LONG-TERM SIMULATED NITROGEN DEPOSITION ALTERS THE COMPOSITION OF FUNGI ACTIVE IN THE FOREST FLOOR

By

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Abstract

Global increases in the rate of atmospheric nitrogen deposition have the potential to alter the composition and function of soil microbial communities. In a long-term field study simulating future rates of atmospheric N deposition, plant litter decay has slowed and soil organic matter has accumulated in conjunction with a decline in both lignolytic enzyme activity and expression of fungal lignolytic genes. Here, I tested the hypothesis that simulated atmospheric N deposition would alter the composition of basidiomycete and ascomycete fungal communities, which may underlie the previously observed biogeochemical responses. The actively metabolizing forest floor fungal community was characterized from cDNA clone libraries constructed from 28S fungal rRNA extracted from the forest floor of two northern hardwoods stands in the lower peninsula of Michigan, USA. The active basidiomycete communities under ambient and simulated atmospheric N deposition differed significantly in terms of membership and the dispersion of members over a phylogenetic tree. Furthermore, suggestive, albeit nonsignificant, differences in the fraction of unique phylogenetic branch length (the UniFrac metric) between simulated and ambient atmospheric N deposition were observed for basidiomycetes. In contrast, the active ascomycete communities under ambient and simulated atmospheric N deposition did not exhibit significant differences in these same metrics. Collectively, these results indicate that chronic N deposition has altered both the composition and function of litter decaying fungi and that these changes have ecosystem-level implications for the cycling and storage of C in forest ecosystems.

Keywords: atmospheric N deposition, fungal communities, forest floor, basidiomycetes, ascomycetes, community composition

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Introduction

Emissions of reactive nitrogen (N) have increased 300-500% over the last century, a biogeochemical change that directly results from anthropogenic activities (Denman et al. 2007). Moreover, atmospheric N deposition in terrestrial ecosystems has been projected to further increase by 250% over the next century (Lamarque et al. 2005). Temperate forests are a globally important carbon (C) sink, and future rates of atmospheric N deposition have the potential to influence their function. The majority of attention has focused on how atmospheric N deposition may enhance net primary productivity in these N-limited ecosystems (Nadelhoffer 1999, Currie et al. 2004, LeBauer and Treseder 2008), albeit there remains considerable debate regarding this response (Magnani et al. 2007). Nevertheless, ecosystem C storage is determined not only by rates of net primary production, but also by rates of decomposition and the formation of soil organic matter. For example, soils globally contain ~75% of the C stored in terrestrial ecosystems (Prentice et al. 2001). If anthropogenic N deposition alters plant litter decay by even a small margin, this response could elicit a large change in the amount of C stored in this globally important pool (Reay et al. 2008).

The depolymerization of lignin by soil fungi is a rate-limiting step in the process of litter decay in forest ecosystems (Osono and Takeda 2005), and there is accumulating evidence that atmospheric N deposition may negatively influence this process (Waldrop and Zak 2006, Berg and Matzner 1997). Decreases in lignin degradation could result in the accumulation of soil organic matter, thereby increasing soil C storage. Fungi are the only organisms capable of metabolizing lignin to CO_2 and they do so by producing oxidative extracellular enzymes, i.e., peroxidases and phenol oxidases (Kirk and Farrell 1987). Interestingly, laboratory studies

demonstrate that high N levels can suppress lignin degradation by the wood-rotting basidiomycete *Phanerochaete chrysoporium* (Weinstein et al. 1980, Fenn and Kirk 1981), although this response is not always consistent across fungal species (Boyle et al. 1992, Leatham and Kirk 1983). Furthermore, N concentrations may regulate fungal production of lignin degrading enzymes and their activity (Boominathan et al. 1990, Vanderwoude et al. 1993). Under field conditions, decreased phenol oxidase activity and reduced laccase gene expression have been reported in soil receiving simulated atmospheric N deposition (DeForest et al. 2004*a,b*, Carreiro et al. 2000, Waldrop et al. 2004, Edwards et al. 2011).

It has long been hypothesized that atmospheric N deposition might alter competitive interactions in favor of decomposers that do not completely degrade lignin (Fog 1988). For example, *Actinobacteria* and most saprotrophic ascomycete and zygomycete fungi have minor lignolytic capabilities, in contrast to the basidiomycete and Xylariaceous ascomycete fungi which mediate the majority of lignin decay in the forest floor (Osono and Takeda 2002); even within the white-rot basidiomycetes (i.e. those capable of the breakdown of both lignin and cellulose) and Xylariaceous ascomycetes, there is substantial variation between taxa in their selectivity for lignin over other constituents of plant litter and in the efficiency of their lignolytic activity (Osono 2007, Osono and Takeda 2002, Hatakka 1994, Steffen et al. 2007). Environmental conditions which inhibit the synthesis of lignolytic enzymes could slow the pace of plant litter decay (Freeman et al. 2001). A previously outlined mechanism (Deforest et al. 2004(b), Blackwood et al. 2007, Hassett et al. 2009) suggested that inhibition of lignolytic enzymes by inorganic N might weaken the competitive ability of powerful lignolytic fungi, thereby enhancing the representation in the microbial community of less efficient lignin degraders.

In a long-term field study simulating future rates of atmospheric N deposition, plant litter decay has slowed and soil organic matter has accumulated in a manner consistent with the microbial mechanisms described above (Zak et al. 2008, Pregitzer et al. 2008). For example, the production of phenolic dissolved organic carbon (DOC) has significantly increased under simulated N deposition (Smemo et al. 2006), consistent with the idea that elevated N deposition could lead to incomplete lignin degradation (Fog 1988). However, the abundance of *Actinobacteria*, which includes some groups capable of partial lignin degradation, has not been altered by chronic N deposition (Eisenlord and Zak 2010), nor has the abundance of basidiomycete laccase gene copies (Hassett et al. 2009). However, reduced lignolytic enzyme activity (Deforest et al. 2004) and reduced transcription of laccase (Edwards et al. 2011) has been observed under elevated atmospheric N deposition, suggesting that lignin degradation has slowed.

I have reasoned that alterations in the composition of basidiomycete and ascomycete communities have occurred in parallel with previously observed declines in enzyme activity and gene expression. Collectively, these changes in fungal community composition and function may underlie the slowing of plant litter decay and the accumulation of soil organic matter observed under simulated N deposition. The objective of my study was to characterize the active forest floor fungal community and observe any changes in its composition in a series of northern hardwoods stands that have received long-term simulated atmospheric N deposition at a rate expected to occur in the near future (3 g NO_3^--N m^{-2} y^{-1}). I have addressed my objective by constructing and sequencing of cDNA libraries of 28S rRNA extracted from the forest floor. This differs from a previous examination of basidiomycete community composition (Hassett et al. 2009), which focused on laccase genes, in that it allows comparisons of diversity and

community composition under simulated and ambient atmospheric N deposition to be conducted within the framework of a broader and better established phylogeny. I used several sequence- and phylogeny-based tools for microbial community analysis to test the hypothesis that the active community of fungi under simulated N deposition differs from that under ambient N deposition.

Methods

Site description

My study sites consisted of two sugar maple (*Acer saccharum* Marsh.) dominated northern hardwoods forest in Lower Michigan (represented by B and D in Figure 1). These two sites represent two of the four sites in the Michigan Gradient Experiment, a long-term elevated atmospheric N deposition experiment spanning a climatic and ambient N deposition gradient in lower and upper Michigan, USA. The gradient extends from south to north, with more southerly sites experiencing warmer mean annual temperatures, longer growing seasons, and higher annual inputs of ambient atmospheric N deposition than more northerly ones (Table 1). Soils are well-drained sandy typic Haplothords of the Kalkaska series. The sites are similar in terms of overstory age and floristic composition. Sites do not significantly differ in soil pH (Table 1), and the forest floor (O_i) is dominated by sugar maple leaf litter.

Each of the 4 sites consists of six 30-m x 30-m plots; three plots receive ambient N deposition, whereas the other 3 receive ambient N deposition plus 3 g NO_3^- -N m^{-2} y^{-1}. This amount is has been added since 1994 and is consistent with levels expected to be reached in northeastern North America and portions of Europe by 2050 (Galloway et al. 2004). Treatments are applied as $NaNO_3^-$ pellets in 6 equal additions of 0.5 g N m^{-2} during the growing season (April-September). Each treatment plot is surrounded by a 10-m treated buffer zone to reduce edge effect, which also receives the aforementioned N deposition treatments.

Sample collection

In each plot at sites B and D, forest floor from the O_e and O_a horizons were collected from 10 random 100-cm^2 quadrats. The ten samples from each plot were combined in the field to make one composite sample per plot, which was homogenized with sterilized scissors. Subsamples from each composite plot sample were placed in 50-mL sterile polypropylene tubes and immediately flash frozen in the field with liquid N_2 and transported to the laboratory, where they were stored at -80 °C until RNA extraction could occur.

RNA extraction and purification

For each composite sample from each plot, total RNA was extracted from 3 g of forest floor using an initial Tris-phenol extraction to separate nucleic acids from contaminants, followed by subsequent extraction of the aqueous phase using a Qiagen RNA/DNA Midi kit following the methods of Luis et al. 2005. Extracted RNA solutions were treated with DNase I to remove any DNA that may be present in the RNA solution and were then stored at -80° C. Prior to reverse transcription, samples were purified using the Plant RNAeasy Mini column kit (Qiagen). Purification was performed according to a modified manufacturer's protocol for isolation of RNA directly from tissue with 2.5 mg of activated charcoal added to 350 µL of the RLC buffer. Purified RNA was quantified using a Quant-iT Ribogreen kit (Invitrogen) and Molecular Devices Fmax fluorescent microplate reader (Sunnyvale, CA), according to Ribogreen manufacturer instructions.

Reverse transcription and amplification of cDNA

First strand cDNA was synthesized from 28S rRNA via a reverse transcription reaction using the reverse primer LR3 (5'CCG TGT TTC AAGAC GGG 3'), 65 ng of purified extracted total RNA, and SuperScript II reverse transcriptase (Invitrogen), according to the manufacturer's protocol. Following first strand synthesis, cDNA of fungal 28S rRNA was amplified via PCR on a Robocycler 96 thermocycler (Stratagene) for 10 cycles with initial denaturation at 95 °C for 3 min, 35 cycles of denaturing at 94 °C for 30 sec, annealing at 50 °C for 45 sec, and elongation at 72 °C for 90sec, and a final elongation at 72 °C for 10 minutes. PCR mixture each contained 1 µL of first strand cDNA, 0.625 µL forward primer of 10 µM LROR (5' ACCC GCT GAA CTT AAGC 3'), 0.625 µL 10 µM reverse primer LR3 , 2.5 µL dNTPs (2 µM), 2.5 µL 10X PCR buffer (1.5 mM $MgCl_2$), 0.2 µL Taq polymerase, and 16.5 µL molecular grade water. Duplicate 25-µL reactions were performed and combined for a total of 50 µL PCR product per plot. PCR products were purified using a MoBio Ultraclean PCR Clean-up kit and stored at -20°C.

Clone library construction and sequencing

Amplified 28S cDNA segments were cloned into vector PCR 2.1 TOPO (Invitogen) using a TOPO TA Cloning kit, with manufacturer's protocol modified to reduce all reagents to one half of recommended volume. Vectors containing inserts were transformed into TOPO TA Cloning TOP10 chemically competent cells (Invitrogen). Ninety-six positive colonies were selected for each sample and grown overnight at 37 ° C in Luria-Bertani broth containing 10% glycerol, 0.025 g L^{-1} ampicilin, 0.0125 g L^{-1} kanamycin. Libraries were screened and frozen at -80° C until sequencing could occur. From each library, 48 clones were submitted for bidirectional sequencing to the DNA Sequencing Lab at the University of Georgia.

