Ecological Monographs, 84(1), 2014, pp. 3–20

A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning

D. Lee Taylor,[1,4] Teresa N. Hollingsworth,[2] Jack W. McFarland,[1,5] Niall J. Lennon,[3] Chad Nusbaum,[3] and Roger W. Ruess[1]

[1]*Institute of Arctic Biology, 311 Irving I Building, University of Alaska, Fairbanks, Alaska 99775 USA*
[2]*USDA Forest Service, PNW Research Station, Boreal Ecology Cooperative Research Unit, Fairbanks, Alaska 99775 USA*
[3]*Broad Institute of MIT and Harvard, 320 Charles Street, Cambridge, Massachusetts 02141 USA*

Abstract. Fungi play key roles in ecosystems as mutualists, pathogens, and decomposers. Current estimates of global species richness are highly uncertain, and the importance of stochastic vs. deterministic forces in the assembly of fungal communities is unknown. Molecular studies have so far failed to reach saturated, comprehensive estimates of fungal diversity. To obtain a more accurate estimate of global fungal diversity, we used a direct molecular approach to census diversity in a boreal ecosystem with precisely known plant diversity, and we carefully evaluated adequacy of sampling and accuracy of species delineation. We achieved the first exhaustive enumeration of fungi in soil, recording 1002 taxa in this system. We show that the fungus : plant ratio in *Picea mariana* forest soils from interior Alaska is at least 17:1 and is regionally stable. A global extrapolation of this ratio would suggest 6 million species of fungi, as opposed to leading estimates ranging from 616 000 to 1.5 million. We also find that closely related fungi often occupy divergent niches. This pattern is seen in fungi spanning all major functional guilds and four phyla, suggesting a major role of deterministic niche partitioning in community assembly. Extinctions and range shifts are reorganizing biodiversity on Earth, yet our results suggest that 98% of fungi remain undescribed and that many of these species occupy unique niches.

Key words: biodiversity; black spruce forest; community assembly; fungi; fungus-to-plant ratio; global species richness; interior Alaska, USA; Picea mariana; *rarefaction; ribosomal internal transcribed spacer; soil horizon; vegetation structure.*

Introduction

The extent of species diversity on Earth is both highly uncertain and controversial (May 1988, 1991, Hawksworth 1991, Mora et al. 2011), yet few questions could be more fundamental given ongoing environmental change. The diversity of large eukaryotes is reasonably well known, whereas the diversities of smaller eukaryotes and prokaryotes are poorly known. Among small organisms such as fungi, only the largest and showiest species (charismatic megamycota) are well known. Fungi provide the global foundation for plant growth as mutualists (e.g., mycorrhizae, endophytes) and decomposers (e.g., soil hyphomycetes, wood-decaying basidiomycetes), and thereby function as key drivers in the carbon cycle. Earth has entered a period of unprecedented extinction (Barnosky et al. 2011), involving species range shifts and overall reorganization of biodiversity. An accurate estimate of the diversity of fungi and improved knowledge of the forces that control the distribution of species and assembly of communities are increasingly critical in light of global climate and land-use changes, loss of biodiversity, and the need to predict microbial functional feedbacks to climate.

The taxonomic diversity of fungi is known mostly from their macroscopic reproductive structures, i.e., sporocarps such as mushrooms. Though there are only roughly 100 000 described species in the Eumycota (Kirk et al. 2008), a true diversity of 1.5 million (Hawksworth 1991), or even 5 million (O'Brien et al. 2005) species has been suggested. Hence, it is clear that much of global fungal diversity remains to be documented. The majority of fungi depend in some way on living or dead plants for their energy supply, suggesting important linkages between aboveground and belowground diversity (Hooper et al. 2000). Hence, there has long been an interest in comparing and relating plant and fungal diversity. Hawksworth (1991) synthesized data from multiple sites in the United Kingdom with known plant diversities and extensive fungal collection records, and found that the ratio of species of fungi to vascular plants was remarkably consistent across sites, and averaged roughly 6 to 1. Based on a global count of 270 000 vascular plants, the UK ratio gave rise to a global

Manuscript received 1 October 2012; revised 26 February 2013; accepted 2 May 2013. Corresponding Editor: D. M. Rizzo.

