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Fungal community homogenization, shift in dominant trophic guild, and appearance of novel taxa with biotic invasion

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Abstract. Invasion by non-native plants may fundamentally restructure the soil fungal community. The invasive plant, *Alliaria petiolata*, produces secondary compounds suppressive to mycorrhizal fungi and may therefore be expected to have generally negative effects on other components of the fungal community. Here, we compared fungal biomass, diversity, community composition, and the relative abundance of fungal trophic guilds, along with edaphic properties of soils collected from uninvaded and invaded plots across six temperate forests. Invaded plots were differentiated from uninvaded plots by lower variation in fungal community composition (beta diversity) and soil properties, higher fungal richness and community evenness (alpha diversity), and a suite of novel saprotrophic and pathotrophic fungi that were consistently present across the invaded landscape and absent from uninvaded forest patches. Invaded plots also had lower ectomycorrhizal but higher saprotrophic and pathotrophic relative abundance, despite there being no difference in fungal biomass between invasion statuses. We hypothesize that shifts in the fungal community with invasion may directly impact plant disease response, soil nutrient cycling processes, and plant performance of the invasive and native plant communities.

Key words: *Alliaria petiolata;* DNA sequencing; fungal community structure; fungi; garlic mustard; microbiome; mycorrhizae.

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INTRODUCTION

Interactions between plants and fungi have developed over millennia of co-evolution (Brundrett 2002); however, introduced plants share no evolutionary antecedence with fungi they encounter in their introduced range. As a result, plant invasions can transform resident fungal communities (Callaway et al. 2008, Lankau 2011, Lekberg et al. 2013), altering symbioses between native plants and fungi (Stinson et al. 2006, Mangla and Callaway 2008, Cantor et al. 2011) and fungal-mediated ecosystem functions such as decomposition (Ashton et al. 2005, Rodgers et al. 2008*a*) and nutrient cycling (Ehrenfeld 2003). Although fungi are not the only soil organisms sensitive to invasive plants (e.g., bacteria: Hawkes et al. 2005, Piper et al. 2015), many fungi associate more intimately with plants than other organisms (Webster and Weber 2007) and play key ecosystem roles as saprotrophs, mycorrhizal symbionts, and plant pathogens and parasites (pathotrophs).

A native species to Europe and Asia, *Alliaria petiolata* (garlic mustard), has been introduced into North America (Rodgers et al. 2008*b*) and is associated with changes in the performance and composition of native plant communities in temperate deciduous forests (Nuzzo 1999, Blossey et al. 2001, Stinson et al. 2007, Rodgers et al. 2008*b*, Poon and Maherali 2015). Garlic mustard is a

member of the mustard family and relative of cabbage crops (Brassicaceae); thus, it produces secondary compounds, particularly glucosinolates and flavonoids, with antimicrobial properties (Zukalová and Vasak 2002, Callaway et al. 2008) and known toxicity to mycorrhizal fungi in the introduced range (Callaway et al. 2008, Lankau 2011). Most notably, garlic mustard suppresses root colonization by arbuscular mycorrhizal fungi (AMF) and is associated with declines in performance for native plants strongly reliant on this symbiosis (Stinson et al. 2006, Callaway et al. 2008). The negative effects of invasion on arbuscular mycorrhizae, either at a single forest site (Stinson et al. 2006, Burke et al. 2011) or at a broader, continental scale (Callaway et al. 2008, Lankau 2011), have been established. AMF diversity tends to decrease as a result of garlic mustard invasion (Barto et al. 2011, Lankau 2011, Lankau and Nodurft 2013), and loss of AMF selects for native plants with low specificity for particular AMF species (Lankau and Nodurft 2013) and reduced mycorrhizal dependencies (Stinson et al. 2006). Since there are clear negative effects of invasion on AMF (Stinson et al. 2006, Barto et al. 2011, Lankau and Nodurft 2013), it is possible that there are also suppressive effects on other fungal functional groups (i.e., saprotrophs, ectomycorrhizal fungi, and pathotrophs). Since AMF only represent a small fraction of the fungal phylogeny, however, and because garlic mustard is itself nonmycorrhizal, other fungal groups, especially free-living saprotrophs, might respond differently than AMF to invasion, but there is little work on the broader fungal ecology of garlic mustard invasions (Lankau 2011).

The primary objective of this study was to comprehensively characterize the fungal community associated with invasion and determine whether and how invaded areas differ from nearby, uninvaded sites across a region of the northeastern USA. To do this, we measured fungal biomass, diversity, community composition, and the relative abundance of saprotrophic, ectomycorrhizal, and pathotrophic guilds in dominant forest types with active *A. petiolata* invasions. Since garlic mustard invasion has previously been shown to be associated with elevated soil nutrient availability and pH (Rodgers et al. 2008*a*), a secondary objective was to assess key soil properties in invaded and uninvaded sites and examine relationships between the fungal community and edaphic properties.

Materials and Methods

Sites, study design, and sample collection

This work was conducted at six temperate, deciduous forests representative of the dominant forest cover of the northeastern USA and that are characterized by active garlic mustard invasion (Table 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*). Dominant native understory plants include tree seedlings of the same species, along with an herbaceous layer of Canada mayflower (*Mianthemum canadense*), trout lily (*Erythronium americanum*), and jack-in-the-pulpit (*Arisaema triphyllum*). Soil type and texture varies across sites, and garlic mustard densities range from ~20 to 38% relative abundance in invaded plots. Although we could not accurately determine how long garlic mustard has been present at each site, we know from previous work that one site (Black Rock) has been invaded for ~63 yr

Table 1. Site locations and characteristics.

Site name (ID)	Location	Soil texture† (soil order)	Garlic mustard‡ (relative abundance)
Black Rock Forest (BR)	Cornwall, New York	Sandy clay loam (Inceptisol)	0.38 (0.02)
West Point (WP)	West Point, New York	Clay loam (Inceptisol)	0.19 (0.01)
Pittsfield State Forest (PF)	Pittsfield, Massachusetts	Silty clay loam (Spodosol)	0.26 (0.07)
Questing Reserve (Q)	New Marlborough, Massachusetts	Sandy clay loam (Spodosol)	0.36 (0.00)
Harvard Forest (HF)	Petersham, Massachusetts	Clay loam (Inceptisol)	0.31 (0.01)
Drumlin Farm Wildlife Sanctuary (DF)	Lincoln, Massachusetts	Clay loam (Entisol)	0.24 (0.05)

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

‡ The number of garlic mustard plants relative to the total number of plants shown as the average of three replicate invaded plots ± 1 SE (in parentheses).