Phylogenetic tree construction

Sequences were edited and contiguous sequences were constructed in Geneious 4.0 (Drummond et al. 2008) and aligned with ClustalW (Larkin et al. 2007). Suspected non-fungal sequences that were amplified by the LROR and LR3 primers were identified and removed from further analysis by constructing a neighbor-joining tree in Geneious as well as performing a search in NCBI BLAST (Altschul et al. 1990, http://blast.ncbi.nlm.nih.gov/Blast.cgi) for sequences that did not appear to group with fungi. Fungal OTUs were generated at the 99% similarity level using DOTUR (Schloss and Handelsman 2005). The resulting OTUs were aligned with a set of selected fungal reference sequences in ClustalW. Preliminary fungal phylogenetic trees were constructed from this alignment by creating distance matrices in MEGA4 (Tamura et al. 2007), boot strapped-neighbor joining trees with a p-substitution model in MEGA4, and boot-strapped maximum likelihood trees using PhyML through the PALM portal with GTR selected as the best substitution model (Guindon and Gascuel 2003, Chen et al. 2009, Posada and Crandall 1998). Based on these trees, fungal OTUs were assigned to phyla. Remaining non-fungal or suspected chimeric OTUs were subsequently identified and removed from analysis. Sequences were suspected to be chimeric if they matched poorly to any sequences in GenBank or produced BLAST matches for fungi for only a small region of the sequence and not for the entire length of the sequence. For each basidiomycete and ascomycete OTU, at least one reference sequence was selected using an NCBI BLAST search.

OTUs identified as belonging to Basidiomycota were aligned with basidiomycete reference sequences and an ascomycete outgroup in Geneious5 using MAFFT multiple sequence alignment (Katoh et al. 2002). Likewise, OTUs belonging to Ascomycota were aligned with

ascomycete reference sequences and a basidiomycete outgroup using similar methods. Maximum-likelihood trees were created using the PhyML (Guindon and Gascuel 2003) with a GTR substitution model through the online portal at www.phylogeny.fr. These trees were used as input for community analyses based on branch length or tree topology described below, e.g. UniFrac and Martin's P-test.

Phylogeny-based community analyses

The basidiomycete and ascomycete maximum likelihood phylogenetic trees were analyzed for unweighted and weighted UniFrac significance at 1,000 permutations (Lozupone and Knight 2005, Lozupone et al. 2006, Lozupone et al. 2007). UniFrac finds the distances between communities using the branch length of the lineages found in only one community but not both, e.g. the unique fraction (Lozupone and Knight 2005, Lozupone and Knight 2008). UniFrac operates on the principal that if two environments are distinct from one another, the lineages of microorganisms therein should be specially adapted to each environment and likewise unique from one another. Conversely, if two environments provide similar microbial habitats, similar lineages microorganisms should be present in both. Phylogenetic branch length between these two similar environments would be shared (Lozupone and Knight 2008). The UniFrac metric was used to compare communities in the two sites, as well as all combinations of treatments and sites through the online UniFrac portal (http://bmf2.colorado.edu/unifrac/). The "each pair of environments" option in UniFrac was employed to determine if *each pair* of libraries differed from one another in the fraction of unique branch length. For basidiomycetes, the "each environment individually" option in UniFrac was also employed to determine if an individual environment had more unique branch length than all other environments combined as

a single tree. For UniFrac significance and Martin's P-test, $P < 0.05$ was considered significant, while a result of $0.05 \leq P < 0.10$ was considered "suggestive" in accordance with the terminology of the developers of the UniFrac online portal (Lozupone et al. 2006); this was not true for other analyses performed in this study (e.g. $\int$-Libshuff) in which $P < 0.05$ was considered significant and $0.05 \leq P < 0.10$ had no special importance.

The P-test (Martin 2002) and UniFrac environmental cluster analysis (Lozupone and Knight 2005) were similarly performed through the UniFrac online portal (Lozupone et al. 2006). The P-test was employed to test whether species from ambient and simulated N deposition libraries were randomly distributed over a phylogenetic tree or whether they exhibited more clustering than expected by chance (Martin 2002, Lozupone and Knight 2008). UniFrac environmental cluster analysis was run without abundance weights using the "cluster environments" option in UniFrac. UniFrac environmental clustering is a hierarchical clustering method based on pair-wise UniFrac distances. It was employed to determine which environments contain similar microbial communities; results may range from 0 if all lineages are shared to 1 if no lineages are shared (Lozupone et al. 2006, Lozupone and Knight 2005).

Sequence-based community analyses, richness estimation, and diversity indices

Basidiomycete and ascomycete sequences were clustered into OTUs using MOTHUR (Schloss et al. 2009) for sequence-based community analysis. To estimate the richness of fungal communities under ambient and simulated N deposition, rarefaction and calculation of abundance-based coverage (ACE) and Chao1 estimators for each treatment (Chao 1984, Chao and Lee 1992, Hughes et al. 2001) were performed on basidiomycete and ascomycete sequences using MOTHUR; these richness estimators were compared with the observed richness of the

clone libraries in order to assess the adequacy of my sampling effort in capturing the diversity of the forest floor fungal communities. Venn diagrams of OTUs were created to assess abundance and overlap of OTUs across sites and treatments. The Shannon diversity index was calculated using MOTHUR in order to compare the level of diversity present in libraries from different treatments.

∫-Libshuff (Schloss et al. 2004) was performed through MOTHUR for basidiomycete and ascomycete fungal communities at both sites. Libshuff is a technique for measuring whether intracommunity relatedness of sequences is higher than intercommunity relatedness (Singleton et al. 2001, Lozupone and Knight 2008). Libshuff compares two coverage curves: a homologous coverage curve and heterologous coverage curve. The homologous coverage curve is the percentage of sequences in library A that are not singletons over genetic distance; the heterologous coverage curve is the fraction of sequences shared by two libraries (A and B) over genetic distance. The Cramer von Mises statistic is used to determine the distance between the two curves. This value is compared to a value generated from a random shuffling of sequences between libraries A and B; if library A is the same as library B, it is expected that the values generated from random shuffling will not significantly differ from the distances between the actual coverage curves (Singleton et al. 2001, Lozupone and Knight 2008). ∫-Libshuff is an implementation of Libshuff that employs the integral form of the Cramer von Mises statistic (Schloss et al. 2004). ∫-Libshuff comparisons were performed in both directions (e.g. community A vs. B and community B vs. A), because a significant difference between community A compared to B does not necessarily imply a difference will be found when community B is compared to A (Singleton et al. 2001, Schloss 2008, Lozupone and Knight 2008).

Results

Sequencing

Of the 576 clones submitted for sequencing, 535 readable sequences containing inserts were returned. Of these, 308 Dikarya sequences were represented in the clone libraries. Of these, the ratio of basidiomycete to ascomycete sequences recovered was 2.2: 1, while the ratio of OTUs at the 99% similarity level was 1.1: 1. The other 227 sequences excluded from further analysis included 41 sequences representing other fungal phyla, 126 sequences representing nonfungal eukaryotes, and 60 sequences representing putative fungal chimeras.

Basidiomycete OTU recovery and diversity

A total of 211 sequences were identified as basidiomycetes, and from these sequences, 67 OTUs were identified at the 99% similarity level using MOTHUR. Of these OTUs, 25 were unique to the ambient N deposition treatment, 30 were unique to the simulated atmospheric N deposition treatment, and 12 occurred in both treatments. The Shannon diversity index for the ambient N deposition basidiomycete library was lower ($H_{ambient}$ =2.79±0.30) than that for simulated N deposition basidiomycete library ($H_{simulated}$=3.18±0.22).

Rarefaction of basidiomycete clone sequences under both ambient and simulated N deposition demonstrated that the OTU recovery revealed a similar sampling effort in both treatments (Figure 2). Additionally, the 95% confidence intervals for the basidiomycete ambient and simulated N deposition rarefaction curves overlapped, demonstrating that both treatments exhibited similar OTU richness at our level of sampling effort (Figure 2). Chao1 estimated the number of basidiomycete "species" (defined as 99% sequence similarity) to be 53 species for

ambient N deposition and 75 species under simulated N deposition; ACE estimated diversity at 59 species under ambient N deposition and 120 species under simulated N deposition. Although the number of observed OTUs approached these estimated asymptotes in rarefaction curves for both treatments (Figure 2), additional sampling would be necessary to fully characterize basidiomycete diversity under both simulated and ambient N deposition.

Basidiomycete OTUs recovered were predominantly species from the Agaricomycotina, particularly the class Agaricomycetes. Of these, Agaricales was the most abundant order with members of the Agaricaceae, Bolbitiaceae, Clavariaceae, Entolomataceae, the Marasmioid clade (Matheny et al. 2006), Mycenaceae, Psathyrellaceae, Stephanosporaceae, and Tricholomataceae represented (Figure 4, Supplemental Table 1). Other OTUs were affiliated with the Agaricomycete orders Atheliales/Amylocorticiales, Auriculariales, Cantharellales, Corticiales, Gomphales, the Polyporoid clade (Binder et al. 2005, Larsson 2007), and Trechisporales. Additionally, representatives of the Tremellomycetes and the Microbotryomycetes were recovered in basidiomycete clone libraries (Figure 4, Supplemental Table 1). Interestingly, most Mycenaceae and Tricholomataceae OTUs were present only under simulated N deposition (Table 5, Supplemental table 1). The abundance of clones from these families was also higher under simulated N deposition; there were 4 times as many Mycenaceae clones and 30 times more Tricholomataceae clones in simulated N deposition libraries (Table 5).

Sequence-based community measurements for basidiomycetes

Basidiomycete clone libraries obtained under ambient and simulated N deposition were compared in terms membership, as assessed by increasing levels of genetic distance using ∫-Libshuff (Schloss et al. 2004). Libraries under simulated N deposition were significantly

different compared to those obtained under ambient N deposition, a result that was consistent in both study sites and when libraries were combined across sites ($P < 0.0001$; Table 2). The converse was also true; ambient N deposition communities were significantly different than communities under simulated N deposition at both sites, ($P_{siteB} = 0.0001$ and $P_{site\ D} = 0.044$; Table 2) and also when libraries were combined across sites ($P = 0.0006$; Table 2).

Phylogeny-based community measurements for basidiomycetes

UniFrac environmental clustering revealed that basidiomycete communities under simulated N deposition did not cluster uniquely from communities under ambient N deposition in my two study sites (Figure 3). For example, the simulated N deposition community in Site B clustered most closely with the community of ambient treatment in site D. All distances were ≥ 0.55, indicating that each community shared less than half of its lineages with any other community. The ambient N deposition treatment in Site B was the most divergent, with all its distance values > 0.7, when compared the communities in the other sites and treatments.

Pair-wise UniFrac significance tests suggested that basidiomycete communities under ambient and simulated N deposition share less phylogenetic branch length than would be expected by chance, both across sites ($P = 0.085$; Table 3) as well as at each site ($P_{site\ B} = 0.092$ and $P_{siteD} = 0.098$; Table 3); however, these differences were not significant. Additional unweighted UniFrac tests were performed using the "each individual community" option in UniFrac to determine whether the simulated or the ambient N deposition treatment was responsible for the differences observed in pair-wise analysis. Ambient N deposition basidiomycete communities contributed more branch length than would be expected by chance at both sites ($P_{siteB} = 0.027$ and $P_{siteD} = 0.036$; Table 4), whereas simulated N deposition

basidiomycete communities did not contribute more branch length than expected by chance at either site (P_{siteB} = 0.68, P_{siteD} = 0.77; Table 4). These same responses also occurred across sites ($P_{ambient}$ = 0.007, $P_{simulated}$ = 0.90; Table 4).