[4] Present address: Department of Biology, MSC03 2020, 1 University of New Mexico, Albuquerque, New Mexico 87131-0001 USA. E-mail: ffllt@unm.edu

[5] Present address: U.S. Geological Survey, 345 Middlefield Road, Menlo Park, California 94025 USA.

extrapolation of 1.5 million species of fungi. While this extrapolation has been controversial (May 1991), it is also the most widely cited estimate of global fungal species richness. Several other studies have also reported statistically significant correlations between diversity within particular fungal functional or taxonomic groups and plant diversity at local to regional scales (Schmit et al. 2005, Sugiyama et al. 2008). However, these compilations have often involved data sets collected by different investigators using differing methods, and have not included molecular data. More systematic and exhaustive evaluations of fungal to plant (F:P) ratios and the mechanistic linkages between aboveground and belowground diversity are needed.

To date, compilations of F:P ratios have been entirely morphological, and thus, have relied upon identification of macroscopic fungal fruiting structures or identification of strains that could be isolated in culture. Previous F:P ratio analyses likely underestimate the true ratio due to the difficulty in culturing many fungi, our inability to distinguish morphological features that might differentiate species in simple organisms, the seasonality of sporocarp production, and the absence of records from non-fruiting stages or taxa (Gardes and Bruns 1996). Rapidly advancing molecular methods offer the prospect of a more thorough documentation of diversity. However, while numerous recent studies (O'Brien et al. 2005, Buée et al. 2009, Jumpponen and Jones 2009) have utilized high-throughput sequencing to examine various aspects of fungal biodiversity, these studies have failed to saturate a sampling (rarefaction) curve. Therefore, these studies have not enumerated total fungal diversity in their samples. Furthermore, most of these studies have analyzed modest numbers of samples over small spatial scales, and have not reported detailed characterization of the associated plant communities, thus leaving F:P ratios unaddressed. While they have not directly addressed F:P ratios, recent molecular surveys in a variety of habitats have suggested that fungal diversity is much higher than previously reported. For example, Bueé et al. (2009) recorded an average of 830 fungal operational taxonomic units (OTUs) per site in temperate forest plantations planted with single tree species, while Jumpponen and colleagues found nearly 700 OTUs on *Quercus* leaf surfaces (Jumpponen and Jones 2009). These observations invite a closer examination of the factors that underlie fungal hyperdiversity.

Fundamental theories to explain community assembly and species coexistence can be divided into those that emphasize deterministic forces (e.g., Lotka-Volterra competition [Gause 1932, Diamond 1978], colonization–recruitment trade-offs [Skellam 1951], resource-ratio theory [Tilman 1982], plant–soil feedback [Bever 1994]) and those that emphasize stochastic factors (e.g., historical contingency [Losos 1994], lottery hypothesis [Sale 1977]). The "unified neutral theory" of Hubbell (Hubbell 2001) has particularly stimulated rigorous comparisons of deterministic vs. stochastic explanations for observed patterns. The greater the numbers of species that coexist without obvious differences in the niche they occupy, the more urgent is the need to search for explanations (Silvertown 2004). Fungal hyperdiversity is thus ripe for careful dissection of coexistence, and the logical starting point is an examination of resource use and evidence for niche partitioning. We recognize that the term *niche* has implied a wide range of meanings among different authors, contributing to a confused and controversial scientific legacy. We use the term in the sense of the range of biotic and abiotic conditions (axes) for which individuals of a species have fitness greater than zero, as conceptualized by Grinnell (1917), Hutchinson (1957), and others. This may be contrasted with definitions of niche that focus more on the impact of a species on its environment, particularly through resource consumption (Elton 1927, MacArthur and Levins 1967) as discussed by Leibold (1995). However, the "requirement" and "role" aspects of niche can be tightly intertwined in fungi, as we discuss later. With respect to *niche partitioning*, we are interested in both alpha and beta niches (Silvertown 2004), the alpha niche encompassing environmental axes that vary at a scale smaller than the extent of the focal community, and the beta niche describing responses to habitat gradients over wider spatial scales within a regional context.