(Lankau and Nodurft 2013) and that this site is comparable to the others in terms of forest composition and garlic mustard cover (Table 1).

We established three replicate uninvaded and three replicate invaded plots (3 m^{-2}) in forest patches at each site with similar overstory vegetation composition, slope, and aspect. All plots were separated by at least 1 m and invaded plots had a minimum of 20 garlic mustard plants/m^2, which is typical for the region (Cavers et al. 1979, Byers and Quinn 1998, Stinson et al. 2007). Soil sampling at all sites was performed in the first two weeks of June 2013. To capture within-plot variability, three soil cores (5 cm wide × 10 cm deep) were collected from different sections of each plot, separated into the organic horizon (~3–5 cm depending on site) and mineral soil (~5–7 cm), composited, and manually homogenized by depth increment. There were a total of 72 samples (6 sites × 2 invasion status × 2 depths × 3 replicates). A subsample from each plot and depth increment was flash-frozen in liquid nitrogen (N) immediately in the field and stored at −80°C for fungal community characterization. The remaining soil was kept on ice until being stored at 4°C in the laboratory within 12 h of sampling. 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. Samples for edaphic characterization and nutrient analyses were processed and analyzed within 48 h of sampling.

Fungal biomass, diversity, and community composition

Fungal biomass was estimated using phospholipid fatty acid (PLFA) analysis on samples that were flash-frozen in liquid N within 24 h of sampling and subsequently freeze-dried (Freezone 6; Labconco, Kansas City, Missouri, USA). 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:v). The polar lipids were isolated and purified using silicic acid chromatography and collected using a methanol wash. Lipids were then methylated by adding 0.2 mol/L methanolic potassium hydroxide (1 mL) and incubating 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. We compared FAME peaks against a standard library of FAMES specific to fungi (18:2ω6, 9c, 18:1ω9c), as well as bacteria (i15:0, a15:0, c15:0, i16:0, 16:1ω7t, 16:1ω7c, i17:0, a17:0, 18:1ω7c, and cy19) and actinobacteria (10Me16:0; Matreya, Pleasant Gap, Pennsylvania, USA). A standard control biomarker (c19:0) was used to convert peak area concentrations into nmol PLFA per g dry soil.

Fungal diversity and community composition were assessed by ITS metabarcoding. DNA was extracted from the organic horizon and mineral soil samples (0.25 g) using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, California, USA). The ITS2 region was amplified using polymerase chain reaction (PCR) and the fungal-specific primer pair fITS7 (Ihrmark et al. 2012) and ITS4 (White et al. 1990). Primers have been available to study the ITS region of fungi since the 1990s (White et al. 1990, Gardes and Bruns 1993) and, more recently, have been modified to more accurately target fungi (Ihrmark et al. 2012). We chose to study the ITS2 region because divergence across this locus permits species-level identification—identification to the genus/species level is necessary for assigning functional guild, and the most robust fungal reference sequence databases are for the ITS region. There are some drawbacks to studying the ITS locus; notably, it is not comprehensive at detecting early-diverging fungal lineages and does not accurately discriminate glomeromycetes (AMF; Stockinger 2010). Nonetheless, the ITS locus has been identified as the universal fungal barcode (Schoch et al. 2012).

Polymerase chain reaction 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, Appendix S1: Table S1). Polymerase chain reaction were performed in triplicate for each sample in 25 μL reaction volume with the following reagents: PCR-grade H_2O (13 μL), Five Prime Hot Master Mix (10 μL; 5 PRIME, Gaithersburg, Maryland, USA), 10 μmol/L fITS7 (0.5 μL), 10 μmol/L ITS4 (0.5 μL), and template DNA (1 μL). Thermocycler conditions followed that of Caporaso et al. (2011). Polymerase chain reaction products were cleaned using the

AxyPrep MAG PCR Clean-up kit (Corning, Tewksbury, Massachusetts, USA). 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, New York, USA). Equimolar libraries of the 72 samples (36 organic horizon and 36 mineral soil samples) were split by soil depth on separate Illumina MiSeq v2 runs (2 × 250 bp chemistry) at the Center for Genomics and Bioinformatics at Indiana University, Bloomington, Indiana, USA. Raw sequences are publically available at the NCBI database using accession number SRP090651.

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) with a 50-bp overlap and allowing 5% mismatch. Chimeric sequences were removed and the ITS2 region was extracted using ITSx (Bengtsson-Palme et al. 2013). 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, and sequences were aligned against the complete UNITE database (10 September 2014 release). Sequences that were not assigned a taxonomy at the phylum level using UCLUST and the UNITE database were parsed from the OTU table and subjected to blastn inquiry against the complete NCBI nucleotide database. From that blastn inquiry, we used MEGAN (v5; Huson et al. 2007) to assign sequences a taxonomy and removed all non-fungal sequences. Since the phylogeny of early-diverging Zygomycota (as formerly known) is not fully resolved (Hibbett et al. 2007), we amended our taxonomic outputs for such clades to reclassify the Zygomycota into two phyla (Mucoromycota and Zoopagomycota), with the glomeromycetes as a clade within the Mucoromycota (Spatafora et al. 2016).

Lastly, we assigned functional annotation to OTUs using a similar approach to FUNGuild (Nguyen et al. 2015). Genera were annotated as saprotrophs or pathotrophs using curation from Tedersoo et al. (2014), ectomycorrhizal using curation from the UNITE ectomycorrhizal database (Kõljalg et al. 2005), and arbuscular mycorrhizal if they were annotated as glomeromycetes. Even though our functional annotation was performed prior to publication of FUNGuild (Nguyen et al. 2015), we subsequently used FUNGuild to assess and compare our assignments. Of the OTUs identified at the genus level, ~95 and 99% of them were assigned to a guild using FUNGuild versus our approach, respectively. The main difference between approaches is that we assigned all glomeromycete OTUs to the arbuscular mycorrhizal guild, even if there was no genus identification, whereas FUNGuild only assigns function for OTUs identified at the genus level. Otherwise, functional assignments were comparable between the two approaches. A list of all identified genera and their functional annotations is available in Appendix S1: Table S2.