The P-test was implemented to examine whether basidiomycete OTUs were randomly distributed or clustered in the phylogenetic tree. Results revealed that basidiomycete OTUs under ambient and N deposition communities were not randomly distributed across the phylogenetic tree. Rather, basidiomycete OTUs displayed more phylogenetic clustering by treatment than would be expected by chance when data was pooled across study site (P = 0.043; Table 3) as well as within Site B alone (P = 0.014; Table 3); however, the result of the P-test for basidiomycetes when Site D was analyzed separately was not significant (P = 0.20; Table 3).

Ascomycete OTU recovery and diversity

Ascomycetes were represented by 97 recovered clone sequences, which were grouped into 59 OTUs using the same approach as described for basidiomycetes. Of these 59 ascomycete OTUs, 25 were only found under ambient N deposition, 22 were recovered only under simulated N deposition, whereas 12 were found in both treatments. Ambient and simulated N deposition ascomycete libraries exhibited a similar degree of Shannon diversity ($H_{ambient}$=3.50±0.22, $H_{simulated}$= 3.44±0.21).

Ascomycetes were represented by a smaller number of recovered sequences compared to basidiomycetes (97 vs. 211 clones); however, the number of ascomycete OTUs present was almost equal to the number of basidiomycete OTUs (59 vs. 67, respectively). Rarefaction curves for ascomycetes under ambient and simulated N deposition demonstrated sharp increases in OTU recovery that did not appear to approach an asymptote (Figure 2). This is not surprising given

that 63% of ascomycete OTUs were singletons. Ascomycete diversity therefore appears to have been undersampled for both ambient and simulated N deposition.

Ascomycete OTUs were all placed within the Pezizomycotina and were affiliated with the Leotiomycetes, Eurotiomycetes, Sordariomycetes, Orbiliomycetes, and Pezizomycetes (Figure 5, Supplemental Table 3). Eleven OTUs, belonging to 3 clades, could not be definitively placed in a class and were labeled "Pleosporales", "Pleosporales and *Ochroconis* spp." and "unresolved basal clade" (Figure 5, Supplemental Table 3). Of particular interest because of their potential for lignolysis were several OTUs affiliated with the Sordariomycete order Xylariales, and the Eurotiomycete family Herpotrichiellaceae (Figure 5, Supplemental Table 3).

Sequence-based community measurements for ascomycetes

Community membership comparisons with ∫-Libshuff demonstrated that ascomycete community membership under simulated N deposition was not different when compared to those under ambient N deposition, when sites were combined ($P = 0.086$; Table 2) or at Site D ($P = 0.44$; Table 2). Site B was the exception, demonstrating a significant difference in ascomycete membership ($P = 0.025$; Table 2) under simulated compared to ambient N deposition. Community membership between ambient N deposition ascomycete communities when compared to simulated N deposition communities showed no differences in either site ($P_{siteB} = 0.46$, $P_{siteD} = 0.48$; Table 2) or when sites were combined ($P = 0.93$; Table 2).

Phylogeny-based community measurements for ascomycetes

Ascomycete communities did not consistently cluster by either site or treatment using UniFrac environmental clustering; communities from simulated N deposition treatments at both sites clustered together, whereas communities from ambient N deposition treatments did not

(Figure 3). Ambient N deposition ascomycete communities, in fact, exhibited the greatest distance of any two communities in the tree (environmental distance = 0.72; Figure 3). Distances between all communities were ≥ 0.56, indicating that even within the same treatment or site, the ascomycete communities shared less than half their lineages.

Ascomycetes exhibited no significant clustering by N deposition treatment when sites were combined (P = 0.35; Table 3) or at Site B (P = 1.00; Table 3) using the P-test. Results for the P-test for Site D suggested clustering by treatment, but this was not statistically significant (P = 0.052; Table 3). Pair-wise UniFrac significance tests displayed a similar pattern, revealing no significant differences in unique branch length between ascomycete communities under ambient and simulated N deposition at Site B (P = 0.44; Table 3) or when sites were combined (P = 0.19; Table 3). However, the community at Site D displayed differences in unique branch length between N deposition treatments at a level that is considered suggestive for UniFrac (P = 0.067; Table 3). Weighting the UniFrac test with OTU abundance resulted in significant differences between treatments in unique branch length at Site D (P = 0.028; Table 3), but this effect was again not apparent at site B (P = 0.76; Table 3) or when sites were combined (P = 0.23; Table 3).

Discussion

Fungal community composition and ecosystem function

Because atmospheric N deposition will continue to increase over the next century (Galloway et al. 2004), it is important to understand the microbial mechanisms by which this agent of global change will alter the cycling and storage of C in soil (Zak et al. 2008, Smemo et al. 2006). Litter-decaying fungi are particularly important, because these organisms transform fresh plant litter into soil organic matter (Osono 2007), thereby mediating the process of soil C storage in forests. In our long-term experiment, simulated N deposition has elicited a change in fungal community membership and the phylogenetic composition of active (i.e., those expressing rRNA genes) saprotrophic fungi, and this response has occurred in parallel with a decline in lignolytic gene expression (Edwards et al. 2011), a decline in extracellular enzyme activity, as well as the slowing of decay and the accumulation of soil organic matter. Collectively, these results indicate that chronic N deposition has altered both the composition and function of litter decaying fungi and that these changes have ecosystem-level implications for the cycling and storage of C in forest ecosystems.

While this is the first observation of which we are aware demonstrating compositional changes in the active basidiomycete community in a northern hardwood ecosystem in response to simulated N deposition, it is not the first observation of impacts of experimental N additions upon decomposer communities. In a boreal ecosystem, increased N inputs reduced the diversity and altered the structure of active fungal communities (Allison et al. 2007). In subalpine spruce forests, experimental N fertilization affected the composition of saprobic sporocarps in the fungal community; some saprobic species responded positively to N fertilization, whereas others

responded negatively or were not affected (Gillet et al. 2010). It should be noted, however, that both Allison et al. 2007 and Gillet et al. 2010 applied N at rates much greater than those applied in our experiment, which was designed to simulate rates of atmospheric N deposition that are predicted to occur by the end of this century.

Interestingly, a significant change in actinobacterial community composition, similar to that observed for here for basidiomycetes, has also been observed under simulated N deposition in this ecosystem (Eisenlord and Zak 2010). Some *Actinobacteria* may play an important role in lignin degradation, metabolizing lignin into soluble polyphenolics (Mason et al. 1988, Godden et al. 1992, Berrocal et al. 1997), the leaching of which has increased under simulated N deposition in the Michigan gradient (Pregitzer et al. 1994). Together, these results demonstrate that changes in the broader decomposer community have simultaneously occurred with slowed decomposition and increased DOC leaching under simulated N deposition.

Changes in community composition have been linked to changes in ecosystem function in other experimental systems, suggesting that compositional shifts have functional implications in soil microbial communities. For, example, in a tropical ecosystem, differences in decomposition rates between monodominant and mixed forests were attributed to differences in microbial community composition and function (McGuire et al. 2010). In a northern hardwoods forest, the composition of a seasonally variable fungal community correlated with different aspects of soil chemistry across seasons, as well as with activities of enzymes involved in C, N, and P cycling (Burke et al. 2011). Furthermore, in a microcosm experiment in which tree and grass litter were inoculated with different communities, microbial respiration from inoculated litter was correlated with the community composition of the inoculum (Strickland et al. 2009*a*).

While changes in community composition potentially mediate changes in function, it is important to caution that some functional redundancy exists across divergent taxonomic groups; additionally, communities of similar taxonomic composition are not necessarily functionally equivalent (Strickland et al. 2009*b*). As such, interpreting compositional shifts resulting from environmental change in terms of ecosystem function is not always straightforward (Strickland et al. 2009*b*). Nonetheless, in our experiment, compositional changes in both fungal and actinobacterial communities have occurred in parallel with the slowing of decay under simulated N deposition, indicating that changes in microbial community composition may underlie biogeochemical responses.

Phylogenetic association and ecology of Basidiomycete OTUs

While many forest floor basidiomycetes are saprotrophs, taxa vary in the rate at which they metabolize leaf litter as well as in their selectivity for the different biochemical constituents of litter (Osono and Takeda 2002, Osono et al. 2003, Osono et al. 2011). Particularly effective decomposers possessing lignolytic capacities occur in the Polyporoid and Marasmioid clades, and in the Mycenaceae and Tricholomataceae families (e.g. *Phanerochaete* spp., *Marasmius* and *Gymnopus* spp., *Mycena* spp., and *Clitocybe* and *Collybia* spp.; Webster and Weber 2010, Osono 2007, Valášková et al. 2007, Šnajdr et al. 2010, Kirk and Farrell 1987). Members of these groups were recovered in this study, and their presence and abundance is important to consider regarding how litter decay might be impacted by compositional changes in the active basidiomycete community. For example, suppression of one or more of these groups under simulated N deposition could lead to the slowing of lignin decay as well as the energy-rich constituents of plant cell wall protected by this decay-resistant polymer. *Marasmius* spp. are

among the most powerful leaf litter decomposers in terms of mass loss, lignin metabolism, and preferential selection for lignin degradation and are therefore an important taxonomic group to consider in relation to slowing decomposition under simulated N deposition (Osono and Takeda 2002, Steffen et al. 2007). Communities under ambient N deposition contained more OTUs associated with the "Marasmioid clade", which includes the family Marasmiaceae as well as *Gymnopus* and the hydropoid clade, a result that is consistent with expectations of slowed decay under simulated N deposition (Matheny et al. 2006). Marasmioid sequences composed ~11% of the basidiomycete sequences in the 28S rRNA cDNA libraries, but were not the dominant basidiomycete clade in these libraries. As such, the overall importance of Marasmioid fungi during litter decay cannot be directly inferred from clone library abundance alone.

Representatives of the Polyporoid clade (sensu Binder et al. 2005) were recovered under both ambient and simulated N deposition; these saprotrophic fungi are also important agents of decay in forest ecosystems. Polypores include both brown-rot species (i.e. those capable of cellulose but not lignin decay) and white-rot species, including the powerful wood-rotter *Phanerochaete chryosporium* (Larsson 2007, Binder et al. 2005). The number of polypore OTUs observed under ambient N deposition was greater than that under simulated N deposition; however, polypores represented only ~3% of basidiomycete clone sequences. The low representation of polypores in clone libraries suggests that they may comprise only a small portion of the biomass of the active community; however, this does not necessarily mean that their contribution to decomposition is negligible in our study sites, given the exceptional white-rot abilities of some polypore species.

