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Organic nitrogen addition suppresses fungal richness and alters community composition in temperate forest soils

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ABSTRACT

Human-driven increases in bioavailable nitrogen over the last century have revealed the critical need to understand how nitrogen availability influences terrestrial ecosystems. In contrast to widespread evidence that increasing inorganic nitrogen strongly influences the diversity and composition of plant and microbial communities, the effect of organic nitrogen addition, the predominant form of nitrogen in soils, remains less clear. In this study, we conducted a field experiment manipulating both the amount and composition of soil organic nitrogen present in soil fungal in-growth bags, followed by molecular characterization of fungal communities after a four-month incubation. Saprotrophic and ectomycorrhizal fungi readily colonized experimental in-growth bags, indicating a broad overlap in the fundamental niches of these two functional guilds. Increases in labile forms of organic nitrogen resulted in sharp declines in species richness and diversity across fungal guilds, as well as notable shifts in fungal community composition. The relative abundance of fungi classified as molds and yeasts peaked where organic nitrogen was both high and most labile, whereas slower-growing saprotrophic and ectomycorrhizal fungi were more abundant in the non-amended treatments. Taken together, our results indicate that similar to inorganic nitrogen, increasing the amount of organic nitrogen can dramatically alter the richness and composition of fungal communities in temperate forest soils.

1. Introduction

Over the last 100 years, human activity has dramatically increased the bioavailable nitrogen in earth's biosphere (Galloway et al., 2008; Vitousek et al., 1997), which has led to major shifts in terrestrial biogeochemical cycling via changes in biotic communities (Liu and Greaver, 2010; Maaroufi et al., 2015). Numerous studies have demonstrated that the addition of inorganic nitrogen suppresses plant and microbial diversity (Bobbink et al., 2010; Borer et al., 2014; Clark and Tilman, 2008; Zak et al., 2011), and alters the composition of communities to favor fast-growing and nitrophilic taxa (Leff et al., 2015; Morrison et al., 2016; Xia and Wan, 2004; Zhang et al., 2018). While these relatively recent shifts in inorganic nitrogen availability have important implications for ecological functioning in many systems, they occur in conjunction with natural gradients of organic nitrogen. Globally, organic nitrogen availability varies notably by latitude (Zinke et al., 1998), representing up to 95% of the soluble nitrogen fraction in some high latitude ecosystems (Chalot and Brun, 1998). Within ecosystems, soil nitrogen often shifts in dominant form from inorganic to organic during vegetational succession (Le Duc et al., 2013) and, at local scales, biomass inputs of dead plants and animals can lead to local 'hotspots' in organic nitrogen abundance (Macdonald et al., 2014). Collectively, this variation in the soil organic nitrogen quantities at multiple spatial and temporal scales likely has important impacts on the distribution of a wide range of organisms.

While inorganic nitrogen is present primarily as ammonia or nitrate, soil organic nitrogen exists as a diverse array of compounds, ranging from simple labile forms such as amino acids to more complex and insoluble aromatic substances (Talbot and Treseder, 2010). A large portion of soil organic nitrogen is unavailable for direct plant uptake (Geisseler et al., 2010), and microbial enzymatic activity is required to depolymerize organic substrates and liberate nitrogen for plant access (Schimel and Bennett, 2004). As mediators of soil biogeochemical cycles and plant nutrient availability, understanding how microbial communities respond to changes in organic nitrogen is key to deciphering how this form of nitrogen influences biological communities. Furthermore, because organic nitrogen has more complex forms relative to inorganic nitrogen, it is likely that microbial responses to

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organic nitrogen depend on the ability of different taxa to metabolize its variable components (Talbot and Treseder, 2010).

Fungi are primary decomposers of complex organic compounds (Baldrian, 2006; Floudas et al., 2012) and thus key determinants of the fate of organic nitrogen in soil (Talbot et al., 2011; van der Wal et al., 2015). Saprotrophic fungi are generally considered efficient decomposers because of their need to extract energy and acquire nutrients directly from complex organic substrates. Within this group, however, there are a number of fast-growing molds and yeasts with limited abilities to utilize complex organic substrates (Frankland, 1998; Hudson, 1968). Root-associated fungi have been thought to have limited abilities to depolymerize complex forms of organic matter due to their reliance on simple sugars provided by their plant hosts. However, evidence is mounting that certain lineages of ectomycorrhizal fungi can decompose organic matter via oxidative mechanisms (Lindahl et al., 2007; Bödeker et al., 2014), most likely to access bound organic nitrogen (Lindahl and Tunlid, 2015). In addition, ectomycorrhizal fungi grow readily with amino acids as their sole nitrogen source in culture (Talbot and Treseder, 2010), indicating they likely have a significant influence on soil organic nitrogen in the ecosystems where they are present.