Many fungal species are known to have unique environmental requirements and functional roles, examples including the numerous host-specific plant pathogens. A smaller number of studies have also demonstrated niche partitioning among species that comprise complex communities (Dickie et al. 2002). However, due to their invisibility belowground and challenges in measuring appropriate environmental variables at appropriate temporal and spatial scales, we know relatively little about which niche axes may be important for fungal communities in soil. Moreover, the evolutionary timescales over which fungi may diverge in resource acquisition strategies or other traits relevant to coexistence is little known. To address the latter issue, analyses that consider phylogeny or taxonomic hierarchy are needed.

The boreal forest is a compelling ecosystem in which to characterize fungal biodiversity and niche dimensions for three reasons. First, the boreal biome has low plant diversity, suggesting that it should be possible to completely census the richness of both plants and fungi. Second, the northern boreal regions are experiencing some of the most rapid and profound changes due to climate warming, including permafrost thaw and changes in fire regime, both factors that have uncertain implications for the massive stores of carbon in boreal soils (Schuur et al. 2009, Chapin et al. 2010). As primary decomposers and plant mutualists, fungi affect rates of both carbon sequestration and emission from soil and are key players in boreal ecosystem feedbacks to climate change. Yet ecological drivers of the abundance, distribution, and function of fungal

species across boreal landscapes are essentially unknown. Third, the boreal forest is one of two biomes that is circumpolar in distribution. *Picea mariana* (black spruce) forests account for ~40% of forested land in Alaska, and are the most widespread vegetation type across North America. Hence, characterization of boreal aboveground–belowground relationships can be extrapolated over a wide geographic area.

Here we report the first robust estimate of both fungal and plant species richness at the same sites based on extensive molecular data from replicated and repeated sampling over a wide spatial scale. In this study, we pose the following hypotheses concerning fungal species richness, the form of aboveground–belowground linkages and axes of fungal niche partitioning:

1) A thorough molecular survey will reveal higher F:P ratios than have been recorded in morphology-based studies of isolates and sporocarps.
2) The consistent regional F:P ratio reported in prior nonmolecular studies will hold when molecular methods are applied, provided that sampling is sufficient.
3) Soil horizon will be the strongest measured axis of niche partitioning, based on the importance of this factor in prior studies.
4) Understory plant community composition will be correlated with fungal community composition due to direct interactions (e.g., mycorrhizae), indirect interactions (e.g., litter chemistry), and shared responses to other environmental drivers (e.g., soil moisture).
5) Closely related species will often occupy different niches with respect to environmental variables due to rapid evolution of microhabitat preferences. If found, such rapid evolution could reflect a lack of evolutionary–genetic constraints on these traits and/or strong selection favoring character displacement.

Methods

Site description

Our study was carried out in black spruce (*Picea mariana*) forests in the greater Fairbanks region of interior Alaska, which occurs at the western edge of the Intermontane Boreal Forest ecoregion of the North American continent (Nowacki et al. 2001). It is characterized by small mountain ranges and associated gently sloping uplands, large areas of flat lowlands, and braided rivers with broad floodplains. Interior Alaska has a relatively dry climate (286-mm annual precipitation, on average), is snow-covered for 6–9 months of the year, and much of the surface is underlain by permafrost. Black spruce stands are the most abundant vegetation type, covering roughly 40% of the landscape (Van Cleve and Dyrness 1983), with dominance on poorly drained lowlands and cold, north-facing slopes. Interior Alaska is an area of discontinuous permafrost, with approximately 75–80% of the ground underlain with permafrost (Osterkamp and Romanovsky 1999). Most of interior Alaska, and our study area, escaped the repeated glaciations of the Quaternary; however, Illinoian and Pre-Illinoian glaciers were present at the southern edge of the study area (along the northern flanks of the Alaska Range) (Péwé et al. 1965). Interior Alaska soils show relatively little morphological development. Silt–loam upland soils have developed from loess laid down during the last glacial maximum and from underlying schist bedrock, and floodplain soils have developed from sandy or silt-textured alluvium, mostly of glacial origin (Rieger et al. 1963).