Fungal sequence processing information

There were 12–18 million sequences in total. Removing low-quality bases and reads removed <1% of the sequences. Merging forward and reverse reads removed 28–29% of the sequences, and ITS extraction removed the 5–10% (ranges represent the difference between the organic horizon and mineral soil samples, respectively). After all quality control steps, we retained 62–65% of the initial reads. The quality control, paired end reads, clustered into 6000–9000 OTUs (see rarefaction curves in Appendix S1: Fig. S1), of which 78–80% could be assigned a taxonomic identity. Operational taxonomic units that had no match to fungi in the NCBI database represented only 0.4% of the total read pool and were removed from the final OTU table. At the genus level for each OTU, we annotated 900–1200 saprotrophic fungal OTUs (23–25% of all sequences), 300–400 ectomycorrhizal OTUs (18–19% of sequences), and 170–250 pathotrophic OTUs (1.5–2% of sequences) for the organic horizon and mineral soil samples, respectively (Appendix S1: Table S2).

Soil chemical properties

Soil samples from organic horizons were analyzed for pH, total organic carbon (C) and N, microbial biomass, and in the mineral soil, only total inorganic N (NO_3^- + NH_4^+) 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, Massachusetts, USA). Total inorganic N was extracted from mineral soil using 2 mol/L KCl (10 g soil:40 mL KCl) and analyzed using a vanadium (III) reduction for NO_3^- and a modified Berthelot reaction for NH_4^+ (Braman and Hendrix 1989). Amino acids were extracted with 0.5 mol/L sodium acetate (4 g soil:10 mL sodium acetate) and their concentrations determined using the fluorometric *o*-phthaldialdehyde and β-mercaptoethanol method with a leucine standard curve (Jones et al. 2002).

Statistical analyses

All statistical analyses were conducted in R 3.0.2 (R Development Core Team 2008), with significance across all tests set at $P \leq 0.05$. Linear mixed-effects models were used to look for effects of site, invasion status, and site × invasion status on univariate fungal and soil response variables 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. All of the edaphic properties were also analyzed together as Euclidean distances and analyzed using principle coordinate analysis (PCoA) with the pcoa function in the ape package. Fungal community analyses were run on a rarified OTU table, with 40,311 sequences per sampling unit. Species (OTU) richness, Shannon's diversity index, and Simpson's index were calculated using the specnumber, diversity, and simp functions within the vegan package (Oksanen et al. 2007). Multivariate analyses of fungal community composition and soil properties were run using resemblance-based permutation methods. Permutation ANOVA (PERMANOVA; Anderson 2001) and heterogeneity of multivariate dispersion (PERMDISP; Anderson and Walsh 2013) were run using the functions adonis and betadisper in the vegan package. Indicator species analysis (Dufrene and Legendre 1997) was run using the multipatt function within the indicspecies package. For indicator species analysis, we examined two individual value components that were used to create the indicator values A and B which corresponded with how specific a fungus was to an invasion status and the probability of finding a fungus in all of the sampling units for a particular invasion status, respectively. Distance-based analyses for fungi were performed on Bray–Curtis dissimilarity matrices calculated from OTU relative abundance. Significance of permutation methods was determined after 1000 permutations. Non-metric multidimensional scaling (NMDS) was used to visually display fungal community composition using the metaMDS function (vegan).

Finally, partial least-squares regression (PLSR) was used to identify covariates (soil properties and garlic mustard relative abundance) most strongly correlated with fungal community richness and fungal guild relative abundance using the plsr function within the pls package. We fit PLSR models to the kernel algorithm and used leave-one out cross-validation. Response variables included total, saprotrophic, ectomycorrhizal, and pathotrophic fungal richness, the relative abundance of saprotrophic and ectomycorrhizal fungi, their ratio, and the relative abundance of pathotrophic fungi. Predictor variables included the relative abundance of garlic mustard and all soil parameters. Unlike multiple regression, PLSR allows for predictor variables to be auto-correlated (Wold et al. 2001). 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). Predictor variables with the highest VIP were then analyzed independently using linear regression. We inspected all parametric models based on QQnorm plots and Shapiro–Wilk tests of normality on model residuals.

Results and Discussion

We characterized fungal communities and edaphic properties (pH, total organic C and N, microbial biomass, total inorganic N, and amino acid concentrations) in the organic and mineral horizons of soils collected from uninvaded and invaded plots at six temperate forest sites (Table 1). There were three replicate plots per invasion status at each site for a total of 72 samples. Garlic mustard relative abundance in invaded plant communities ranged from 19 to

38%, a density typical for the region (Cavers et al. 1979, Byers and Quinn 1998, Stinson et al. 2007).

Relative abundance of taxa, functional guilds, and dominant fungi between invasion statuses

Basidiomycetes dominated the fungal community in both uninvaded and invaded soils, ranging from 31 to 50% relative sequence abundance (Appendix S1: Fig. S2). Ascomycetes averaged 19% across invasion statuses, and Mucoromycota averaged 12%. Other phyla (Zoopagomycota, Chytridiomycota, Rozellomycota, and glomeromycetes within the Mucoromycota) made up <1–5% of identified sequences. The relative abundance of basidiomycetes did not vary between uninvaded and invaded plots, but the relative abundance of ascomycetes was higher in invaded plots ($F_{1,60} = 4.34$, $P = 0.005$), as was that of Mucoromycota ($F_{1,60} = 5.21$, $P = 0.001$). Since general ITS primers are biased against glomeromycetes (AMF; Stockinger et al. 2010), AMF were not included in subsequent analyses of functional groups.

The relative abundance of broad functional groups was significantly different in association with invasion (Fig. 1). Saprotrophic and ectomycorrhizal fungi had similar relative abundance in uninvaded plots (20–28%), but invaded plots contained 41–43% higher saprotrophic relative abundance and 66–82% lower relative abundance of ectomycorrhizal fungi. The ratio of saprotroph to ectomycorrhizal sequences was 0.49 in uninvaded soil and 1.1 in invaded soil ($F_{1,60} = 3.95$, $P = 0.05$). The relative abundance of pathotrophic fungi was less than that of saprotrophic and ectomycorrhizal fungi but contained 55% higher relative abundance in invaded plots compared to uninvaded plots. Since sequence datasets permit estimates of relative abundance only, as the abundance of one group decreases, abundance of another group must increase. However, there were no differences in fungal biomass between invasion statuses (Table 2), suggesting that differences in relative abundance reflect different dominant fungal trophic guilds between uninvaded and invaded soils. That is to say, of the sequences annotated to a functional guild, invaded soils appear to be dominated by saprotrophic taxa, while soils without active garlic mustard invasion have a near-equal representation by saprotrophic and ectomycorrhizal taxa, with pathotrophic fungi comprising a smaller proportion of the community. While our results demonstrate that saprotrophs and pathotrophs co-exist with active garlic mustard invasion, our study does not provide a clear mechanism as to why ectomycorrhizal fungi would be uniquely sensitive relative to the other two fungal groups. Even though previous work has shown that garlic mustard invasion suppresses ectomycorrhizal colonization (Wolfe et al. 2008, Castellano and Gorchov 2012), these studies did not compare ectomycorrhizal fungi to other fungal groups. Since ectomycorrhizal fungi are distributed throughout the fungal phylogeny, it is unclear what specific inhibitory effect of garlic mustard would impact the various clades of ectomycorrhizal fungi but not their saprotrophic and pathotrophic relatives. This may then be an indirect response to an ecosystem-level change caused by invasion or a reflection of garlic mustard habitat preferences. Since garlic mustard invasion is associated with enhanced soil nutrient availability (see *Relationships among fungal and soil parameters and garlic mustard abundance*), ectomycorrhizal plants in invaded soils may be less reliant on their symbiotic partners for nutrient acquisition, leading to a decline in ectomycorrhizal fungal abundance.