It is well documented that inorganic nitrogen enrichment can significantly affect fungal community structure (Morrison et al., 2016, and references therein). By comparison, the influence of organic nitrogen addition on fungal community structure is less well understood. Boberg et al. (2011) demonstrated that adding labile nitrogen (i.e., glycine) consistently enhanced the growth of saprotrophic fungi colonizing forest litter, a pattern not observed with more recalcitrant organic nitrogen forms. These results are consistent with other studies demonstrating that additions of more complex organic substrates have mixed effects on saprotrophic fungal community structure, stimulating certain taxa at the expense of others (Allison et al., 2009). Over short time scales (i.e., months), the addition of labile organic nitrogen (glutamic acid) has been shown to stimulate ectomycorrhizal fungal colonization, although this effect appears to vary depending on whether the tree host is a gymnosperm versus angiosperm (Avolio et al., 2009) as well as which ectomycorrhizal fungal species are present (Hedwall et al., 2017). Over longer time scales (i.e., decades), significant changes in ectomycorrhizal fungal communities have been directly linked with soil organic nitrogen levels (Kyaschenko et al., 2017; Le Duc et al., 2013), suggesting the addition of organic nitrogen may be a stronger driver of shifts in ectomycorrhizal fungal community composition relative to changes in species richness.

Key to elucidating how organic nitrogen affects fungal community structure is to decouple changes in availability (low versus high quantity) from changes in form (i.e., soluble forms versus insoluble forms that require depolymerization for nitrogen access). Doing so will help to identify which component of organic nitrogen addition is most influential, and the extent to which responses vary among different groups of fungi. To address this knowledge gap, we conducted a field study in Minnesota, USA that manipulated both the amount and composition of soil organic nitrogen present in soil in-growth bags. The bags were incubated *in situ* for four months at eight sites with varying tree species composition (*Pinus* or *Quercus* canopy dominance). Using high-throughput molecular methods, fungal communities were characterized by α- and β-diversity, the relative abundance of fungal guilds (e.g., saprotrophs, pathogens, mycorrhizae), and life history strategy (i.e., fast-growing ruderals versus slow-growing non-ruderals). Parallel to microbial richness suppression observed during experimental addition of inorganic nitrogen, we hypothesized that increased organic nitrogen availability would decrease fungal community richness by favoring fast-growing fungi that are able to access labile organic N substrates (e.g., amino acids, proteins) and proliferate. Because of the relatively short duration of the incubation, we expected significant colonization by fast-growing molds in particular. However, we hypothesized this group would have lower relative abundance when organic nitrogen was supplied as soil organic matter versus as a suite of relatively labile organic nitrogen compounds because of this group's limited ability to depolymerize complex organic substrates. In contrast, we expected that saprotrophic and at least some ectomycorrhizal fungi with the capacity to enzymatically depolymerize soil organic matter would increase in relative abundance when organic nitrogen was supplied as soil organic matter.

2. Materials and methods

2.1. Experimental design

To manipulate the amount and composition of organic nitrogen, soil was harvested from the A and C horizons of an undisturbed forest in Wellston, Michigan, (typic haplorthod of the Kalkaska series; 44°33′00” N 85°46′48” W) in July 2015. We chose soils from this location for two reasons. First, the absence of ectomycorrhizal plant hosts (canopy dominated by *Acer saccharum*) decreased the likelihood that members of this fungal guild were initially present in the soils. More importantly, soils from two different horizons enabled us to create two treatments

Table 1
Organic components added to create the labile N treatment. Percent ON (organic nitrogen) in natural soil was determined from Talbot and Treseder (2010).

Type of ON	% ON in Natural Soil	Representative Compound(s)	% of total ON added to labile N	Compound mass added to labile N treatment (5L soil)
Proteins	13–85%	BSA	25%	16.41 g
		Tryptone	15%	12.75 g
Aromatic (cyclic; includes Purines/Pyrimidines)	18–58%	Imidazole	10%	2.55 g
		Uracil	15%	4.21 g
		Guanine	5%	1.13 g
Amino sugars	≤30%	Chitin	10%	16.59 g
		N-Acetyl-D-glucosamine	10%	16.59 g
N-containing Phospholipids (e.g., phosphotidycholine)	≤20%	(none)[a]	–	–
Others (includes free amino acids)	5-10%[a]	Urea	5%	1.13 g
		Glutamine	4%	2.19 g
		Glutamate	2.50%	3.17 g
		Threonine	2.50%	2.23 g
		Tryptophan	1%	0.77 g

[a] To avoid adding additional organic P, phospholipids were not included, leading to an increase in the “other” category.

Table 2
Characterization of soil treatments and forests for soil pH, moisture and total C and N content. Forest values were averaged across forest sites.

	Sample	pH	Moisture	total C (mg C g^{-1} soil)	total N (mg N g^{-1} soil)	C:N
Soil treatments	low SOM	6.19	0.0003	0.74	0.10	7.01
	high SOM	5.61	0.0003	27.40	2.24	12.24
	labile N	7.00	0.0014	7.06	2.27	3.11
Forest	*Pinus*	5.37	0.10	12.7	0.97	13.1
	Quercus	4.32	0.20	36.8	2.30	16.0