In the present study we sampled 12 of the 151 black spruce sites studied by Hollingsworth et al. (2006, 2008). All sites have a primary overstory canopy of mature black spruce, and are thus representative of the most widespread boreal vegetation type in Alaska and Canada, but vary with respect to tree density, stand age, understory vegetation, mineral soil pH, soil moisture, topography and other biotic and abiotic features. The 12 sites were visited in two successive years, and all soil samples divided into organic and mineral horizon composites; thus, 48 samples form the foundation of subsequent analyses. Site locations are shown in Appendix A: Fig. A1.

Vegetation description and measurement of environmental variables

Vegetation was classified into two communities and two subtypes related to floristic composition of the sites and correlations with environmental factors. These communities are described in detail elsewhere (Hollingsworth et al. 2006). Acidic black spruce/lichen forests are dominated by black spruce, fruticose lichens, and many species of mosses. This community type occurs in both uplands and lowlands of interior Alaska and in areas of low mineral-soil pH (<5.5). Within this community type we find wet and dry sites related to topography and presence of permafrost. Wet acidic black spruce muskeg is an open black spruce subtype that occurs on low-nutrient soils, often in lowland areas of shallow active layer. Black spruce trees are often stunted, and the subtype tends to be dominated by shrubs, sedges, and acidic *Sphagnum*. The nonacidic black spruce/Rose/Horsetail forest plant community is dominated more by vascular species than the acidic black spruce forest, with the occasional presence of *Picea glauca* (white spruce). The wet nonacidic black spruce/larch fen is restricted to minerotrophic lowlands and is usually co-dominated by larch (*Larix laricina*). The dry nonacidic black spruce forest subtype occurs in xeric conditions, mostly in the uplands. Like the acidic black spruce forest, this subtype is dominated by lichens, but the lichen species are different.

Soil sampling and processing for fungal communities

Sites were visited between 25 August and 26 September in 2004 and again in 2005. On each visit to a site, a total of 50 soil cores 1.8 cm in diameter × 20 cm in depth were collected along four parallel transects with approximately 10-m spacing between cores. Subsamples of the O_i (organic) and A (mineral) horizons (Soil Survey Division Staff 1993) from each core were pooled within sites and deposited in 50mL Falcon tubes (BD Biosciences, San Jose, California, USA) then stored at −80°C until lyophilization. The soils were then ground on a ball mill at −20°C, with 0.8-cm steel beads. Genomic DNA was extracted from 1–5 g of soil from each composite sample using the Mo Bio Powersoil kit (MO BIO Laboratories, Carlsbad, California, USA) following the manufacturer's instructions. The soil DNA extracts were normalized to 2.5 ng/μL after Picogreen (Molecular Probes, Eugene, Oregon, USA) quantification.

PCR and sequencing

Polymerase chain reactions (PCRs) utilized 25-μL Amersham Ready-To-Go beads, 0.5-μm primers ITS1-FL and tagged versions of TW13 (Taylor et al. 2008), and the following cycling conditions: initial denaturation at 96°C for 2 min followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 40 s and extension at 72°C for 3 min, with a final extension at 72°C for 10 min. For each soil DNA extract seven replicate PCRs were performed and pooled. We utilized a low number of cycles and a large number of replicate PCRs to reduce chimera formation and stochastic amplification biases. We applied a molecular-tagging strategy to mark PCR products from various sources with DNA tags, which were then pooled before library sequencing (Taylor et al. 2008). To minimize the cloning of primer-dimers and other short inserts, 100 μL of the pooled fragments were size-fractionated over Chroma Spin 400 columns (BD Biosciences, San Jose, California, USA), then concentrated through DNA Clean and Concentrator-5 columns (Zymo Research, Irving, California, USA). The templates were quantified on a Nanodrop spectrophotometer and normalized to 25ng/μL prior to ligation. The pooled PCR products were then cloned into the Invitrogen PCR4-TOPO (Life Technologies Corporation, Carlsbad, California, USA) vector. The resulting PCR libraries were shipped frozen to the Broad Institute, Cambridge Massachusettss (USA), where transformation, plating, colony picking, Templiphi reactions, and sequencing were carried out on automated equipment, using M13 forward and reverse vector primers. These molecular methods have been partially described elsewhere (Taylor et al. 2007, 2008, Geml et al. 2008, 2009). Site coordinates and numbers of soil cores and sequences generated are given in Appendix A: Tables A1 and A2.