Saprotrophic fungi

Dominant members of the saprotrophic community included the Mucoromycota and Agaricomycetes, with the former significantly more abundant in invaded plots alongside the less common ascomycotan classes Pezizomycetes and Sordariomycetes (Fig. 1A, B). Some saprotrophic genera were novel to invaded plots, including *Antrodiella, Dactylella, Arthrobotrys*, and *Junghuhnia*, which were among 14 significant saprotrophic indicator taxa associated with invasion (Table 3). In contrast, there were only three significant saprotrophic indicator taxa associated with uninvaded plots. The invaded plots did not favor one particular group of saprotrophs, with *r*-strategists within the Mucoromycota (e.g., genus: *Mortierella, Umbellopsis*; Appendix S1: Fig. S3; Brabcová et al. 2016), yeasts (e.g., genus: *Leucosporidium, Mastigobasidium;* Tedersoo et al. 2014), weak ascomycotan decomposers (e.g., class: Pezizomycetes, Sordariomycetes; Morrison et al. 2016), and white rot basidiomycotan decomposers (e.g., genus: *Antrodiella, Junghuhnia;*

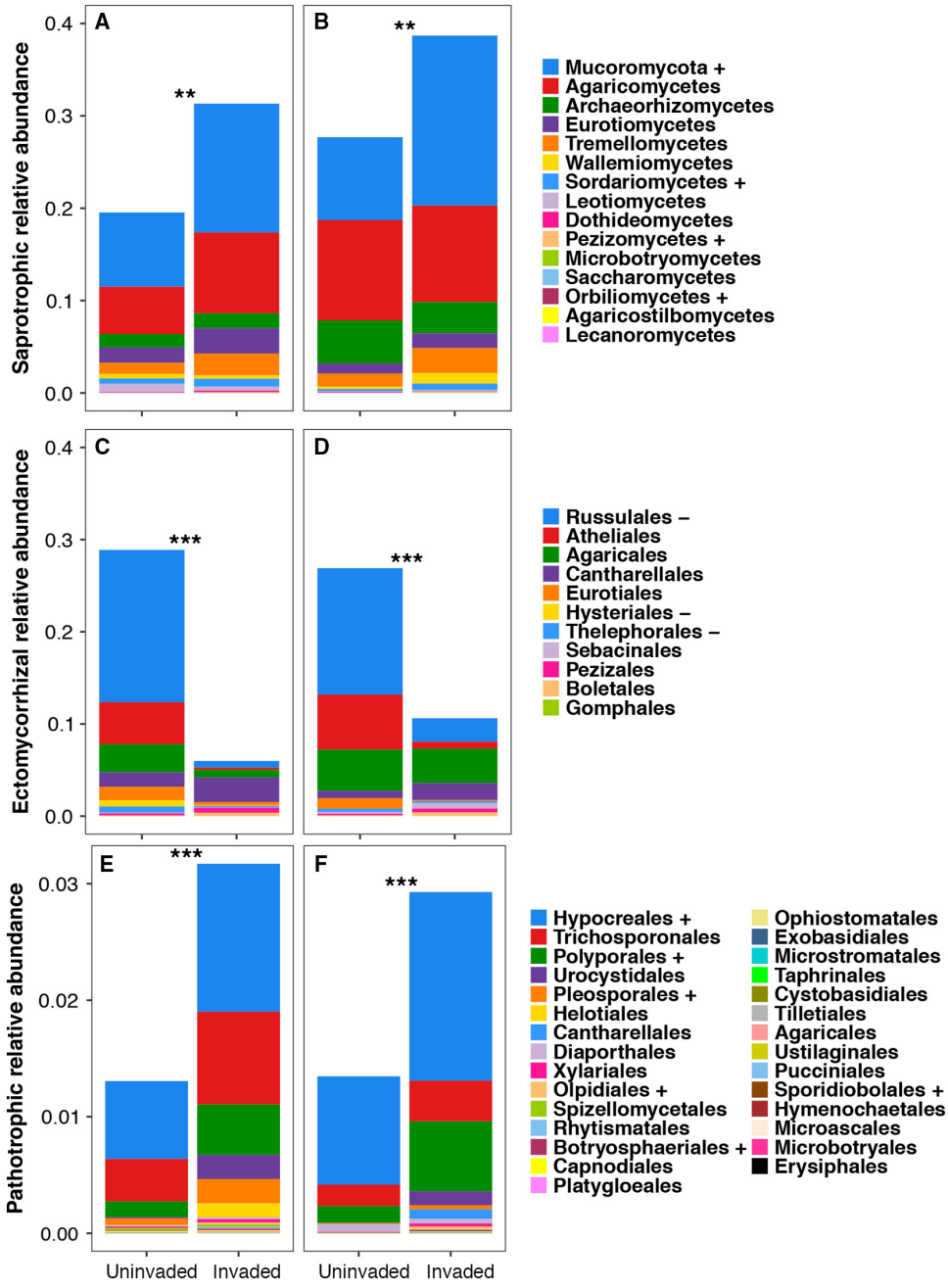

Fig. 1. The relative abundance of saprotrophic, ectomycorrhizal, and pathotrophic fungal taxa for uninvaded and invaded plots in the organic horizon (A, C, E) and mineral soil samples (B, D, F). Saprotrophic fungi are shown at the class level due to their high diversity, whereas ectomycorrhizal and pathotrophic taxa are displayed at the order level. Total bar height represents the mean relative abundance of each functional group (n = 18). Relative abundance is stacked in rank abundance for each soil horizon and invasion status. Significant differences between uninvaded and invaded soils are indicated with asterisks where $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. In the figure key, significant increases and decreases in the relative abundance of a specific taxon between uninvaded and invaded soils averaged across soil horizons are indicated using + and − signs, respectively ($P \leq 0.05$).