The Mycenaceae and Tricholomataceae were well represented in this ecosystem, together comprising 30% of all basidiomycete sequences, suggesting these families are both abundant and active in decomposition. Tricholomataceae and Mycenaceae families contain organisms with the physiological capacity for lignin decomposition and may be important mediators of changes in ecosystem function. Although closely related, fungi in these two families display different decay physiologies, distinguishing them from each other in terms of ecological function. *Clitocybe* spp. are selective delignifiers that primarily act upon partially decayed litter (Osono et al. 2011). *Mycena* spp., in contrast to *Clitocybe* spp., primarily colonize freshly fallen litter and often simultaneously decompose both lignin and cellulose (Cannon and Kirk 2007, Osono et al. 2011, Osono 2007, Osono et al. 2003). Mycenaceae are capable of causing a higher degree of mass and lignin loss on litter than most other basidiomycetes, but may be less effective at overall mass loss or break down of recalcitrant material than either Marasmiacieae and *Clitocybe* spp., depending on the substrate (Osono and Takeda 2002, Osono et al. 2003, Steffen et al. 2007). Therefore, a compositional shift involving these taxa could impact how and when particular biochemical constituents are attacked during the decay process, suggesting the potential for changes in both functional gene expression and enzyme activity in the forest floor.

Given the important role of *Mycena* spp. and some members of the Tricholomataceae in lignin decay, we might anticipate a decline in representation of these families under simulated N deposition to co-occur with a slowing of decomposition; however, we observed the opposite, with greater diversity and abundance of Mycenaceae and Tricholomataceae sequences present under simulated N deposition. BLAST searches demonstrated that the Tricholomataceae OTUs we recovered matched more closely to *Clitocybe* and *Lepista* than to *Tricholoma* (Supplemental Table 2), suggesting that these OTUs represent saprotrophic and not ectomycorrhizal species.

Interestingly, simulated N deposition has been documented to favor to *Clitocybe* and *Mycena* spp. in other experiments; in one such study, the abundance of fruiting bodies for *Mycena* spp. and *Clitocybe* spp. increased under simulated N deposition in a beech forest (Rühling and Tyler 1991).

Better knowledge of the ecology of these families would facilitate the understanding of both their response to N deposition and their function during litter decay. For example, *Mycena* and *Marasmius* spp., which responded in an opposing manner to simulated N deposition in this study, have been observed to be exhibit niche partitioning of the litter resource by occupying different litter depths (Frankland 1998). Furthermore, these two genera demonstrate antagonism towards one another in culture, with the outcomes of these competitive interactions altered by invertebrate grazing (Frankland 1998). Collectively, these kinds of interactions suggest that the community composition changes we observed could be mediated through a variety of mechanisms. Although some of the responses we observed run counter to our initial expectations, the fact that changes in the community of active fungi includes shifts favoring fungi with unique decomposing abilities suggests that changes in community composition have the potential to alter the process of litter decay and organic matter formation.

Ascomycete community response

I did not observe a consistent ascomycete response to simulated N deposition. While phylogenetic and community membership analyses revealed site-specific changes, we failed to detect a consistent change in community composition under simulated N deposition. Furthermore, we did not sample the community to saturation, which may have prevented our ability to detect differences between ascomycete communities under simulated N deposition;

conversely, undersampling could also result in detection of phylogenetic or membership changes that are not a reflection of the true community, but merely an artifact of insufficient coverage of the entire ascomycete community. Thus, we have no evidence that simulated N deposition affected the ascomycete communities in a uniform way at our level of sampling intensity. In contrast, Edwards et al. (2011) found a compositional change in the active ascomycete community at Site D; however, here we used a different reverse transcription technique to create cDNA (specific vs. random primers), sampled during a different season (spring vs. fall), and used different statistical approaches.

The ascomycete community likely plays a secondary function in lignin decay in the forest floor compared to basidiomycetes. Ascomycetes represented a minority of the sequences in our libraries, suggesting they comprise a lower proportion of the metabolically active community in the forest floor in the spring. In general, ascomycete species are responsible for a lower amount of litter and lignin decay than basidiomycete species (Osono and Takeda 2002, Osono 2007). Furthermore, only a small subset of ascomycete taxa are known to possess white-rot capabilities. In particular, the ascomycete genera *Xylaria* and *Geniculosporium* in the family Xylariaceae and the species *Phialophora lignicola* (synonymn *Lecytophora lignicola*) in the family Herpotrichiellaceae (class Eurotiomycetes, order Chaetothyriales) are known for unusually high amounts of ligninolytic activity in comparison to other ascomycetes, with rates of lignocelluloses decay for some of these species on par with those of many basidiomycetes (Osono and Takeda 2001, Osono and Takeda 2002, Osono et al. 2003, Osono et al. 2009). If decay under simulated N deposition has slowed due to reduced ligninolysis, we would anticipate a decline in these families under simulated N deposition. However, OTUs affiliated with Xylariaceae and Herpotrichiellaceae, the ascomycete families that include lignocellulose degraders, were

recovered under both simulated and ambient N deposition; however, they were not dominant components of the ascomyete community under either N deposition treatment. Xylariaceae and Herpotrichiellace sequences represented ~5% and ~7% of ascomycete sequences, respectively. Not all members of these families necessarily share the same propensity for litter decay; therefore, the representation of potentially lignolytic ascomycetes in our libraries is likely even lower than these percentages suggest (Osono and Takeda 2002, Osono et al. 2003, Osono et al. 2009, Badali et al. 2008).

The majority of ascomycetes recovered in this study do not possess known ligninolytic abilities and may attack other constituents of plant litter. Some ascomycetes species are cellulolytic, so we cannot discount an important role for ascomycetes in cellulose decay in the forest floor (Osono et al. 2003, 2009). One OTU represented in this study was associated with the Myxotrichiaceae, a family known to have powerful cellulolytic abilities (Cannon and Kirk 2007). Additionally, saprotrophs compose all or part of other represented families including the Dermataceae, Helotiacieae, Hyaloscyphaceae, and Orbiliaceae; however, the ecological roles of some of these families are not fully understood (Cannon and Kirk 2007). If ascomycetes in our libraries are primarily cellulose decomposers, this may explain why the ascomycete community showed no consistent changes under simulated N deposition. Fungal lignolytic activity can be stimulated by nitrogen limitation and suppressed by high availability of nitrogen (Keyser et al. 1978, Fenn and Kirk 1981, Osono and Takeda 2001); therefore, the activity of nonlignolytic ascomycetes may not necessarily be suppressed by increased N availability through the same mechanisms as white-rot fungi, although the possibility still exists that cellulolytic ascomycete composition could be altered by indirect effects of simulated N deposition.

Summary and conclusions

In summary, my results demonstrate that the composition of the active basidiomycete fungal community has been altered under simulated N deposition, a change which has co-occurred with a decline in litter decay and the accumulation of organic matter in surface soil. The outcome for the active ascomycete community is less clear, but this might be resolved through further sampling. As such, my results partially support the hypothesis that simulated N deposition alters the composition of forest floor fungal communities. Whether the changes in the active basidiomycete community reflect underlie the decline in litter decay is unclear, because some highly lignolytic taxa were favored under simulated N deposition, whereas others declined. Further information about the ecology of the species in our system would aid in understanding why species composition has changed and how this mediates slower decomposition and DOC leaching. Nevertheless, my results are consistent with the idea that compositional shifts in the fungal community may underlie biogeochemical responses to atmospheric N deposition.

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Table 1. Climatic, floristic, and edaphic properties of four sugar maple dominated forests receiving simulated N deposition.

Characteristic	Site	
	B	D
Location		
Latitude (N)	45°33	43°40
Longitude (W)	84°52’	86°09’
Climate		
Mean annual precipitation (mm)*	874	824
Mean annual temperature (°C)**	6.2	7.7
Wet + dry total N deposition (g N m^{-2} yr^{-1})†	0.91	1.18
Vegetation		
Overstory age (2008)	95	100
Soil Chemistry††		
Exchangeable calcium (cmol(+) kg^{-1})	3.43	2.36
Exchangeable magnesium (cmol(+) kg^{-1})	0.49	0.44
Exchangeable aluminum	0.19	0.63
Base Saturation (%)	69	82
pH (10 cm mineral soil)	4.92	4.60

*Mean annual precipitation, for the years 1994 to 2008, was recorded using weighing rain gages (Model 5-780, Belfort Instrument Co., Baltimore, MD) located in open areas within 5 km of each site.

**Mean annual temperature, for the years 1994 to 2008, was recorded on site at 2 m using thermistors which were read every 30 minutes throughout the year, with averages recorded every 3 h using data loggers (EasyLogger Models 824 and 925, Data Loggers, Inc., Logan UT).

†MacDonald et al. 1992

††D.R. Zak, *unpublished data*

Table 2. ∫-Libshuff change in coverage scores and significance results for basidiomycete and ascomycete sequences recovered from the forest floor under ambient and simulated N deposition at two northern hardwood forest sites.

Phyla	ΔC_{XY} Libshuff	Libshuff *P* value XY	ΔC_{YX} Libshuff	Libshuff *P* value YX
Basidiomycetes				
Site B†	0.015	<0.0001***	0.015	0.0001***
Site D†	0.024	<0.0001***	0.0018	0.044*
Both sites combined	0.0067	<0.0001***	0.0017	0.0006***
Ascomycetes				
Site B†	0.016	0.025*	0.0054	0.46
Site D†	0.0042	0.44	0.0025	0.48
Both sites combined	0.0022	0.086	0.00020	0.93

XY compares simulated vs. ambient N deposition libraries. YX compares ambient vs. simulated N deposition libraries.
* Significant at the 0.05 level
** Significant at the 0.01 level
*** Significant at the 0.001 level
† Significant difference between sites.

Table 3. UniFrac unique fraction metric significance and P-test significance for basidiomycete and ascomycete communities under ambient and simulated N deposition. Values compare communities under ambient and simulated N deposition in each site and combined across both sites. Analyses were run 10 ten times at 1000 permutations using maximum likelihood phylogenies.

Phyla	UniFrac significance (unweighted)	UniFrac significance (weighted)	P-test
Basidiomycetes			
Site B	0.092*	0.41	0.014**
Site D	0.098*	0.54	0.20
Both sites combined	0.085*	0.56	0.043**
Ascomycetes			
Site B	0.44	0.76	1.00
Site D	0.067*	0.028**	0.052*
Both sites combined	0.19	0.23	0.35

* Suggestive at the 0.1 level; ** Significant at the 0.05 level; *** Significant at the 0.01 level

Table 4. UniFrac unique fraction metric significance (unweighted) for basidiomycete communities under ambient and simulated N deposition. Values compare the contribution in terms of unique branch length of each individual community to the entire branch length of a tree constructed using maximum likelihood containing all basidiomycete OTUs recovered. Separate analyses were conducted with sequences identified by treatment alone and by both site and treatment. Analyses were run 10 times for 1000 permutations.

	N deposition treatment	
	Ambient	Simulated
	P	*P*
Site B	0.027**	0.68
Site D	0.036**	0.77
Both sites combined	0.0066***	0.90

* Suggestive at the 0.1 level; ** Significant at the 0.05 level; *** Significant at the 0.01 level

Table 5. Clone occurrence and abundance for OTU sequences associated with the basidiomycete families Mycenaceae and Tricholomataceae (order Agaricales).