Sequence analyses

Our approaches to sequence bioinformatics have also been described in detail elsewhere (Taylor et al. 2008, 2010, Geml et al. 2009, 2010, Taylor and Houston 2011). In brief, sequence data obtained for both strands were edited and assembled for each clone using CodonCode Aligner version 1.3.4 (CodonCode Corporation, Centerville, Massachusetts, USA). Primer tags were identified, bases with phred scores below 20 were masked, and clone orientations were corrected using Perl scripts (*available online*).[6] We then removed primer and vector sequences and isolated the internal transcriber space (ITS) region of each clone by locating the positions of ITS1-F and ITS4 (White et al. 1990, Gardes et al. 1991) in multiple sequence alignments generated using ClustalW (Thomson et al. 1994). The dirty ends of ITS sequences were removed using the program EMBOSS TrimSeq (Rice et al. 2000), and sequences with greater than 2% Ns remaining (i.e., base calls of such poor quality that they are undetermined) were deleted from the data set. Sequences were grouped into operational taxonomic units (OTUs) using the single-linkage genome assembly program Cap3 (Huang and Madan 1999) with the following non-default settings: overlap percent identity = 96.9; match score factor = 5; maximum overhang percent length = 60; clipping range = 6. No simple ITS sequence identity threshold can perfectly distinguish species boundaries across diverse fungi since the molecular evolution of the ITS region varies across fungal lineages. However, a number of focused phylogenetic studies suggest that a threshold of 97% may lump discrete sister species (Kindermann et al. 1998, Horton 2002, Geml et al. 2006, 2009); on the other hand, a more rigorous threshold would begin to artificially divide species due to sequencing errors and intraspecific variation (O'Brien et al. 2005, Quince et al. 2009, Kunin et al. 2010). Thus we view 97% as the best available approximation when a single standard must be applied to large data sets spanning wide fungal diversity. Furthermore, this threshold is likely to be conservative with respect to diversity of biological species. To evaluate whether our 97% sequence identity threshold and single-linkage clustering using Cap3 accurately captured species-level boundaries and whether our OTU counts might have been inflated by pseudogenes or chimeras, we carried out detailed phylogenetic analyses of selected OTUs. We independently analyzed OTUs that fell into three distinct genus-level clusters, one representing the Basidiomycota (*Cortinarius*), one the Ascomycota (*Leptodontidium*), and one the former Zygomycota (*Mortierella*). For each phylum, these genera represent one of the most species-rich clusters of OTUs seen in our data set, which were chosen because these are the most likely to exhibit artifactually high

[6] www.borealfungi.alaska.edu

numbers of OTUs. We searched GenBank for close matches to all OTUs in each of the three groups, created multiple sequence alignments using MAFFT EINSI (Katoh et al. 2002) and/or MUSCLE (Edgar 2004) and inferred evolutionary relationships using maximum likelihood with rapid bootstrap under the GTRCAT model in RAxML 7.0.4 (Stamatakis et al. 2005, 2008).