Table 2. Soil microbial biomass, fungal diversity, and chemical properties for the organic horizon and mineral soil samples at uninvaded and invaded plots averaged across six temperate forests.

Characteristics	Organic horizon		Mineral soil	
	Uninvaded	Invaded	Uninvaded	Invaded
Microbial biomass (nmol PLFA/g soil)				
Total microbial biomass	320 (46)a	297 (41)a	136 (12)a	140 (13)a
Fungal biomass	39.5 (9.2)a	34.0 (8.5)a	17.1 (2.6)a	19.3 (3.7)a
Bacterial biomass	211 (38)a	200 (39)a	103 (12)a	105 (13)a
Fungal/bacterial ratio	0.17 (0.02)a	0.15 (0.02)a	0.16 (0.01)a	0.18 (0.02)a
Fungal diversity (OTUs)				
Total richness (*S*)	888 (117)a	1,229 (102)b	570 (63)a	979 (65)b
Saprotrophic *S*	222 (19)a	307 (17)b	166 (13)a	254 (8)b
Ectomycorrhizal *S*	74 (8)a	109 (7)b	34 (4)a	50 (4)b
Pathotrophic *S*	23 (4)a	33 (6)b	31 (2)a	58 (3)b
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
Soil chemical properties				
Ammonium (μg/g soil)	–	–	17.3 (9.70)a	17.0 (9.84)a
Nitrate (μg/g soil)	–	–	1.95 (0.72)a	4.82 (1.56)b
Amino acids (μg/g soil)	–	–	65.4 (13.0)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

Notes: Values represent the mean ± 1 SE (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. PLFA, phospholipid fatty acid; OTUs, operational taxonomic units.

Tedersoo et al. 2014) all exhibiting higher relative abundance in soil collected from invaded compared to uninvaded plots. This diverse assemblage of saprotrophic fungal groups represents a broad capacity for mineralizing C substrates of varying chemical complexities (Floudas et al. 2012) leading to the hypothesis that soil organic C contents, which were lower in invaded than in uninvaded plots (Table 2), differed between invasion statuses due to enhanced saprotrophic capacities for decay. Although we did not measure decomposition per se, previous work has shown that leaf litter decay is more rapid in garlic mustard-invaded soils (Rodgers et al. 2008*a*).

Ectomycorrhizal fungi

The Russulales dominated the ectomycorrhizal community in uninvaded plots (13–16% relative abundance); however, the relative abundance of this group declined with invasion to <1–3% (Fig. 1C, D). This group was comprised predominantly of *Russula*, which was the most abundant genus in our dataset (Appendix S1: Fig. S3). *Russula* exhibited a ~90% lower relative abundance in invaded soil (Appendix S1: Fig. S3), which is consistent with earlier work showing that this genus colonized host plant roots less in garlic mustard-invaded forests (Castellano and Gorchov 2012). As an ectomycorrhizal indicator taxon of uninvaded plots, *Cenococcum* was less abundant than *Russula*, but was present in every uninvaded plot while being nearly absent from invaded plots. Since key forest trees such as oak, pine, and birch form ectomycorrhizae, loss of both abundant (e.g., *Russula*) and regionally ubiquitous (e.g., *Cenococcum*) ectomycorrhizal symbionts may explain why ectomycorrhizal root tip colonization of native tree seedlings has been shown to decline with garlic mustard invasion (Wolfe et al. 2008, Castellano and Gorchov 2012). Dead ectomycorrhizal mycelia are also hotspots for other microorganisms, including saprotrophic fungi and pathotrophs (Brabcová et al. 2016). Since living mycorrhizal fungi also deter plant pathogens that colonize roots (Newsham et al. 1995), loss of living ectomycorrhizal fungi might open niche space for pathotrophic clades.

Table 3. Indicator species analysis for fungal genera that were significantly associated with uninvaded or invaded plots.

Genus	Guild	A	B	IndVal	*P*
Organic horizon					
Uninvaded					
Cenococcum	Ectomycorrhizal	0.91	1	0.95	0.001
Gallerina	White rot	0.98	0.76	0.87	0.04
Invaded					
Gliocladium	Mycoparasite	0.96	1	0.98	0.001
Dendryphion	Plant pathogen	1	0.63	0.79	0.001
Olpidium	Plant pathogen	1	0.44	0.66	0.004
Dothidea	Saprotroph	0.81	0.75	0.78	0.01
Cosmospora	Mycoparasite	0.87	0.69	0.77	0.01
Scopuloides	White rot	0.92	0.56	0.72	0.02
Armillaria	White rot	0.76	0.56	0.72	0.02
Lophiostoma	Saprotroph	0.84	0.94	0.89	0.03
Boletinellus	Brown rot	0.89	0.88	0.89	0.03
Scutellinia	Saprotroph	0.91	0.44	0.63	0.03
Boletus	Ectomycorrhizal	0.94	0.38	0.6	0.03
Gautieria	Ectomycorrhizal	0.92	0.38	0.59	0.03
Clitopilus	Saprotroph	0.99	0.63	0.79	0.04
Peziza	Saprotroph	0.82	0.56	0.68	0.04
Steccherinum	White rot	0.82	0.5	0.64	0.04
Leucosporidium	Yeast	0.82	0.38	0.55	0.04
Epicoccum	Plant pathogen	0.87	0.75	0.81	0.04
Mollisia	Plant pathogen	1	0.25	0.5	0.04
Antrodiella	White rot	1	0.25	0.5	0.04
Mineral soil					
Uninvaded					
Megacollybia	Saprotroph	1	0.28	0.53	0.04
Xenasmatella	White rot	1	0.28	0.53	0.05
Invaded					
Psathyrella	Saprotroph	0.97	0.83	0.9	0.001
Fusarium	Plant pathogen	0.93	0.72	0.82	0.001
Olpidium	Plant pathogen	1	0.67	0.81	0.001
Cystofilobasidium	Facultative yeast	0.88	1	0.93	0.002
Clonostachys	Plant pathogen	0.99	0.61	0.79	0.002
Pseudotomentella	Ectomycorrhizal	0.9	0.56	0.71	0.003
Dendryphion	Plant pathogen	1	0.5	0.71	0.004
Calvatia	Saprotroph	0.84	0.78	0.81	0.005
Diplodia	Plant pathogen	0.92	0.56	0.71	0.006
Hypoxylon	Saprotroph	0.87	0.72	0.79	0.007
Nemania	Saprotroph	0.84	0.78	0.81	0.009
Hypholoma	White rot	0.81	0.72	0.77	0.01
Boletus	Ectomycorrhizal	0.99	0.56	0.74	0.01
Hypochnicium	White rot	0.85	0.61	0.72	0.01
Eutypella	Plant pathogen	0.91	0.5	0.68	0.01
Dactylella	Saprotroph	1	0.33	0.58	0.02
Neonectria	Plant pathogen	0.82	0.61	0.71	0.02
Mastigobasidium	Yeast	0.91	0.5	0.68	0.03
Lopadostoma	Saprotroph	0.93	0.39	0.6	0.03
Pochonia	Animal parasite	0.84	0.56	0.68	0.03
Arthrobotrys	Saprotroph	1	0.28	0.53	0.04
Cylindrocladiella	Plant pathogen	0.95	0.33	0.56	0.05
Junghuhnia	White rot	1	0.28	0.53	0.05