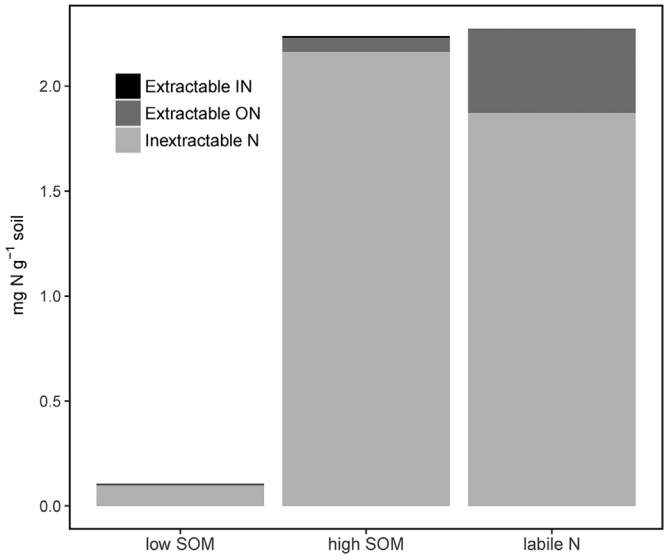

Fig. 1. Nitrogen (N) pools comprising the three experimental soil treatments, ordered across the x-axis from lowest to highest organic N content. Pools include extractable inorganic N (IN), extractable organic N (ON) and inextractable N, calculated as the difference between total N and extractable N pools. Extractable IN was present in very low amounts, and can be visually detected in high SOM treatment. Values represent the average of three replicates per soil treatment. SOM represents soil organic matter.

that differed in the total amount of soil organic matter, one with high SOM and one with low SOM, while minimizing other differences in physical or chemical soil properties between treatments (Table 2; Zak et al., 1986; Zak and Pregitzer, 1990). Hereafter, we refer to these treatments as the 'high SOM' and 'low SOM' treatments, respectively (Fig. 1). To experimentally isolate the effects of soil organic nitrogen from those of soil organic matter, we generated a third experimental soil treatment (i.e., the 'labile N' treatment), in which we supplemented the C-horizon soil (i.e., low SOM) with a variety of organic nitrogen compounds naturally found in soils (Table 1; Talbot and Treseder, 2010). We enriched this C-horizon soil with enough organic nitrogen to match the total nitrogen abundance in the 'high SOM' treatment. While this type of nitrogen addition made it impossible to track responses to specific forms of organic nitrogen, we chose this approach because it more closely approximated the diverse array of organic nitrogen forms present in natural soils. Furthermore, we did not supplement the high SOM treatment with organic nitrogen, as we did not believe this experimental treatment represented an ecologically relevant environment.

Prior to experimental initiation, we assessed the size of the nitrogen pools in the three soil treatments (i.e., organic nitrogen, inorganic nitrogen, total nitrogen). As expected, the total nitrogen pool was equivalent between the high SOM and labile N treatments, and ~20x higher than the low SOM treatment (Fig. 1). The organic nitrogen addition in the labile N treatment increased the K_2SO_4-extractable organic nitrogen to 17.6% of total nitrogen, relative to 3.0% and 7.5% of high SOM and low SOM treatments, respectively (Fig. 1). In choosing the organic nitrogen compounds for our experimental amendment, we were careful to avoid substrates containing significant amount of phosphorus to avoid a secondary fertilization effect. However, the added compounds unavoidably contained carbon, so our organic nitrogen supplementation did increase carbon content of the labile N treatment relative to low SOM and also decreased the carbon:nitrogen ratio in this soil treatment (Table 2). The total nitrogen pool size was estimated via combustion using an elemental analyzer (Elementar varioPro, Mt. Laurel, NJ, USA) after drying soils for 48 h at 60 °C. Extractable organic nitrogen was measured by extracting soils with 0.5 M K_2SO_4, filtering (Whatman No. 42), and analyzing extracts on a Shimadzu TOC-V (Shimadzu Corporation, Kyoto, Japan). Dissolved inorganic nitrogen was quantified spectrophotometrically through a reaction with salicylate and hypochlorite, in the presence of sodium nitroprusside, for ammonium content (Bower and Holm-Hansen, 1980) and a reaction with vanadium chloride in the presence of sulfanilamide and N-(1-naphthyl)-ethylenediamine for nitrate content (Doane and Horwath, 2003). The ammonium and nitrate assays were measured following a 4 or 18-hour incubation, respectively, at an absorbance of 640 nm or 540 nm using a BioTek Synergy H1 hybrid microplate reader (Winooski, Vermont, USA). Eight technical replicates were conducted for each sample and inorganic nitrogen assays were carried out in 96-well plates. The extractable inorganic nitrogen pool was calculated as the sum of nitrate and ammonium values per soil treatment, and the insoluble organic nitrogen pool was calculated as the difference between total nitrogen and extractable organic and inorganic nitrogen pools.