We ran all sequences through the UCHIME (Edgar et al. 2011) chimera-detection program using the de novo option and including close relatives from GenBank, as implemented on our public web tool (*available online*).[7] All sequences with scores above 3.0 were discarded as likely chimeras, while all sequences with scores between 0.8 and 3 were manually investigated via construction of multiple alignments containing all most closely related sequences derived from separate ITS1 and ITS2 BLAST searches (Taylor and Houston 2011). Several additional sequences were discarded. In all cases, chimeric sequence types were of very low abundance (1–5 clones), all but one being recovered from a single soil sample.

The best representative sequence from each OTU was roughly identified through nucleotide BLAST (Altshul et al. 1997) searches of our local fungal ITS database (footnote 2) that is updated from the GenBank nr nucleotide database weekly (Taylor and Houston 2011), with and without environmental (unidentified) sequences included. If the match with the highest bit score was an unidentified sequence, both the top identified and unidentified accessions are given in the Supplement: file 1, Blast_IDs.csv. Otherwise, only the top identified accession is given. These identifications allowed us to assign guild membership to many OTUs. We used the following criteria to evaluate the level of identification for each OTU. A score of A, indicating identification to the species level, was given when a hit with >97% similarity was found to a well-identified fungus. A score of B was given when no match >97% was found to a well-identified fungus, but a match >97% was found to another uncultured/environmental sequence. A score of C was given when the best match to a well-identified or uncultured sequence was between 90 and 96.9%. A score of D was given, indicating a poor identification, when only matches below 90% identity were found, or only overlaps of less than 200 base pairs (bp) were found. These identification levels are listed in the Supplement: file 1, Blast_IDs.csv. The latter scenario usually indicates that BLAST has found significant similarity only to the highly conserved 5.8S region. Note that we likely overestimate the proportion of species-level matches for two reasons: first, many of the hits with >97% identity overlapped only a portion of the query sequence; second, we accepted in category A 97% hits to fungal isolates or sporocarps that had a genus, but not a species name (e.g., *Phialophora* sp.), and thus were not "fully identified" according to previous criteria (Nilsson et al. 2009). The representative sequence for each OTU has been submitted to GenBank under accessions KF617225–KF618373. The full OTU-by-site matrix is given in Supplement: file 2, OTU_by_site_matrix.csv.

[7] www.borealfungi.uaf.edu

Statistical analyses

Species-accumulation curves and bootstrap estimates of total richness were computed using EstimateS, version 7.5 (Colwell 1997). Curves are based on means of 50 randomized subsamplings. We performed additional rarefaction analyses by randomly adding each of the 12 sites 50 times and tabulating numbers of vascular plant species and fungal OTUs observed, then calculating the corresponding fungus-to-plant (F:P) ratios. Our estimate of the minimum ratio utilized the 95% confidence interval (CI) upper bound for plant species combined with the 95% CI lower bound for fungal species. The maximum ratio was calculated similarly, using the lower CI for plants and upper CI for fungi.

To compare community composition among sites and samples, we removed fungal taxa occurring in fewer than two samples, relativized the abundances by site totals, and calculated dissimilarity among the 48 samples using the abundance-based version of the Bray-Curtis index. We then tested whether fungal communities differed across soil horizon, sampling year, and the four vegetation types using multiple-response permutation procedures (MRPP) (Berry et al. 1983). This method calculates the dissimilarity among samples grouped by a categorical variable and compares this value to a distribution generated by randomly permuting species observations across samples. Relationships between individual OTUs and selected categorical environmental factors were tested using "indicator species analyses" (Dufrene and Legendre 1997). In this approach, an index that combines frequency and abundance of an OTU in a target group of samples relative to its overall frequency and abundance is compared to a randomized distribution. OTUs that are more frequent and abundant (i.e., concentrated) in a particular group of samples (e.g., organic horizon samples) than expected by chance are considered to be indicator species for that environment. To visualize relationships between fungal community composition and measured environmental variables, we ordinated the 48 site–date–horizon combinations using nonmetric multidimensional scaling (NMS) (Kruskal 1964), a method of indirect gradient analysis (Whittaker and Niering 1965) that makes relatively few assumptions about the forms of species–environment relationships (McCune et al. 2002). We started with a random configuration to estimate the minimum supported dimensionality. Using this dimensionality, a final solution was estimated via 50 runs started from random seeds and 500 Monte Carlo resamplings to test