		Clone abundance			
		Ambient N deposition		Simulated N deposition	
OTU	Family	Site B	Site D	Site B	Site D
3	Mycenanceae	3	1	1	10
14	Mycenaceae	1	-	3	-
30	Mycenaceae	-	-	1	1
46	Mycenaceae	-	-	1	1
47	Mycenaceae	-	-	1	1
100	Mycenaceae	-	-	1	-
9	Mycenaceae?	-	1	4	-
97	Mycenaceae?	-	-	1	-
2	Tricholomataceae	-	-	-	21
6	Tricholomataceae	-	-	-	7
45	Tricholomataceae	-	-	-	2
105	Tricholomataceae	1	-	-	-

- Indicates absence in clone libraries at our level of sequencing effort

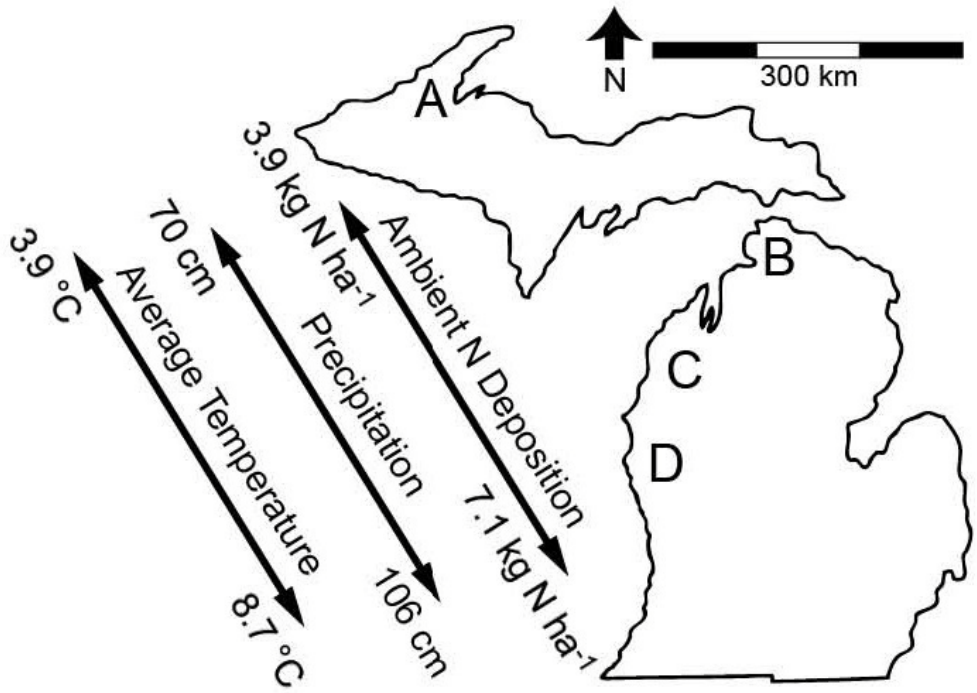

Figure 1. Location of four study sites, floristically and edaphically similar, but differing in ambient N deposition rates and climactic characteristics.

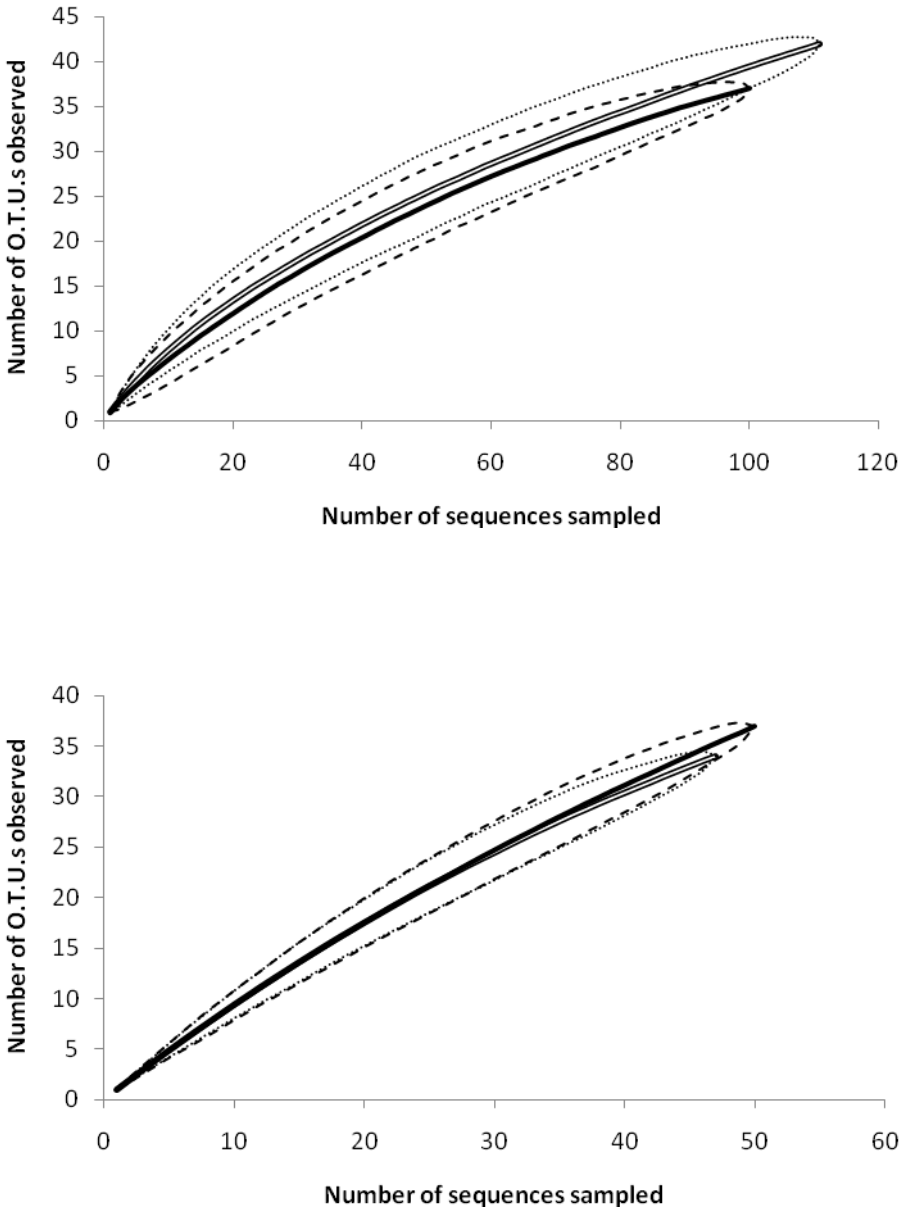

Figure 2. Rarefaction curve of observed OTU richness of basidiomycetes (top) and ascomycetes (bottom) under ambient and simulated N deposition in a northern hardwood forest. OTUs were defined at the 99% similarity level. Rarefaction curves were generated by a re-sampling without replacement approach in MOTHUR. Points on the curve represent average richness obtained for 1000 iterations. Solid line represents the rarefaction for ambient N deposition; thick dashed lines represent the upper and lower 95% confidence intervals for the ambient N deposition curve. Hollow line represents rarefaction curve for simulated N deposition; thin dashed lines represent the upper and lower 95% confidence intervals for the simulated N deposition rarefaction curve.

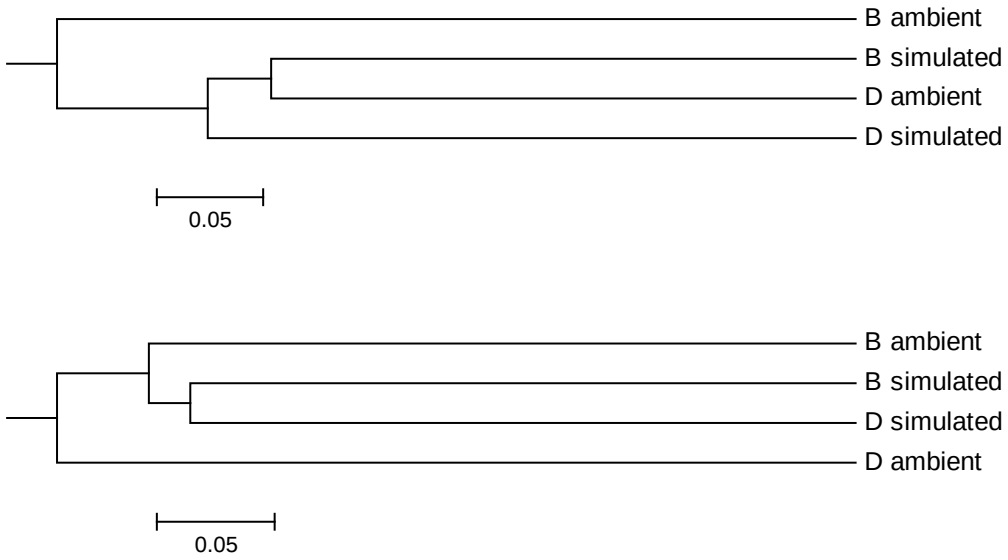

Figure 3. Unweighted UniFrac environmental clustering for basidiomycetes (top figure) and ascomycetes (bottom figure). A distance of 0 indicates that two environments are identical, while a distance of 1 indicates that two environments share no lineages. Figure was generated using the cluster environments option in UniFrac and a maximum likelihood phylogeny constructed with PhyML. Letters refer to sites B and D; ambient and simulated refer to N deposition treatments.

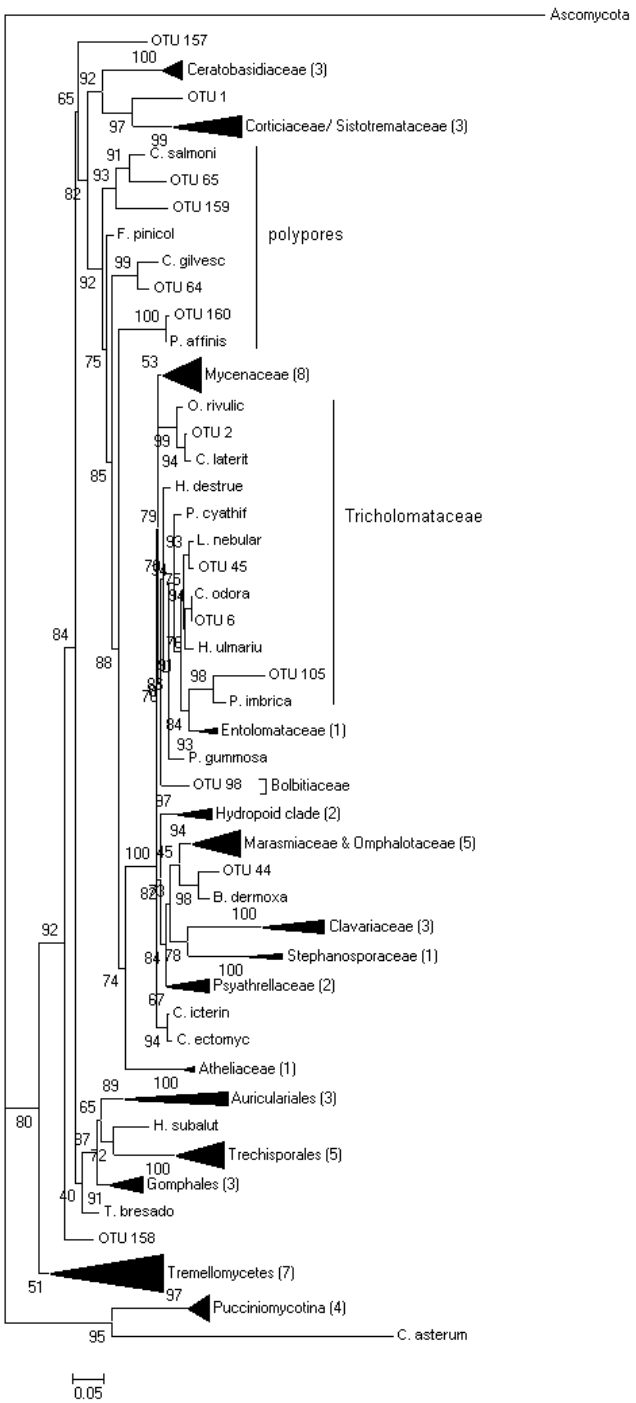

Figure 4. Maximum likelihood tree of basidiomycete OTUs with reference sequences. Tree was constructed in PhyML. Numbers on branches are approximate likelihood-ratio test (aLRT) values. In labels for collapsed subtrees, the number in parentheses indicates how many OTUs are contained in the collapsed subtree. Tricholomataceae and polypore species are not collapsed because they are polyphyletic. OTUs 157 and 158 are of uncertain taxonomy and are, therefore, not labeled. A description of the taxonomic assignment of OTUs in the tree is found in Supplemental Table 1.