Note: The guild, specificity (A, where 1 = found exclusively in one treatment), fidelity (B, where 1 = observed in all samples belonging to one treatment), indicator value (Indval), and *P* values are shown for each significant indicator genus ($P < 0.05$).

Pathotrophic fungi

Pathotrophic fungal relative abundance was significantly higher in association with invasion. Pathotrophic taxa within the orders Hypocreales (genera: *Fusarium* and *Volutella*), Polyporales (genus: *Ganoderma*), Pleosporales, Olpidiales, Botryosphaeriales, and Sporidiobolales all exhibited significantly higher relative abundance in invaded compared to uninvaded plots. Although we know fungal pathogens can affect invasive plant performance and the resident plant community (Flory and Clay 2013), surprisingly little work has addressed how pathogens themselves respond to plant invasions (Mangla and Callaway 2008, Stricker et al. 2016). There is some evidence for plant invasions to increase abundance of specific plant pathogens (Mangla and Callaway 2008, Stricker et al. 2016), such as the tropical shrub, *Chromolaena odorata*, which can increase *Fusarium semitectum* abundance upward of 250% (Mangla and Callaway 2008), but our results suggest that a broader assemblage of pathogens accumulate in soils with biotic invasion. Notably, we identified a suite of pathotropic indicator taxa that were novel to invaded soils, including *Olpidium*, *Dendryphion*, and *Mollisia* (Table 3). Of considerable interest, *Olpidium* was comprised of a single OTU that was present in nearly all invaded plots but none of the uninvaded plots and was identified as *Olpidium brassica*, a fungal vector for a number of deadly plant viruses (Hartwright et al. 2010) with spores that can remain dormant and infective for up to 20 yr (Campbell 1985). We should note that despite being assigned a function of plant pathogen by comparison to the functional database we used (Tedersoo et al. 2014), fungi in this group may also act as saprotrophs when their hosts are not present. Here, 29% of the genera designated pathotrophic are known to behave as parasites when their hosts are present and saprotrophs when their hosts are absent, including the indicator taxon, *Mollisia* (Nguyen et al. 2015). Nevertheless, while our current study does not provide evidence that pathotrophic fungi are causing disease or affecting plant performance, our results do provide the first survey of pathotrophic diversity in garlic mustard-invaded soil and suggest that a diverse community of pathotrophs (or facultative pathotrophs) accumulate in soils with biotic invasion, making this an important area for future research.

Fungal diversity and community composition

Taxonomic (OTU) richness was consistently higher in invaded compared to uninvaded plots (Table 2). Saprotrophic fungi exhibited higher OTU richness than the other functional groups, but richness for all groups was higher in association with invasion. This included rare and endemic ectomycorrhizal fungi, which may be more opportunistic and weaker symbionts than abundant and ubiquitous taxa (Tedersoo et al. 2010), like *Russula* and *Cennoccocum*. In terms of relative abundance, there were fewer dominant fungal taxa and greater fungal community evenness in association with invasion, as estimated by Shannon's and Simpson's indices of diversity (Table 2). Higher fungal richness and greater evenness in association with invasion suggest that the absence of dominant (particularly ectomycorrhizal) taxa creates open niches for proliferation by different groups and is congruent with established invasion theories that predict proliferation of disturbance-affiliated taxa at invasion "hotspots" (Stohlgren et al. 2003). Though this theory was established for plant communities, it may also apply belowground, as one mechanism for the theory of "hotspots" posits that the availability of resources in invaded habitats supports high levels of diversity (Stohlgren et al. 2003). Belowground, we found higher soil nitrate availability and lower soil C:N ratios in invaded compared to uninvaded plots (see *Relationships among fungal and soil parameters and garlic mustard abundance*), suggesting that N is less limiting in invaded soils. Ectomycorrhizal fungi also compete with saprotrophic fungi for soil N (Averill and Hawkes 2016) and, as discussed earlier, with pathogens for root space (Newsham et al. 1995). Loss of dominant ectomycorrhizal fungi from invaded soils may therefore allow proliferation of disturbance-affiliated fungi that could increase fungal diversity.

In contrast to alpha diversity, fungal beta diversity was higher in uninvaded than in invaded plots (PERMDISP: organic: $F_{1,24} = 11.91$, $P = 0.002$; mineral: $F_{1,24} = 19.15$, $P < 0.0001$). Beta diversity, which describes variation in community composition across a given landscape (Anderson et al. 2006), was not only lower in association with invasion, but distinct between invasion statuses for the entire fungal community (Fig. 2A, B; PERMANOVA: organic: $F_{1,24} = 1.89$, $P = 0.002$; mineral: $F_{1,24} = 0.63$, $P = 0.001$) and the saprotrophic,