Soils from the three treatments were individually added to 53 μm nylon mesh bags (Elko, Minneapolis, Minnesota, USA), permitting ingrowth by fungi and other microbes, but excluding plant roots. Each bag was 3 cm diameter x 10 cm length and encased in a 50 ml polypropylene centrifuge tube with two large 'windows' cut out, allowing the bag to maintain physical shape during vertical burial (Fig. S1). In April 2016, in-growth bags containing each soil treatment were buried at the organic-mineral soil interface to a depth of 10 cm in four forest sites dominated by *Quercus ellipsoidalis* and four sites dominated by *Pinus strobus* at the Cedar Creek Ecosystem Science Reserve in East Bethel, MN, USA (45°25′12" N 93°11′50" W). Further site details are provided in Fig. S2. At each site, eight locations along the perimeter of an 8 × 8 m plot, spaced 4 m apart, were determined. One in-growth bag from each treatment was buried in a triangle cluster at each location (i.e., block; Fig. S3). Following burial, the leaf litter layer was replaced and each bag was wetted with de-ionized water to facilitate initial soil contact. In total, 192 bags were buried (eight in-growth bag replicates per soil treatment in four replicate sites per forest type). Three soil cores (3 × 10 cm) from the center of each forest site were also collected in order to quantify soil characteristics (i.e., total carbon and total nitrogen, inorganic nitrogen, extractable organic nitrogen following identical protocols; Table 2), and to characterize initial soil fungal communities. Soil in-growth bags were harvested 110 days (~four months) after deployment (August 2016) and stored at −80 °C until molecular characterization and nutrient analysis was initiated.

2.2. Molecular characterization of fungi

Total genomic DNA was extracted from 0.5 g of soil from each ingrowth bag as well as from forest soil sample using the Qiagen Power Soil DNA extraction kit (Hilden, Germany). PCR amplification of the ITS1 gene region was conducted using primers ITS1F and ITS2 (Smith and Peay, 2014). PCRs for each sample contained: 200 μM primers, 200 μM each dNTP, 2 mM $MgCl_2$, and Hot-Start GoTaq DNA polymerase (Promega, Madison, WI, USA). Following an initial denaturation step at 95 °C for 10 min, PCR was cycled 30 times at 95 °C for 30 s, 52 °C annealing temperature for 20 s, 72 °C for 30 s and a final extension at 72 °C for 8 min. The ITS1 gene was also amplified from a mock community (Nguyen et al., 2015), as well as initial soil treatments, a PCR blank and a DNA extraction blank. PCR amplification was confirmed via gel electrophoresis and libraries were cleaned and normalized to the same DNA concentrations using the Charm 'Just-A-Plate' kit (Charm Biotech, San Diego, CA, USA), followed by pooling in equimolar concentrations. Sequencing was performed on the MiSeq platform (Illumina, San Diego, CA, USA) with 250 bp paired end reads at the University of Minnesota Genomic Sequencing Center.

The raw demultiplexed sequences were processed using the FAST pipeline (https://github.com/ZeweiSong/FAST). Briefly, primers were trimmed using cutadapt (Martin, 2011) and forward and reverse reads were paired using PEAR (Zhang et al., 2014). Low quality sequences were removed (maximum expected error rate = 1), singletons were discarded, and chimeras were detected and eliminated using VSEARCH (Rognes et al., 2016) and the UNITE database (Kõljalg et al., 2013). Sequences were truncated to 160 bp and clustered into operational taxonomic units (OTUs) at 97% sequence similarity using VSEARCH. Rare OTUs (i.e. those with fewer than four sequences) were removed from the analysis based on results from the mock community, where spurious OTUs were found at an abundance of 3 sequences and fewer. Raw sequences were uploaded to the NCBI Sequence Read Archive (SRP126330).

To normalize sampling effort, we then rarefied each library to 2102 sequences. The resulting sample x OTU matrix was further quality filtered by subtracting sequence read counts present in any negative controls from those in the experimental samples (10 and 252 sequences from DNA extraction and PCR blanks, respectively), as well as OTUs detected in initial soil treatments (low SOM blank: 17 OTUs; high SOM blank: 863 OTUs; labile N blank: 51 OTUs). Taxonomy was assigned using the BLASTn algorithm (Altschul et al., 1990) against the UNITE database (v.7.0.; Kõljalg et al., 2013), and functional assignments (i.e., trophic mode and guild) were made in FunGuild using taxonomic assignments (Nguyen et al., 2016a). Representative sequences from the 50 most abundant OTUs with unknown functional classifications were manually checked against the UNITE database. We updated the OTU trophic mode classification as ectomycorrhizal in the absence of taxonomy, if the four of the five best hits from each functionally unclassified OTU were sourced from ectomycorrhizal root tips. Additionally, we designated all sequences classified to Eurotiales, Hypocreales, Mortierellales, Mucorales, Saccharomycetales, Tremellales within the saprotrophic guild 'yeasts and molds', as well as the genus *Cryptococcus* (Bödeker et al., 2016). We further refined the trophic classification of ectomycorrhizal genera into ruderal and slow-growing ectomycorrhizal guilds, representing taxa with generally high reproductive rates typically present in early successional stages relative to more slowly growing taxa that tend to be more efficient nutrient foragers (Newton, 1991; Cairney and Chambers, 2013; Dickie et al., 2013). Specifically, *Amphinema, Cenococcum, Genea, Hebeloma, Humaria, Laccaria, Meliniomyces, Peziza, Thelephora, Tomentellopsis, Tuber,* and *Wilcoxina* were classified as ruderal. *Amanita, Cantharellales, Clavulina, Cortinarius, Hydnum, Inocybe, Lactarius, Leccinum, Naucoria, Piloderma, Pseudotomentella, Russula, Sebacina, Tomentella, Tylospora,* and *Scleroderma* were classified as slow-growing ECM fungi.