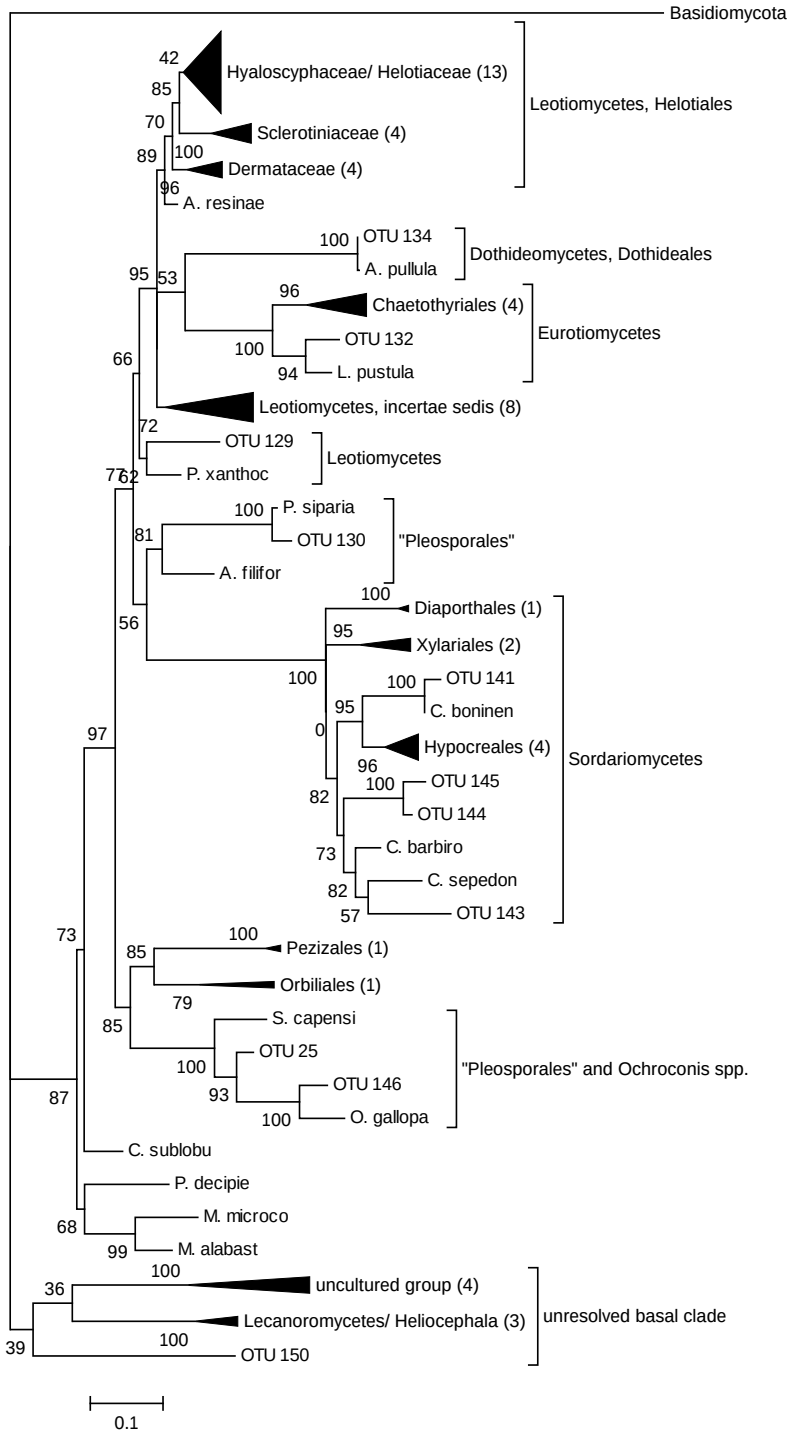

Figure 5. Maximum likelihood tree of ascomycete OTUs with reference sequences. Tree was constructed in PhyML. Numbers on branches are approximate likelihood-ratio test (aLRT) values. In labels for collapsed subtrees, the number in parentheses indicates how many OTUs are contained in the collapsed subtree. A description of the taxonomic assignment of OTUs in the tree is found in Supplemental Table 3.

Supplemental Table 1. Taxonomic assignment of basidiomycete OTUs and their relative abundance within basidiomycete clone libraries.

OTU	Subdivision	Class	Order	Family	Relative abundance (%)†			
					Ambient N deposition		Simulated N deposition	
					Site B	Site D	Site B	Site D
44	Agaricomycotina	Agaricomycetes	Agaricales	Agaricaceae	4.8	0.0	0.0	0.0
98	Agaricomycotina	Agaricomycetes	Agaricales	Bolbitiaceae	0.0	0.0	2.9	0.0
102	Agaricomycotina	Agaricomycetes	Agaricales	Clavariaceae	2.4	0.0	0.0	0.0
103	Agaricomycotina	Agaricomycetes	Agaricales	Clavariaceae	2.4	0.0	0.0	0.0
104	Agaricomycotina	Agaricomycetes	Agaricales	Clavariaceae	2.4	0.0	0.0	0.0
99	Agaricomycotina	Agaricomycetes	Agaricales	Entolomataceae	0.0	0.0	0.0	1.3
41	Agaricomycotina	Agaricomycetes	Agaricales	Hydropoid clade of Marasmioid clade††	4.8	0.0	0.0	0.0
42	Agaricomycotina	Agaricomycetes	Agaricales	Hydropoid clade of Marasmioid clade††	4.8	0.0	0.0	0.0
95	Agaricomycotina	Agaricomycetes	Agaricales	Hydropoid clade of Marasmioid clade††	0.0	0.0	0.0	1.3
5	Agaricomycotina	Agaricomycetes	Agaricales	Marasmiaceae & Omphalotaceae††	4.8	0.0	0.0	7.9
10	Agaricomycotina	Agaricomycetes	Agaricales	Marasmiaceae & Omphalotaceae††	7.1	0.0	0.0	1.3
13	Agaricomycotina	Agaricomycetes	Agaricales	Marasmiaceae & Omphalotaceae††	9.5	0.0	0.0	0.0
93	Agaricomycotina	Agaricomycetes	Agaricales	Marasmiaceae & Omphalotaceae††	2.4	0.0	0.0	0.0
94	Agaricomycotina	Agaricomycetes	Agaricales	Marasmiaceae & Omphalotaceae††	0.0	0.0	2.9	0.0
3	Agaricomycotina	Agaricomycetes	Agaricales	Mycenaceae	7.1	1.7	2.9	13.2
14	Agaricomycotina	Agaricomycetes	Agaricales	Mycenaceae	2.4	0.0	8.6	0.0
30	Agaricomycotina	Agaricomycetes	Agaricales	Mycenaceae	0.0	0.0	2.9	1.3
46	Agaricomycotina	Agaricomycetes	Agaricales	Mycenaceae	0.0	0.0	2.9	1.3

47	Agaricomycotina	Agaricomycetes	Agaricales	Mycenaceae	0.0	0.0	2.9	1.3
100	Agaricomycotina	Agaricomycetes	Agaricales	Mycenaceae	0.0	0.0	2.9	0.0
9	Agaricomycotina	Agaricomycetes	Agaricales	Mycenaceae?	0.0	1.7	11.4	0.0
97	Agaricomycotina	Agaricomycetes	Agaricales	Mycenaceae?	0.0	0.0	2.9	0.0
43	Agaricomycotina	Agaricomycetes	Agaricales	Psathyrellaceae	4.8	0.0	0.0	0.0
96	Agaricomycotina	Agaricomycetes	Agaricales	Psathyrellaceae	0.0	0.0	0.0	1.3
101	Agaricomycotina	Agaricomycetes	Agaricales	Stephanosporaceae	0.0	1.7	0.0	0.0
2	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	0.0	0.0	0.0	27.6
6	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	0.0	0.0	0.0	9.2
45	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	0.0	0.0	0.0	2.6
105	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	2.4	0.0	0.0	0.0
28	Agaricomycotina	Agaricomycetes	Atheliales/ Amylocorticiales	Atheliaceae	0.0	0.0	0.0	3.9
152	Agaricomycotina	Agaricomycetes	Auriculariaceae or Exidiaceae		2.4	0.0	0.0	0.0
153	Agaricomycotina	Agaricomycetes	Auriculariaceae or Exidiaceae		0.0	0.0	0.0	1.3
161	Agaricomycotina	Agaricomycetes	Auriculariaceae or Exidiaceae		2.4	0.0	0.0	0.0
7	Agaricomycotina	Agaricomycetes	Cantharalleles	Ceratobasidaceae	7.1	3.4	5.7	0.0
162	Agaricomycotina	Agaricomycetes	Cantharellales	Ceratobasidaceae	2.4	0.0	0.0	0.0
163	Agaricomycotina	Agaricomycetes	Cantharellales	Ceratobasidaceae	2.4	0.0	0.0	0.0
1	Agaricomycotina	Agaricomycetes	Corticiales	Corticiaceae	0.0	65.5	20.0	5.3
66	Agaricomycotina	Agaricomycetes	Corticiales	Corticiaceae	0.0	0.0	0.0	2.6
67	Agaricomycotina	Agaricomycetes	Corticiales	Corticiaceae	0.0	0.0	0.0	2.6
164	Agaricomycotina	Agaricomycetes	Corticiales	Corticiaceae	0.0	0.0	2.9	0.0
18	Agaricomycotina	Agaricomycetes	Gomphales	Gomphaceae	0.0	0.0	5.7	2.6
27	Agaricomycotina	Agaricomycetes	Gomphales	Gomphaceae	2.4	0.0	0.0	2.6
156	Agaricomycotina	Agaricomycetes	Gomphales	Gomphaceae	0.0	0.0	0.0	1.3
64	Agaricomycotina	Agaricomycetes	Polyporoid clade†††		0.0	3.4	0.0	0.0