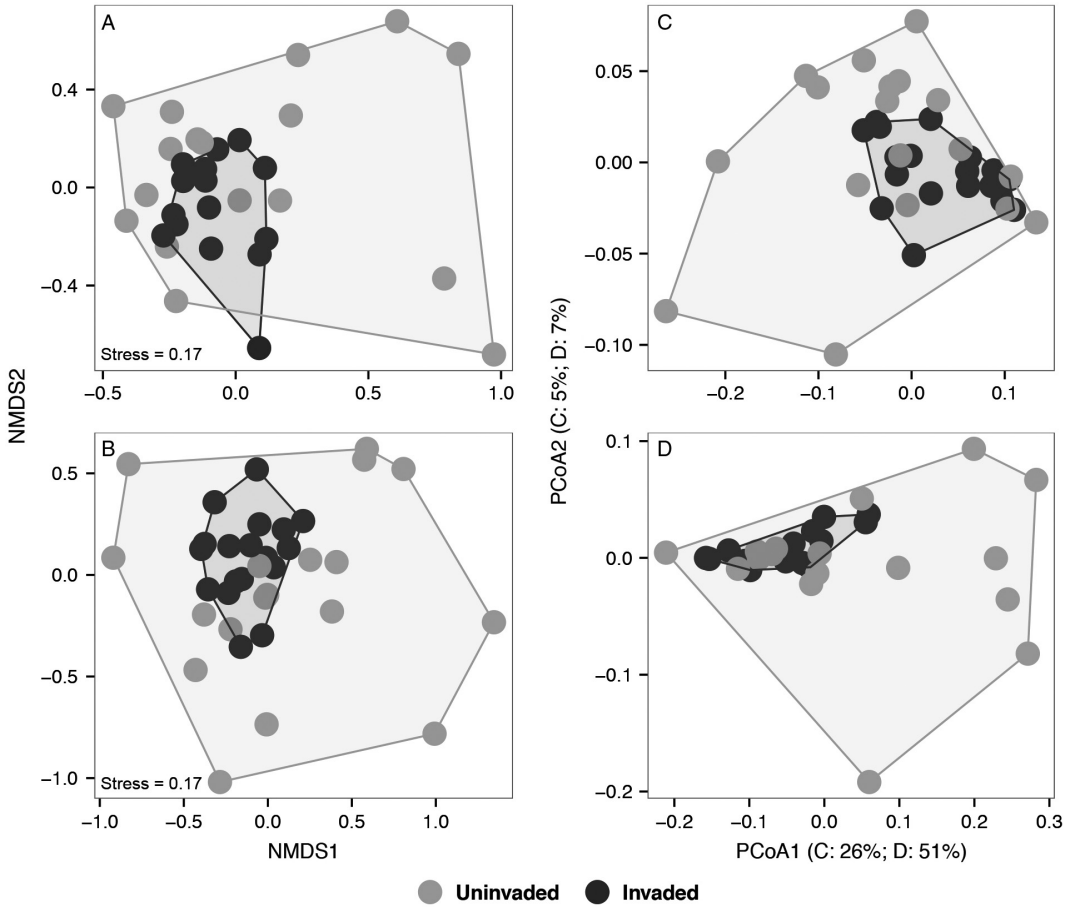

Fig. 2. Fungal community composition (A, B) and soil property composition (C, D) in uninvaded and invaded plots at six temperate forests for the organic horizon (A, C) and mineral soil samples (B, D). The relative abundance of fungal operational taxonomic units was converted to Bray–Curtis distances, collapsed into two nonmetric multidimensional scaling (NMDS) axes, and displayed with the ordination stress. Soil properties were converted to Euclidean distances, analyzed using principle coordinates analysis (PCoA), and displayed with percent variation explained for the first two PCoA axes. Convex hulls represent the range in compositions across each invasion status.

ectomycorrhizal, and pathotrophic groups when examined independently (Appendix S1: Table S3). Homogeneity in the fungal community was also present at two distinct spatial scales, with less variation in replicate invaded plots at a given site, as well as convergence in replicate invaded sites across the region. Notably, reduced heterogeneity in invaded plots was observed at the site invaded for ~63 yr (Black Rock) and the invaded plots at this site converged with invaded plots across the other sites (Fig. 2), suggesting that differences between uninvaded and invaded plots are sustained even when garlic mustard has been present for many decades. At regional scales, soil fungal communities are largely assembled through environmental filtering (Lekberg et al. 2007, Kivlin et al. 2014), with community convergence occurring where environmental conditions are relatively homogenous (Caruso et al. 2012). In our study, invaded soils were more homogeneous in both fungal community composition (Fig. 2A, C) and soil property composition (Fig. 2B, D), suggesting that reduced environmental heterogeneity in invaded soils may restrict variation in fungal

community composition (Maaß et al. 2014). We specifically compared the relationship between homogeneity in soil properties and fungal communities, finding that in the invaded plots only, homogeneity in soil properties was significantly correlated with homogeneity in fungal communities (R^2 = 0.26–0.43, P = 0.001–0.05; Appendix S1: Fig. S4). Determining whether garlic mustard invasion homogenizes fungal communities and soil properties or whether invasion more readily occurs in habitats with distinct fungal communities and soil properties is important for understanding the functional consequences of invasion or predicting which landscapes have greater susceptibility to garlic mustard invasion.

Relationships among fungal and soil parameters and garlic mustard abundance

Plant invasions often alter soil N availability and soil pH (see Ehrenfeld 2003, 2010). Here, we found that invaded plots had higher soil pH and nitrate concentrations and lower C:N ratios than uninvaded soils (Table 2). Previous work has reported similar effects of garlic mustard on pH and inorganic N (Rodgers et al. 2008*a*). There is also experimental evidence that the presence of garlic mustard increases soil pH and that higher soil pH promotes garlic mustard growth (Anderson and Kelley 1995). Thus, it seems likely that the higher nitrate levels and lower C:N ratios that we observed in invaded soils also benefit the proliferation of garlic mustard by increasing soil N availability (Hawkes et al. 2005, Rodgers et al. 2008*a*, *b*).

To characterize relationships between the fungal community, soil properties, and garlic mustard abundance, we used PLSR to identify covariates (garlic mustard abundance, soil moisture, soil pH, organic C and N contents, C:N ratio, and total inorganic N, ammonium, nitrate, and amino acid concentrations) of fungal community parameters (total, saprotrophic, ectomycorrhizal, and pathotrophic fungal richness, the relative abundance of saprotrophic and ectomycorrhizal fungi, and their ratio, and the relative abundance of pathotrophic fungi). Significant predictors of total fungal and saprotrophic richness were the relative abundance of garlic mustard in invaded plots, soil pH, and soil C:N ratio (Fig. 3). These results are consistent with continuum theory, suggesting that diversity gets filtered along specific environmental gradients (Austin 1985). Notably, fungal richness is often correlated with soil pH and C:N ratios at regional (Lauber et al. 2008, Thomson et al. 2015) and global scales (Tedersoo et al. 2014), and this is consistent with our results. Within the continuum concept, the diversity and abundance of particular fungal groups may vary across specific environmental gradients. We found that saprotrophic fungal richness was positively correlated with garlic mustard relative abundance and that this relationship was not observed for ectomycorrhizal or pathotrophic fungi. Since garlic mustard is non-mycorrhizal, saprotrophic fungi may achieve higher diversity with increasing garlic mustard densities because garlic mustard does not host mycorrhizal fungi that use resources saprotrophic fungi also require (Shah et al. 2009, Fernandez and Kennedy 2015).