2.3. Statistical analysis

The contributing effects of soil treatment and forest type on fungal OTU richness and Shannon diversity were assessed using linear mixed effects models. In each case, site was included as a random effect. The mixed effects models were estimated using lmer () function in R package lme4 (Bates et al., 2015), fit by REML, and t-tests were conducted using Satterthwaite approximations to degrees of freedom in package lmerTest (Kuznetsova et al., 2016). Post-hoc tests conducted using the glht () function in R package multcomp (Hothorn et al., 2008), with Bonferonni-Holm corrections for multiple comparisons (Hsu, 1996). For both OTU richness and Shannon diversity, models for all fungal OTUs as well as those classified as saprotrophic and ectomycorrhizal fungi were generated. Principal coordinates analyses (PCoA) were conducted to visualize variation in fungal community composition (i.e., β-diversity, with all fungal OTUs considered) by soil treatment and forest type. The visualizations were based on Bray-Curtis distances using Hellinger-transformed OTU abundances. Significant differences in centroid location and dispersion by soil treatment and forest type were determined using permutational multivariate analyses of variance (PERMANOVA) and dispersion (betadisper) analyses. These analyses were conducted for all soil fungi, as well as for saprotrophic and ectomycorrhizal fungi individually. In the PERMANOVA models, site was incorporated as a blocking factor, and 1000 permutations were run. Finally, to assess whether soil treatment and forest type contributed to differences in the abundance between and within fungal

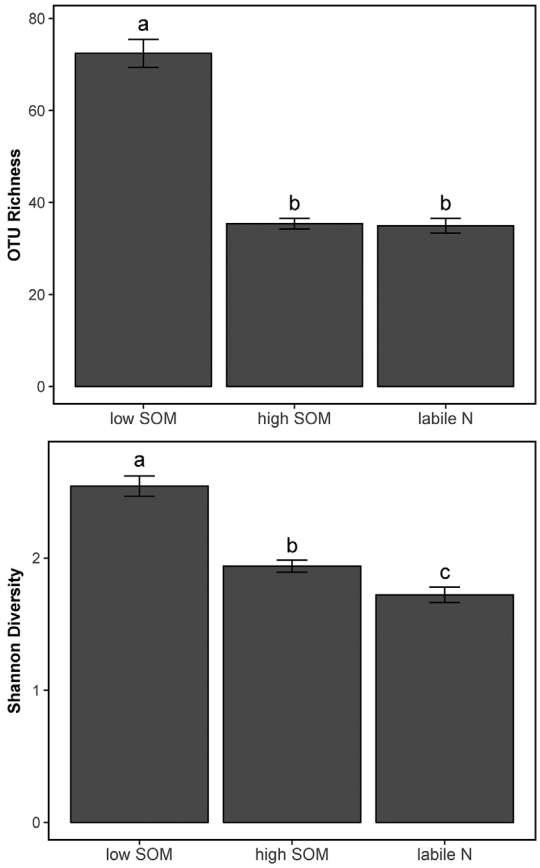

Fig. 2. Total fungal OTU richness and Shannon diversity by experimental soil treatment. Treatments are ordered across the x-axis from lowest to highest organic N content. Treatment significance ($\alpha < 0.05$) was determined in a linear mixed effects model. Letters indicate statistical differences following posthoc analysis, conducted from the ghlt function in R package multcomp with holm-bonferonni P-value corrections for all pairwise comparisons test. SOM represents soil organic matter.

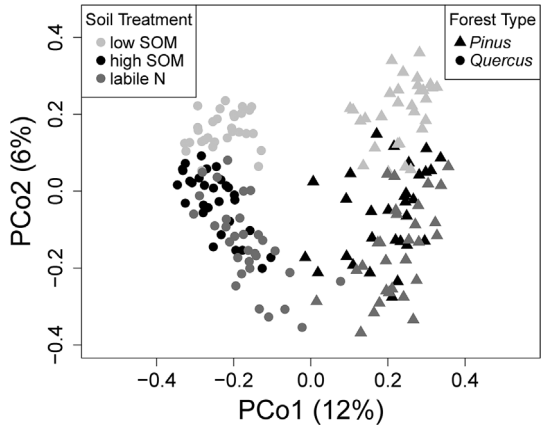

Fig. 3. Visualization of total fungal OTU β-diversity between soil treatments and forest types in a principal coordinates analysis. Fungal β-diversity was calculated from Bray-Curtis distances of Hellinger-transformed abundances of OTUs clustered at 97% sequence similarity. SOM represents soil organic matter.

guilds, we utilized linear mixed effects models to estimate overall saprotrophic and ectomycorrhizal abundance as well as relative abundances within saprotrophic (fast-growing yeasts and molds, non-molds) and ECM fungi (ruderal, slow-growing).

