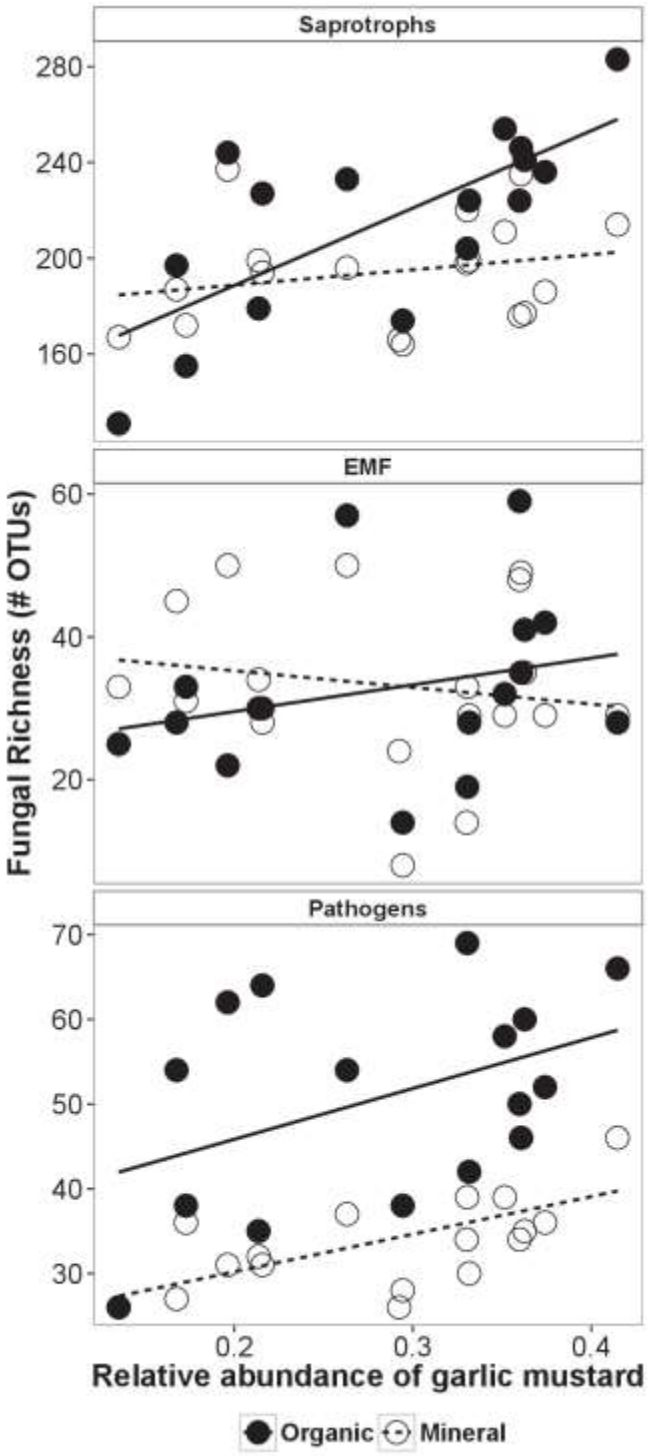
Supplementary Figure 1. Microbial biomass in garlic mustard invaded and uninvaded plots at six northeastern deciduous forests. Bacterial and fungal biomass varied across sites and there was a significant invasion x site interaction for both soil horizons ($F_{bacteria}$ = 4.35, $P_{bacteria}$ = 0.002, F_{fungi} = 4.44, P_{fungi}= 0.002). Bars represent the mean of three replicate plots per site x invasion status combination and error bars are one standard error.



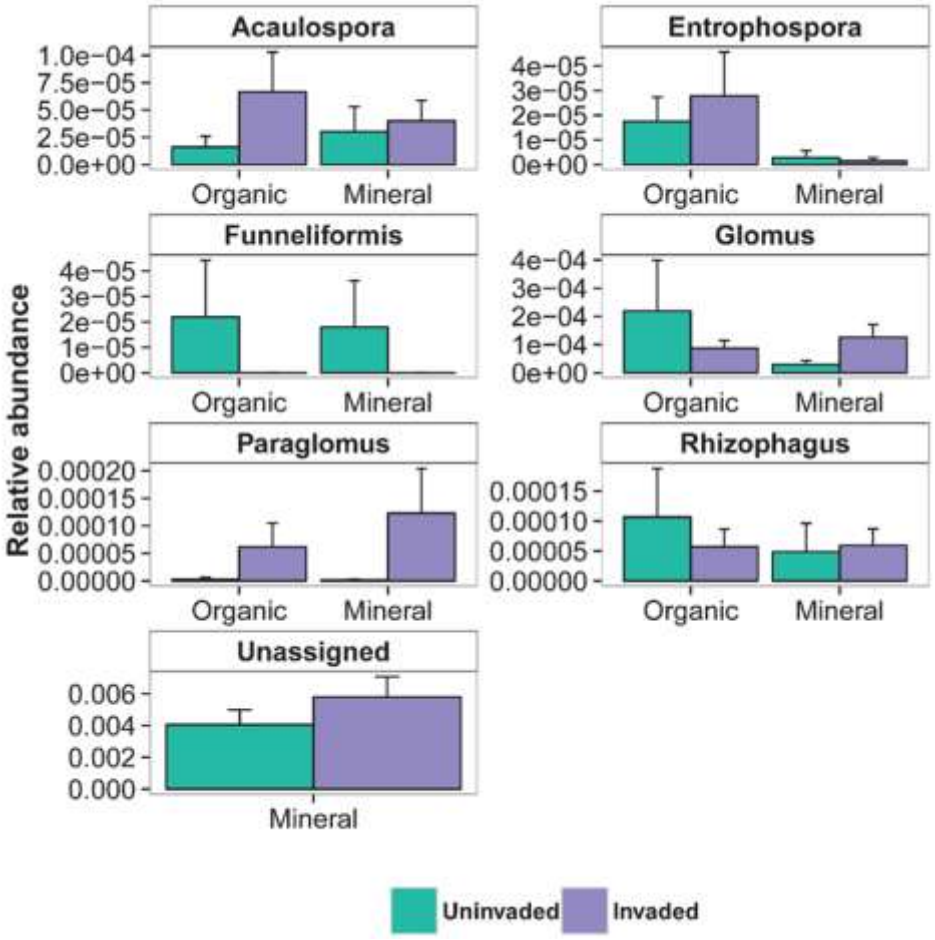
Supplementary Figure 2. The degree of variation in fungal community composition exhibited as the homogeneity of dispersion in fungal community Bray-Curtis distances. The relative abundance of OTUs were converted to Bray-Curtis distances and analyzed using PERMDISP. The boxplots represent the distance from the mean Bray-Curtis distance (centroid) comparing uninvaded and invaded fungal community compositions. The organic horizon (**A**) and mineral soil (**B**) are displayed separately.



Supplementary Figure 3. A correlation loading plot showing the relationship between garlic mustard abundance (# plants m^2) and the soil covariables included in the final PLSR model output. Only two soil variables are significant univariate predictors of invasion, soil nitrate concentration and soil silt content, which are labelled in the plot.



Supplementary Figure 4. The correlation between fungal richness and the relative abundance of garlic mustard across the different functional groups.



Supplementary Figure 5. The relative abundance of arbuscular mycorrhizal fungal genera from uninvaded and invaded plots across six northeastern forests. Bars represent the mean relative abundance and error bars are the standard error.