65	Agaricomycotina	Agaricomycetes	Polyporoid clade†††		4.8	0.0	0.0	0.0
159	Agaricomycotina	Agaricomycetes	Polyporoid clade†††		0.0	0.0	2.9	0.0
160	Agaricomycotina	Agaricomycetes	Polyporoid clade†††	Phanerochaetaceae	0.0	1.7	0.0	0.0
157	Agaricomycotina	Agaricomycetes	Thelephorales? Polyporales?		0.0	0.0	0.0	1.3
158	Agaricomycotina	Agaricomycetes	Thelephorales? Geastrales?		2.4	0.0	0.0	0.0
26	Agaricomycotina	Agaricomycetes	Trechisporales	"Trechisporaceae"	0.0	1.7	5.7	0.0
63	Agaricomycotina	Agaricomycetes	Trechisporales	"Trechisporaceae"	0.0	1.7	0.0	1.3
154	Agaricomycotina	Agaricomycetes	Trechisporales	"Trechisporaceae"	0.0	0.0	2.9	0.0
155	Agaricomycotina	Agaricomycetes	Trechisporales	"Trechisporaceae"	0.0	0.0	0.0	1.3
17	Agaricomycotina	Agaricomycetes	Trechisporales	'"Trechisporaceae"	9.5	0.0	0.0	0.0
49	Agaricomycotina	Tremellomycetes	Cystofilobasidiales	mitosporic Cystofilobasidiales or Cystofilobasidiaceae	0.0	3.4	0.0	0.0
20	Agaricomycotina	Tremellomycetes	Filobasidiales	mitosporic Filobasidiales or Filobasidiaceae	0.0	3.4	2.9	0.0
112	Agaricomycotina	Tremellomycetes	Filobasidiales	mitosporic Filobasidiales or Filobasidiaceae	0.0	0.0	2.9	0.0
19	Agaricomycotina	Tremellomycetes	Tremellales	mitosporic Tremellales	0.0	3.4	0.0	1.3
48	Agaricomycotina	Tremellomycetes	Tremellales	mitosporic Tremellales	0.0	3.4	0.0	0.0
110	Agaricomycotina	Tremellomycetes	Tremellales	mitosporic Tremellales	2.4	0.0	0.0	0.0
111	Agaricomycotina	Tremellomycetes	Tremellomycetidae insertae sedis or Filobasidiales	Tremellomycetidae inserta sedis or Filobasidiaceae	0.0	0.0	2.9	0.0

72	Pucciniomycotina	Microbotryomycetes	mitosporic Microbotryomycetidae incertae sedis	mitosporic Microbotryomycetidae incertae sedis	0.0	0.0	2.9	1.3
107	Pucciniomycotina	Microbotryomycetes	mitosporic Microbotryomycetidae incertae sedis	mitosporic Microbotryomycetidae incertae sedis	0.0	0.0	0.0	1.3
109	Pucciniomycotina	Microbotryomycetes	mitosporic Microbotryomycetidae incertae sedis	mitosporic Microbotryomycetidae incertae sedis	0.0	1.7	0.0	0.0
108	Pucciniomycotina	Microbotryomycetes	Sporidiobolales	mitosporic Sporidiobolales	0.0	1.7	0.0	0.0

†Percentage of clones in total basidiomycete clones for each library. Total number of basidiomycete clones for each site, N deposition treatment were as follows: Site B ambient, 42; Site B simulated, 35; Site D ambient, 58, Site D simulated, 76.

†† Marasmioid clade as described by Matheny et al. 2006

††† Polyporoid clade as described by Binder et al. 2005 and Larsson 2007

Supplemental Table 2. Top NCBI BLAST match information for basidiomycete OTUs, including description and accession number of BLAST match and the percent coverage and identity with the OTU sequence.

OTU	Accession	Description	Query coverage (%)	Maximum identity (%)
1	EU489986	Uncultured basidiomycete clone 4S1_D11 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
2	U66431	Clitocybe lateritia small subunit ribosomal RNA gene, partial sequence, internal transcribed spacers ITS1 and ITS2, complete sequence, 5.8S ribosomal RNA gene, complete sequence, large subunit ribosomal RNA gene, partial sequence.	100	98
3	FJ040357	Uncultured fungus clone LSUTypeUS11 28S large subunit ribosomal RNA gene, partial sequence.	100	100
5	AY639411	Gymnopus bicolor voucher AWW116-SFSU 28S ribosomal RNA gene, partial sequence.	99	99
6	AF261390	Clitocybe odora strain RV98/145 25S large subunit ribosomal RNA gene, partial sequence.	99	100
7	FJ040343	Uncultured fungus clone LSUTypeUS1 28S large subunit ribosomal RNA gene, partial sequence.	100	100
9	EU365678	Sarcomyxa serotina strain ACCC 50309 28S ribosomal RNA gene, partial sequence.	97	95
10	DQ457686	Marasmius rotula isolate AFTOL-ID 1505 25S large subunit ribosomal RNA gene, partial sequence.	99	99

13	DQ156126	Marasmius oreades isolate AFTOL-ID 1525 25S ribosomal RNA gene, partial sequence.	100	97
14	FJ040355	Uncultured fungus clone LSUTypeUS9 28S large subunit ribosomal RNA gene, partial sequence.	100	100
17	AF347090	Trechispora hymenocystis isolate 362 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	98
18	FJ040364	Uncultured fungus clone LSUTypeUS18 28S large subunit ribosomal RNA gene, partial sequence.	100	99
19	AJ876781	Uncultured basidiomycete partial 28S rRNA gene. clone 12.1.	100	99
20	HQ433141	Uncultured Basidiomycota clone bas07010 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	100
26	EU490099	Uncultured basidiomycete clone C32_A10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
27	AF347099	Clavariadelphus ligula 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	96
28	GU174279	Uncultured fungus clone Oc_O_MayF03 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	100
30	FJ040356	Uncultured fungus clone LSUTypeUS10 28S large subunit ribosomal RNA gene, partial sequence.	100	99

41	JF519439	Uncultured Hydropus clone RELIS_G4_F11 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	97	99
42	EU292445	Uncultured fungus clone IH_Tag067_4485 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	96
43	FJ040353	Uncultured fungus clone LSUTypeUS7 28S large subunit ribosomal RNA gene, partial sequence.	100	98
44	HM488785	Lepiota neophana 25S large subunit ribosomal RNA gene, partial sequence.	99	97
45	AY645055	Clitocybe candicans isolate AFTOL-ID 541 25S ribosomal RNA gene, partial sequence.	97	99
46	EU292285	Uncultured fungus clone IH_Tag064_3086 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	97
47	FJ040356	Uncultured fungus clone LSUTypeUS10 28S large subunit ribosomal RNA gene, partial sequence.	100	98
48	AB086382	Trichosporon terricola genes for ITS1, 5.8S rRNA, ITS2, 26S rRNA, partial and complete sequences.	99	98
49	FJ040365	Uncultured fungus clone LSUTypeUS19 28S large subunit ribosomal RNA gene, partial sequence.	100	99

63	GU174402	Uncultured fungus clone Oc_O_MayC06 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
64	AY684166	Albatrellus higanensis isolate AFTOL-ID 774 25S ribosomal RNA gene, partial sequence.	98	98
65	GU055621	Uncultured Corticium clone NG_P_B05 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	94	96
66	GU174401	Uncultured fungus clone Oc_O_MayG07 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	98
67	GU174281	Uncultured fungus clone Oc_O_MayH12 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
72	EU692074	Uncultured soil fungus clone MWCtl3T0_3B 28S ribosomal RNA gene, partial sequence.	98	97
93	DQ156126	Marasmius oreades isolate AFTOL-ID 1525 25S ribosomal RNA gene, partial sequence.	100	97
94	AF042630	Crinipellis maxima isolate DAOM196019 25S large subunit ribosomal RNA gene, partial sequence.	92	98
95	DQ457673	Porotheleum fimbriatum isolate AFTOL-ID 1725 25S large subunit ribosomal RNA gene, partial sequence.	98	99

96	FJ185160	Coprinellus aff. radians 1-2PS small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	100	99
97	EU365678	Sarcomyxa serotina strain ACCC 50309 28S ribosomal RNA gene, partial sequence.	97	96
98	DQ389731	Conocybe siliginea voucher LO93-04 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	100	98
99	EU489915	Uncultured basidiomycete clone 4M1_E06 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	96
100	HQ433184	Uncultured Basidiomycota clone bas07065 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	98
101	DQ341941	Uncultured Basidiomycota clone 140a_03c KBS-LTER large subunit ribosomal RNA gene, partial sequence.	95	97
102	HQ433218	Uncultured Basidiomycota clone bas07166 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	98
103	AF115333	Clavicorona taxophila 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	100	92

104	GQ159940	Uncultured fungus clone JDUBC_698_SCHIRP10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	93	99
105	HQ179664	Clitocybe hesleri voucher TENN:008084 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 25S ribosomal RNA gene, partial sequence	100	95
107	FN428971	Rhodotorula sp. AY207 partial 26S rRNA gene, isolate HEW-1-23.	96	98
108	AM039679	Rhodotorula sp. HB1139 partial 26S rRNA gene, strain HB1139.	97	97
109	FN401524	Rhodotorula yarrowii partial 26S rRNA gene, strain KBP 3848.	96	99
110	AF444702	Cryptococcus sp. CBS 8366 26S ribosomal RNA gene, partial sequence.	96	99
111	HQ433195	Uncultured Basidiomycota clone bas07088 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	97
112	FN428974	Cryptococcus sp. AJ200 partial 26S rRNA gene, isolate AJ200.	92	98
152	AF291295	Basidiodendron cinereum 28S large subunit ribosomal RNA gene, partial sequence.	83	99
153	DQ341805	Uncultured Basidiomycota clone 087a_03 KBS-LTER large subunit ribosomal RNA gene, partial sequence.	95	99

154	EU490099	Uncultured basidiomycete clone C32_A10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	98
155	EU490099	Uncultured basidiomycete clone C32_A10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	98
156	AF139973	Ramaria eumorpha RGT 930825/01 25S ribosomal RNA gene, partial sequence.	95	96
157	FJ040363	Uncultured fungus clone LSUTypeUS17 28S large subunit ribosomal RNA gene, partial sequence	100	100
158	HQ604795	Sphaerobolus stellatus isolate ST-10 voucher UBC F19770 internal transcribed spacer 2 and 28S ribosomal RNA gene, partial sequence.	98	99
159	EF537893	Corticium roseum isolate AFTOL-ID 1943 25S large subunit ribosomal RNA gene, partial sequence.	100	93
160	EU118652	Phanerochaete affinis voucher KHL 11839 (GB) internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
161	JF449746	Uncultured Auriculariales clone OS_2w_H05 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	97	94
162	FJ040343	Uncultured fungus clone LSUTypeUS1 28S large subunit ribosomal RNA gene, partial sequence.	100	98

163	AY293171	Ceratobasidium sp. GEL5602 25S ribosomal RNA gene, partial sequence.	99	99
164	DQ341948	Uncultured Basidiomycota clone 141a_08 KBS-LTER large subunit ribosomal RNA gene, partial sequence.	94	99

Supplemental Table 3. Taxonomic assignment of ascomycete OTUs and their relative abundance within ascomycete clone libraries.