3. Results

From rarefied sequence libraries, we sampled a total of 874 fungal OTUs across all soil treatments and sites. Total fungal community richness was significantly different among soil treatments (Fig. 2A; Table S1; Fig. S4), being approximately two-fold higher in low SOM treatment compared to either the high SOM or labile N treatments, respectively ($z = 14.4–14.5$; $P < 0.001$). In contrast to soil treatment, forest type had no significant effect on fungal community richness, and there were no significant interactions between forest type and soil treatment. Similar to richness, treatment significantly influenced fungal Shannon diversity, while forest type and the interaction between main factors were not significant (linear mixed effects model; Table S1). Shannon diversity was highest in fungal communities colonizing the low SOM, followed by high SOM, and the lowest Shannon diversity in fungal communities in labile N (Fig. 2B).

Both soil treatment and forest type significantly influenced total fungal community composition (PERMANOVA; Table S1). Principal coordinates analysis (PCoA) mirrored these results, with PCo1 separating fungal communities by forest type while fungal communities were differentiated by soil treatment along PCo2 (Fig. 3). There was also a significant interaction between soil treatment and forest type (Table S1), with the compositional differences between forest types being notably less distinct in labile N treatment (Fig. 3). Multivariate dispersion analysis indicated that variation between replicate fungal communities was significantly lower in labile N relative to other soil treatments (dispersion analysis; Soil treatment: $F_{2,177} = 5.3$; $P = 0.006$), as well as in the *Quercus*-dominated sites relative to the *Pinus*-dominated sites (Forest Type: $F_{1,178} = 7.6$; $P = 0.006$). Finally, fungal communities from initial forest soils (i.e., time 0) were closer in multivariate space to the fungal communities assembling in the low SOM soil treatment and most distant from communities in labile N (Fig. S5).

Saprotrophic and ectomycorrhizal fungi represented the main fungal guilds colonizing in-growth bags, with a smaller proportion of fungal pathogens or unclassified trophic modes present (Fig. S6). The ten most abundant fungal genera were *Mortierella, Scleroderma, Pseudogymnoascus, Tomentella, Russula, Sebacina, Inocybe, Cryptococcus, Humaria,* and *Laccaria* (in descending order; Figs. S7 and S8). *Mortierella*, a fast-growing mold, stood out as the dominant genus, comprising an average of 37.2–68.6% of fungal sequences across soil treatments and forest types. Overall, saprotrophic fungal relative abundance was significantly influenced by soil treatment but not forest type (linear mixed effects model; Table S1), being highest in labile N treatment (0.74 ± 0.03) and lowest in the low SOM treatment (0.41 ± 0.02). This result was primarily driven by the responses of the yeast/mold guild, which varied significantly across soil treatments (Fig. 4; $z = 3.62–8.3$; $P < 0.001$). In contrast, the relative abundance of ectomycorrhizal fungi was dependent on both soil treatment and forest type (linear mixed effects model; Table S1). In the *Quercus*-dominated sites, ectomycorrhizal fungal relative abundance was highest in high SOM treatment (0.36 ± 0.04); whereas, in the *Pinus*-dominated sites, ectomycorrhizal fungal relative abundance was highest in the low SOM (0.51 ± 0.04) treatment. Soil treatment did not significantly influence the relative abundance of ruderal ectomycorrhizal fungi; whereas, the relative abundance of slow-growing ectomycorrhizal fungi was significantly lower in labile N treatment relative to both the high SOM and low SOM treatments, respectively (Fig. 4; $z = -3.1$ to -3.8; $P < 0.004$).

4. Discussion

Our results provide strong experimental evidence that variation in the amount and form of organic nitrogen can influence the richness and composition of fungal communities in forest soils. Similar to results obtained from a large number of studies that have examined effects of inorganic nitrogen (Morrison et al., 2016, and references therein), increases in organic nitrogen resulted in sharp declines in fungal species richness. This result was consistent for saprotrophic and ectomycorrhizal fungi, the dominant fungal guilds colonizing in-growth bags in our study. In addition to declines in overall number of species in the soil treatments with higher organic nitrogen, we observed significant declines in fungal species diversity and notable shifts in composition with increasing organic nitrogen availability. Given the consistency of our results between gymnosperm- and angiosperm-dominated forests, it is clear that changes in soil organic nitrogen availability, like inorganic nitrogen, can significantly alter fungal community structure.

With regard to the species richness patterns observed, we found that the richness of saprotrophic fungi was significantly suppressed with organic nitrogen addition, regardless of form. This negative richness effect has been documented with the experimental addition of inorganic nitrogen (Allison et al., 2007; Freedman et al., 2015; Frey et al., 2004), although other studies have found that adding inorganic nitrogen stimulates saprotrophic fungal richness in tropical and temperate forest soils (Kerekes et al., 2013; Morrison et al., 2016). One challenge in comparing our results to many inorganic nitrogen addition studies is that field experiments tracking fungal communities are typically measured over much longer time periods (i.e., years rather than months). We speculate that saprotrophic richness may have been higher in the treatments with greater organic nitrogen had the incubation run longer, due to the potential for more efficient decomposers to outcompete fast-growing yeasts and molds once soluble nutrient sources were accessed, a frequently observed pattern in successional studies (Frankland, 1998; Voříšková and Baldrian, 2013). However, due to the single application of organic nitrogen, it is important to note that our study was not adequately designed to test successional processes. Elevating organic nitrogen, in either labile or recalcitrant forms, also did not increase ectomycorrhizal fungal richness. Rather, richness of this fungal group was doubled in the low OM treatment, the experimental condition where organic nitrogen was most limited and carbon:nitrogen ratio was intermediate relative to the other two treatments. This result differs from observational studies showing that ectomycorrhizal fungal richness increases where soil organic matter is high (Peay et al., 2010; Tedersoo et al., 2014). Taken together, it appears that ecological factors that co-vary with increased soil organic matter, including vertical niche partitioning, host density, and host phylogenetic diversity, may be more