Saprotrophic fungi, competition with mycorrhizal fungi, and plant invasions

Although we know that saprotrophic fungi are sensitive to abiotic global change stressors, including simulated N deposition (Morrison et al. 2016), soil warming (Geml et al. 2015), and land-use change (Lauber et al. 2008), to our knowledge, our study is the first to explicitly examine saprotrophic fungi within the context of invasion. Saprotrophs play a particularly important role in nutrient cycling as the dominant decomposers in forest soils (Talbot et al. 2014) and they interact with ectomycorrhizal fungi to decompose soil organic matter (Fernandez and Kennedy 2015). To this latter point, we found that there was a negative correlation between the ratio of saprotrophic to ectomycorrhizal fungi and soil C:N ratios (Table 2). Saprotrophs and ectomycorrhizal fungi compete for soil N (Fernandez and Kennedy 2015), and this competition can influence soil C concentrations and thus C:N ratios. Since C:N ratios were lower in invaded soil due to lower organic C contents, the negative correlation between the ratio of ectomycorrhizal to saprotrophic fungi and soil C:N ratio might be explained by the Gadgil effect, which posits that ectomycorrhizal fungi facilitate the accumulation of organic C pools since they do not use soil organic C for energy (Gadgil and Gadgil 1975), while saprotrophic fungi deplete organic C pools through their

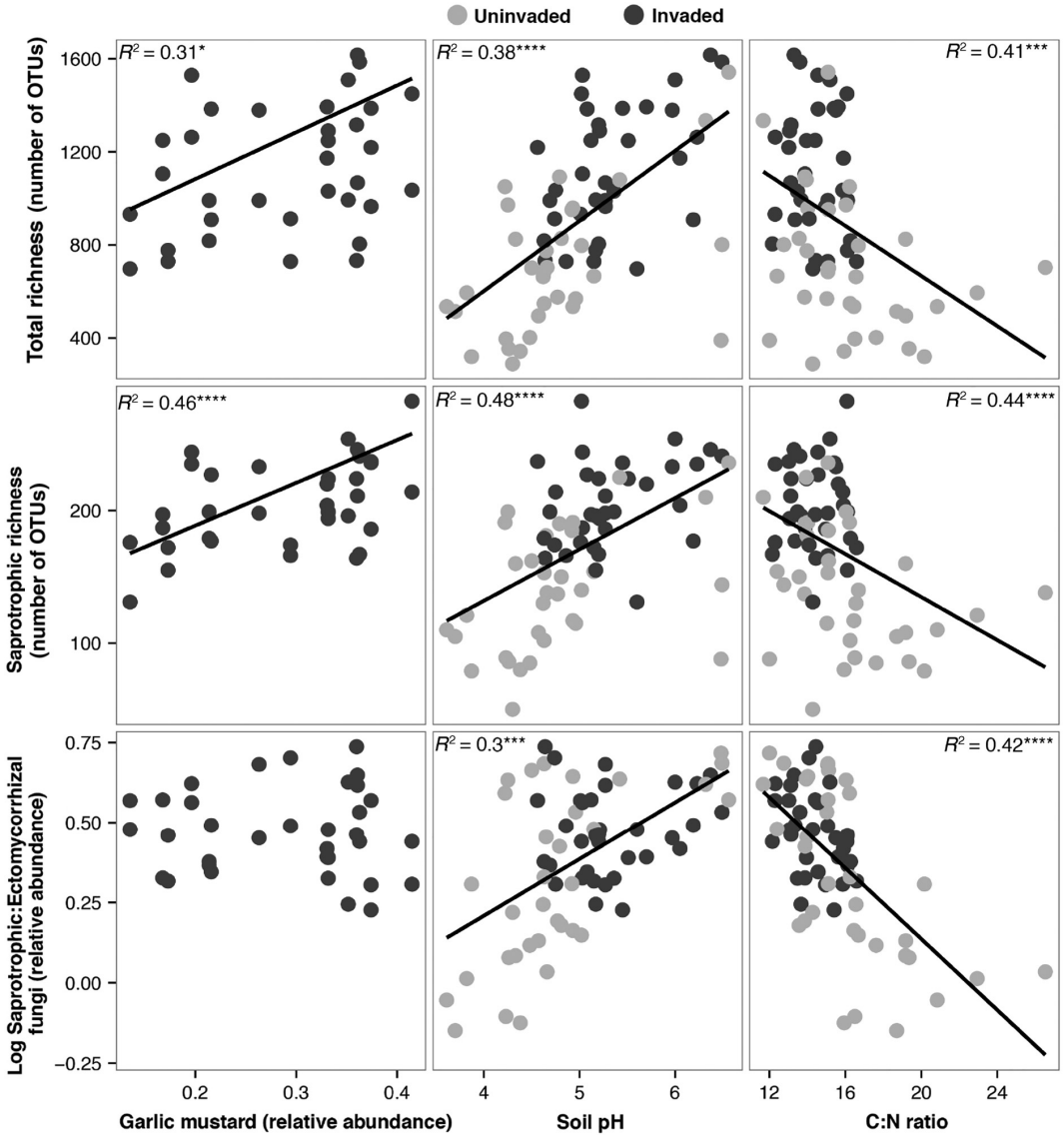

Fig. 3. Correlations between garlic mustard relative abundance, soil properties, and fungal community parameters. Significant correlations are indicated with asterisks where $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, $^{****}P \leq 0.0001$.

metabolism (Averill and Hawkes 2016). Thus, contexts in which ectomycorrhizal fungal abundance decreases and saprotrophic abundance increases, such as under active garlic mustard invasions observed here, may reduce soil C concentrations. But as noted previously, it is also plausible that garlic mustard invades soils with already-depleted soil C stocks and enriched soil N pools.

Conclusions

Garlic mustard invasion was associated with a distinct fungal community dominated by saprotrophic taxa and depleted of ectomycorrhizal fungi. Some ectomycorrhizal fungi common in uninvaded soils were virtually absent from invaded soils, while a suite of saprotrophic and pathotrophic taxa were exclusively found in

invaded soils. Garlic mustard invasion was also associated with increased OTU richness, mostly due to higher saprotrophic fungal richness, but significantly reduced community heterogeneity (beta diversity), the latter corresponding to a reduced heterogeneity in soil chemical properties as well. While species richness was greater in the invaded plots compared to the uninvaded plots, loss of beta diversity across the invaded landscape indicates that invasion will homogenize diversity in the region overall. The authors declare no conflict of interest.

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