OTUs assigned to taxonomic groups								
OTU	**Subdivision**	**Class**	**Order**	**Family**	**Relative abundance (%)†**			
					Ambient N deposition		Simulated N deposition	
					Site B	Site D	Site B	Site D
134	Pezizomycotina	Dothideomycetes	Dothideales		5.9	0.0	0.0	0.0
24	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	0.0	0.0	10.3	0.0
56	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	5.9	0.0	3.4	0.0
131	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	0.0	0.0	3.4	0.0
133	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	0.0	0.0	0.0	5.6
132	Pezizomycotina	Eurotiomycetes			5.9	0.0	0.0	0.0
113	Pezizomycotina	Leotiomycetes	Helotiales	Dermataceae	5.9	0.0	0.0	0.0
114	Pezizomycotina	Leotiomycetes	Helotiales	Dermataceae	0.0	3.0	0.0	0.0
21	Pezizomycotina	Leotiomycetes	Helotiales	Dermateaceae	0.0	3.0	0.0	11.1
50	Pezizomycotina	Leotiomycetes	Helotiales	Dermateaceae	0.0	0.0	0.0	11.1
22	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae/ Helotiaceae	0.0	3.0	0.0	11.1
23	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae/ Helotiaceae	17.6	0.0	0.0	0.0
52	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae/ Helotiaceae	0.0	3.0	0.0	5.6
53	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae/ Helotiaceae	5.9	0.0	3.4	0.0
54	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae/ Helotiaceae	0.0	3.0	3.4	0.0

121	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae/ Helotiaceae	0.0	3.0	0.0	0.0
122	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae/ Helotiaceae	0.0	0.0	3.4	0.0
123	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae/ Helotiaceae	5.9	0.0	0.0	0.0
124	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae/ Helotiaceae	0.0	0.0	0.0	5.6
125	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae/ Helotiaceae	0.0	0.0	0.0	5.6
126	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae/ Helotiaceae	0.0	0.0	3.4	0.0
127	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae/ Helotiaceae	0.0	3.0	0.0	0.0
128	Pezizomycotina	Leotiomycetes	Helotiales	Hyaloscyphaceae/ Helotiaceae	0.0	3.0	0.0	0.0
8	Pezizomycotina	Leotiomycetes	Helotiales	Sclerotiniaceae	5.9	9.1	3.4	5.6
57	Pezizomycotina	Leotiomycetes	Helotiales	Sclerotiniaceae	5.9	0.0	3.4	0.0
135	Pezizomycotina	Leotiomycetes	Helotiales	Sclerotiniaceae	0.0	0.0	0.0	5.6
136	Pezizomycotina	Leotiomycetes	Helotiales	Sclerotiniaceae	0.0	0.0	0.0	5.6
51	Pezizomycotina	Leotiomycetes	incertae sedis		0.0	0.0	6.9	0.0
55	Pezizomycotina	Leotiomycetes	incertae sedis		0.0	0.0	6.9	0.0
115	Pezizomycotina	Leotiomycetes	incertae sedis		0.0	0.0	0.0	5.6
116	Pezizomycotina	Leotiomycetes	incertae sedis		0.0	0.0	3.4	0.0
117	Pezizomycotina	Leotiomycetes	incertae sedis		0.0	0.0	0.0	5.6
118	Pezizomycotina	Leotiomycetes	incertae sedis		0.0	0.0	3.4	0.0
119	Pezizomycotina	Leotiomycetes	incertae sedis		0.0	3.0	0.0	0.0
120	Pezizomycotina	Leotiomycetes	incertae sedis		5.9	0.0	0.0	0.0
129	Pezizomycotina	Leotiomycetes			0.0	3.0	0.0	0.0
59	Pezizomycotina	Orbiliomycetes	Orbiliales		0.0	0.0	6.9	0.0

147	Pezizomycotina	Pezizomycetes	Pezizales	5.9	0.0	0.0	0.0
58	Pezizomycotina	Sordariomycetes	Diaporthales	0.0	6.1	0.0	0.0
137	Pezizomycotina	Sordariomycetes	Hypocreales	5.9	0.0	0.0	0.0
138	Pezizomycotina	Sordariomycetes	Hypocreales	0.0	0.0	3.4	0.0
139	Pezizomycotina	Sordariomycetes	Hypocreales	0.0	3.0	0.0	0.0
140	Pezizomycotina	Sordariomycetes	Hypocreales	0.0	0.0	3.4	0.0
11	Pezizomycotina	Sordariomycetes	Xylariales	0.0	3.0	6.9	5.6
142	Pezizomycotina	Sordariomycetes	Xylariales	0.0	3.0	0.0	0.0
141	Pezizomycotina	Sordariomycetes		0.0	3.0	0.0	0.0
144	Pezizomycotina	Sordariomycetes		0.0	0.0	3.4	0.0
145	Pezizomycotina	Sordariomycetes		0.0	0.0	3.4	0.0
143	Pezizomycotina	Sordariomycetes		5.9	0.0	0.0	0.0

OTUs of uncertain taxonomy

OTU	Subdivision	Clade	Subclade	Relative abundance (%)†			
				Ambient N deposition		Simulated N deposition	
				Site B	Site D	Site B	Site D
130	Pezizomycotina	"Pleosporales"		0.0	3.0	0.0	0.0
25	Pezizomycotina	"Pleosporales" and Ochroconis spp.		0.0	6.1	0.0	5.6
146	Pezizomycotina	"Pleosporales" and Ochroconis spp.		0.0	3.0	0.0	0.0
150	Pezizomycotina	unresolved basal clade		0.0	3.0	0.0	0.0
61	Pezizomycotina	unresolved basal clade	Lecanoromycetes/ Heliocephala	0.0	0.0	6.9	0.0

62	Pezizomycotina	unresolved basal clade	Lecanoromycetes/ Heliocephala	0.0	6.1	0.0	0.0
149	Pezizomycotina	unresolved basal clade	Lecanoromycetes/ Heliocephala	0.0	3.0	0.0	0.0
15	Pezizomycotina	unresolved basal clade	uncultured group	0.0	6.1	3.4	5.6
16	Pezizomycotina	unresolved basal clade	uncultured group	5.9	6.1	3.4	0.0
60	Pezizomycotina	unresolved basal clade	uncultured group	5.9	3.0	0.0	0.0
148	Pezizomycotina	unresolved basal clade	uncultured group	0.0	3.0	0.0	0.0

† Percentage of clones in total ascomycete clones for each library. Total number of ascomycete clones for each site, N deposition treatment were as follows: Site B ambient, 17; Site B simulated, 29; Site D ambient, 33, Site D simulated, 18.

Supplemental Table 4. Top NCBI BLAST match information for ascomycete OTUs, including description and accession number of BLAST match and the percent coverage and identity with the OTU sequence.

OTU	Accession	Description	Query coverage (%)	Maximum identity (%)
8	FJ040388	Uncultured fungus clone LSUTypeUS40 28S large subunit ribosomal RNA gene, partial sequence.	100	99
11	GU174272	Uncultured fungus clone Alb_O_MayB04 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	100
15	FJ040367	Uncultured fungus clone LSUTypeUS21 28S large subunit ribosomal RNA gene, partial sequence.	100	99
16	FJ040368	Uncultured fungus clone LSUTypeUS22 28S large subunit ribosomal RNA gene, partial sequence.	100	100
21	GU174270	Uncultured fungus clone Oc_O_MayG10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
22	GU174300	Uncultured fungus clone Alb_O_MayE08 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	98

23	EU292504	Uncultured fungus clone IH_Tag102_2407 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	96
24	GU174353	Uncultured fungus clone Alb_O_AugH07 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
25	EU035425	Fusicladium amoenum strain CBS 254.95 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	97
50	FJ040395	Uncultured fungus clone LSUTypeUS47 28S large subunit ribosomal RNA gene, partial sequence.	100	100
51	GU174334	Uncultured fungus clone Oc_O_AugF03 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
52	GU174292	Uncultured fungus clone Oc_O_AugG12 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
53	GU174310	Uncultured fungus clone Alb_O_MayE06 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99

54	GU174269	Uncultured fungus clone Alb_O_AugC08 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
55	EU692738	Uncultured soil fungus clone MWSol3T8_5E 28S ribosomal RNA gene, partial sequence.	98	99
56	HQ432995	Uncultured Ascomycota clone asc07038 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
57	EU489981	Uncultured ascomycete clone 4S1_D05 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	97
58	FJ040376	Uncultured fungus clone LSUTypeUS30 28S large subunit ribosomal RNA gene, partial sequence.	100	100
59	GU174293	Uncultured fungus clone Alb_O_MayG11 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
60	HQ433120	Uncultured Ascomycota clone asc07198 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	89
61	EU691365	Uncultured soil fungus clone BPAGM2T0_1H 28S ribosomal RNA gene, partial sequence.	98	99
62	EU691365	Uncultured soil fungus clone BPAGM2T0_1H 28S ribosomal RNA gene, partial sequence.	98	92

113	HQ433015	Uncultured Ascomycota clone asc07059 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	100
114	FJ040383	Uncultured fungus clone LSUTypeUS37 28S large subunit ribosomal RNA gene, partial sequence.	100	99
115	GU174406	Uncultured fungus clone Oc_A_MayF05 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	100	99
116	GU174333	Uncultured fungus clone Oc_O_AugB04 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	100
117	GU174282	Uncultured fungus clone Alb_O_MayH03 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
118	JF519259	Uncultured Alatospora clone RELIS_K6_A11 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	97	99
119	GU174313	Uncultured fungus clone Oc_O_MayH06 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99

120	GU174271	Uncultured fungus clone Alb_O_AugG05 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	96
121	HQ433125	Uncultured Ascomycota clone asc07203 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	99	97
122	FJ040392	Uncultured fungus clone LSUTypeUS44 28S large subunit ribosomal RNA gene, partial sequence.	100	97
123	HQ433080	Uncultured Ascomycota clone asc07129 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	99	98
124	GU174300	Uncultured fungus clone Alb_O_MayE08 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
125	GU174403	Uncultured fungus clone Oc_O_AugA12 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	100
126	GU174291	Uncultured fungus clone Alb_O_MayE11 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
127	GU552534	Fungal sp. mh3463.2 28S large subunit ribosomal RNA gene, partial sequence.	100	99
128	HM595595	Lachnellula sp. ZLY-2010b isolate M118 internal transcribed spacer 2 and 28S ribosomal RNA gene, partial sequence.	100	94

129	HQ432990	Uncultured Ascomycota clone asc07033 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	98	99
130	GU174276	Uncultured fungus clone Oc_O_MayG09 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	98
131	EU940120	Fungal sp. M175 isolate M175 28S large subunit ribosomal RNA gene, partial sequence.	89	99
132	EU292609	Uncultured fungus clone IH_Tag126_1883 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	94
133	EU691414	Uncultured soil fungus clone BPAGM2T8_1H 28S ribosomal RNA gene, partial sequence.	98	100
134	GU174335	Uncultured fungus clone Oc_O_MayG03 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	100
135	AY789407	Scleromitrula shiraiana strain Hirayama062001 25S large subunit ribosomal RNA gene, partial sequence.	98	98
136	AY789347	Sclerotinia sclerotiorum strain WZ0067 25S large subunit ribosomal RNA gene, partial sequence.	98	94
137	HQ433119	Uncultured Ascomycota clone asc07197 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	97

138	HQ433045	Uncultured Ascomycota clone asc07091 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	99
139	FN859457	Hypomyces sp. TFC 201334 genomic DNA containing ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene, strain TFC 201334.	100	95
140	FJ040373	Uncultured fungus clone LSUTypeUS27 28S large subunit ribosomal RNA gene, partial sequence.	100	99
141	GU552491	Fungal sp. mh1970.24 28S large subunit ribosomal RNA gene, partial sequence.	100	99
142	EU552100	Anthostomella leucospermi culture-collection CBS:110126 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	98
143	GU174378	Uncultured fungus clone Alb_A_AugC05 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	100
144	DQ273476	Uncultured sordariomycete clone F41 28S ribosomal RNA gene, partial sequence.	90	99
145	EU292361	Uncultured fungus clone IH_Tag067_1414 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	99	94
146	FJ176263	Chalara sp. OC0010 isolate OC0010 28S ribosomal RNA gene, partial sequence.	93	97
147	AJ698473	Gyromitra infula partial 28S rRNA gene, isolate GyrUSA.	100	96

148	HQ433038	Uncultured Ascomycota clone asc07083 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	100	88
149	EU691365	Uncultured soil fungus clone BPAGM2T0_1H 28S ribosomal RNA gene, partial sequence.	98	97
150	FJ040371	Uncultured fungus clone LSUTypeUS25 28S large subunit ribosomal RNA gene, partial sequence.	100	86