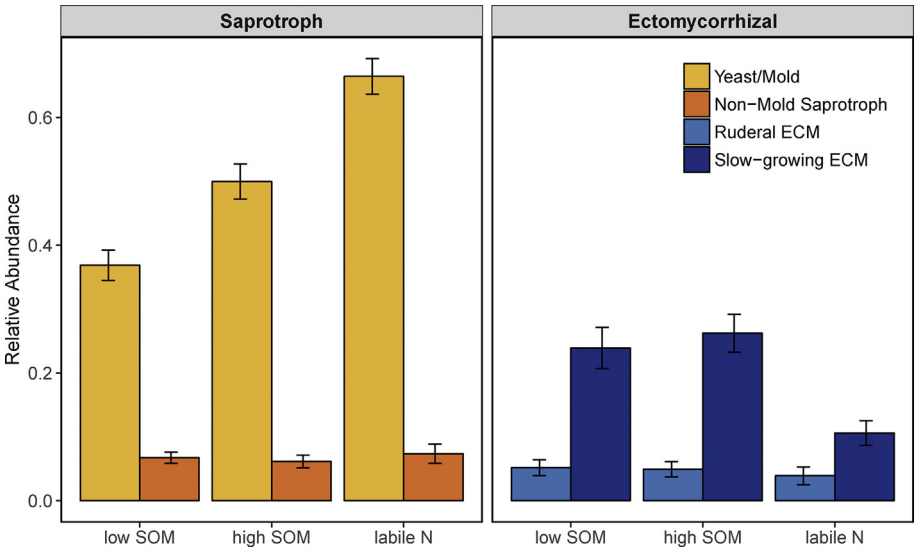

Fig. 4. Relative abundance saprotrophic and ectomycorrhizal fungi by guild across soil treatments, ordered from lowest to highest organic N content along the x-axis. Relative abundance was calculated as the average proportion of total reads. Significance was calculated using linear mixed effects models for each trophic mode of fungi (i.e., saprotroph and ectomycorrhizal fungi separately). Error bars represent standard error, SOM indicates soil organic matter.

important in promoting ectomycorrhizal fungal richness than increased organic nitrogen availability (Looney et al., 2016; Nguyen et al., 2016b; Smith et al., 2011; Tedersoo and Nara, 2010).

The co-colonization by saprotrophic and ectomycorrhizal fungi of all the experimental in-growth soil treatments, which included a wide range of forms of organic nitrogen, and consequently soil carbon:nitrogen ratios, suggests broad overlap in the fundamental niches of these functional guilds (Bödeker et al., 2016). Not surprisingly, given the limited duration of the experiment, fast-growing molds and yeasts dominated the community regardless of soil nitrogen treatment. This trend matches with previous work indicating that forest floor disturbances alter fungal community composition, particularly opportunistic molds that can quickly capitalize on new resource inputs (Lindahl et al., 2010). Interestingly, mold relative abundance peaked where organic nitrogen was both high and most labile. The response of this group could be related to the acquisition of labile forms of organic nitrogen or the associated decrease in carbon:nitrogen ratio within the soil treatment. We suspect that the peak abundance of genus *Mortierella* (the dominant genus across all treatments) in the labile N treatment was due to our addition of chitin and its primary precursor, which represented 20% of the labile ON addition. Species in this group have well demonstrated chitinolytic capacities (Kim et al., 2008), and exhibited rapid colonization of substrates containing chitin (De Boer et al., 1999). Surprisingly, a similar increase for the non-mold saprotrophic fungi in the treatments with higher carbon and organic nitrogen was not observed. While the reasons for this response are not immediately clear, it may simply reflect the low overall colonization by this group, which represented less than 10% of the sequence reads in all three soil treatments.

Among ectomycorrhizal fungi, those classified as ruderal showed little response to treatment. This could suggest that this group of ectomycorrhizal fungi are good competitors for soil organic nitrogen, but this runs counter to observations that ruderal ectomycorrhizal fungi are favored in disturbed habitats, where soil nitrogen is typically present in mostly inorganic forms (Wan et al., 2001). Instead, similar to non-mold saprotrophic fungi, low overall colonization of ruderal ectomycorrhizal fungi may have muted their response to the experimental treatments. In contrast, slow-growing ectomycorrhizal fungi were more abundant in both the low SOM and high SOM treatments, making up approximately 20% of total fungal sequences. Their relative abundance, however, was reduced by over 50% in the labile N treatment. Given the well-documented ability of ectomycorrhizal fungi to grow on a variety of organic nitrogen substrates (Abuzinadah and Read, 1988; Lilleskov et al., 2002), we believe that slow-growing ectomycorrhizal fungi were likely suppressed by the high abundance of fast-growing molds. This interpretation aligns with the results of Bödeker et al. (2016), who found that molds dominated the first four months of colonization of litter- and humus-containing in-growth bags at the expense of both non-mold saprotrophic fungi and ectomycorrhizal fungi in a Swedish boreal forest. Despite their lower abundance relative to molds, we did observe that ectomycorrhizal fungi were consistently more abundant than non-mold saprotrophic fungi in all soil treatments. This latter finding parallels the colonization patterns recently documented on decomposing fungal residues in the same study system (Fernandez and Kennedy, 2018). Collectively, these results indicate that ectomycorrhizal fungi appear to be effective foragers for both labile and recalcitrant forms of organic nitrogen, and thereby likely are direct competitors with non-mold saprotrophic fungi for these resources (Talbot et al., 2013; Baskaran et al., 2017).

Although this study is the first to our knowledge to directly examine the response of multiple fungal guilds to manipulations of ecologically realistic amounts and forms of soil organic nitrogen, we recognize that our results have notable limitations. We used in-growth bag soils that kept physical properties and pH as similar as possible (Zak et al., 1986; Zak and Pregitzer, 1990) to better isolate the effects of changes in soil organic matter and organic nitrogen among treatments, but introducing soils with different physical properties than those around the bags may have altered fungal in-growth patterns. Specifically, the slightly lower soil moisture and pH of the experimental soil treatments, relative to forest soil, could have stimulated a subset of the forest soil community to colonize the in-growth bags. Similarly, while we chose a diverse range of organic nitrogen forms for the labile N treatment (following the classification of Talbot and Treseder, 2010), our combination remained a simplification relative to the diverse forms of organic nitrogen present in forest soils. For example, this treatment did not include phospholipids, which have been shown to important sources of organic nitrogen and phosphorus for ectomycorrhizal fungi (Talbot and Treseder, 2010). Furthermore, an unavoidable consequence of our experimental nitrogen supplementation was a corresponding addition of carbon, and the subsequent decline in substrate carbon:nitrogen ratio. Despite this caveat, increased carbon content in the low SOM treatment was likely not the key factor structuring soil fungal communities, as we would have expected a considerable increase in abundance of the non-mold saprotrophs. Instead, the richness, evenness and compositional response of saprotrophs largely mirrored those of ectomycorrhizal fungi across treatments. Along with changes in carbon:nitrogen ratios, variation in pH between the experimental soil treatments due to natural variation in soil organic matter content (low SOM v. high SOM

treatments) also had the potential to influence fungal colonization patterns. Finally, it is possible that there is a mismatch between the fungal community shifts we observed and those occurring at the ecosystem scale, due to the fact that in this experimental set-up (i.e., localized in-growth bags), fungi can translocate carbon and nitrogen from the surrounding non-amended soil.

With regard to our quantification of fungal responses, we focused on shifts in relative sequence reads as a proxy for changes in abundance. This was necessary because the most common measure of fungal biomass (i.e., ergosterol content) does not capture Mucormycotoan molds because of the unique composition of their membrane lipids (Weete et al., 2010). Although the quantitative use of sequence reads has been questioned (Amend et al., 2010), multiple studies indicate that relative read counts can accurately track non-molecular metrics of fungal abundance (Ihrmark et al., 2012; Nguyen et al., 2015; Taylor et al., 2016). An additional outstanding question is the extent to which our results may have differed had we used a longer incubation period. Bödeker et al. (2016) observed a significant increase in both non-mold saprotrophic fungi and ectomycorrhizal fungi in a second season of incubation and we suspect that similar patterns would be present in our system. This warrants caution for the conclusion that increases in organic nitrogen result in long-term decreases in fungal richness, although studies of fungal and plant communities suggest that species richness can be suppressed long after nitrogen inputs are stopped (Choma et al., 2017; Isbell et al., 2013) (but see, Hasselquist and Högberg, 2014; Högberg et al., 2014).

In summary, these results add to a well-established body of literature indicating that forest fungal communities are strongly affected by soil nitrogen levels. Importantly, our study demonstrates that local increases in organic nitrogen, the dominant form of nitrogen in forest soils (Talbot and Treseder, 2010), can have similarly large effects on fungal community structure as increases in inorganic nitrogen, albeit over a shorter time scale. The latter form of nitrogen has received much greater attention due to anthropogenic alterations to nitrogen cycles (Vitousek et al., 1997), but given that the dominant form of nitrogen in most soils is organic (Tamm, 1991; Inselsbacher and Näsholm, 2012), quantifying how changes in the amount and form of organic nitrogen influences fungal community richness and abundance has similarly important ecological implications. In addition, our results add to the growing recognition that ectomycorrhizal fungi likely play an underappreciated role in the decomposition of soil organic matter, particularly deeper in soils and at later stages of decay, where saprotrophic fungal growth may be limited by decreased carbon lability (Talbot et al., 2008; Lindahl and Tunlid, 2015). Moving forward, future experimental studies using additional diverse mixtures of organic nitrogen and examining responses of longer time scales will help refine our understanding of exactly how fungal community changes and soil organic nitrogen availability are linked.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2018.07.008.

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