for significance. We performed a parallel ordination of plant community composition and stored the resulting plant axes as a way to summarize vegetation variation across the sites. We carried out principal-components analysis (PCA) of the 12 sites in order to reduce the 76 environmental variables measured at each site (Hollingsworth et al. 2006) to a few sets of intercorrelated variables aligned with axes that best explain the variation among sites. Correlations between fungal species ordination axes and potential drivers were evaluated in two ways. First, we examined the Kendall correlations between each individual environmental variable and fungal species ordination axes. Second, the site scores derived from the environmental PCA and vegetation NMS ordinations were used to create a second environmental matrix that was compared to fungal ordination axes, again by Kendall correlations. Calculations were carried out using PC-Ord 4.0 (McCune and Mefford 1999).

Results

Fungal diversity

We initially obtained 36 864 clone sequences, and then applied rigorous quality, length, taxonomic, and chimera filters, reducing the final data set to 28 884 clones. When clustered at 97% sequence identity across the internal transcriber space (ITS) region, these clones yielded 1002 operational taxonomic units (OTUs). Rarefaction analysis revealed a distinct inflexion with a slope that neared zero as our full sample size was reached (Fig. 1A). Further, the Chao 1 estimator, which adds a calculated number of "unseen" species to arrive at an estimate of true richness, was also asymptotic, reaching a maximum of 1044 OTUs. This saturation of the rarefaction curve indicates that we obtained a complete accounting of fungal OTU richness in these samples, which has not previously been accomplished in a forested ecosystem, to our knowledge. Additionally, only 117 of these 1002 OTUs were sequenced only once (singletons), meaning that our results are not driven by a long "tail" of exceptionally rare taxa that might represent polymerase chain reaction (PCR) and/or sequencing artifacts (Kunin et al. 2010).

The observed hyperdiversity of the fungal community is in stark contrast to the low plant diversity found in interior Alaskan *Picea mariana* forests. Only 60 of the 97 plant species documented at the 12 sites (Appendix A: Fig. A1) are vascular, giving a fungus-to-vascular plant multiplier of approximately 17:1. This result confirms our first hypothesis that molecular methods would lead to higher F:P ratios than reported in prior nonmolecular studies. The F:P ratio varied from site to site, with a mean of 16.0 (± 6.1 SD) (Appendix A: Table A1). However, when sites were added sequentially via rarefaction, the mean ratio rises from 14.5 for a single site to 16.35 with five sites, and is essentially constant at 16.8 with eight or more sites (Fig. 1B). Hence, the estimated F:P ratio was consistent across the 200-km study area. Furthermore, our sampling of sites and DNA sequences was adequate to reach a stable plateau in the F:P ratio. These findings lend support to our second hypothesis of a consistent regional F:P ratio, at least within our study area and forest type.

Our focused phylogenetic analyses of three of the most diverse genus-level clusters of OTUs in our data set demonstrate a strong correspondence between OTUs and species-level phylogenetic groups. For each genus, nearly all of our OTUs fell into distinct clades, and many were closely related to sequences obtained in other studies. In the case of *Cortinarius*, a mushroom-forming ectomycorrhizal basidiomycete, most OTUs fell into clusters together with sequences derived from sporocarps, with each cluster containing a different taxonomically defined species (Fig. 2). In *Leptodontium*, a group of root-associated dark septate endophytes (DSE) in the Ascomycota, several of our OTUs were closely related to well-characterized strains, but many other sequences were closely related only to other uncultured/environmental sequences (Appendix B). Similar patterns were seen in our analysis of *Mortierella*, a diverse group of saprotrophic soil "sugar fungi" that have historically been placed in the zygomycetes (Appendix B). With only two exceptions across 89 OTUs, different OTUs did not belong to a shared species-group, with terminal species-group defined by (1) branch length equivalent to 3% or greater from the nearest taxon, and/or (2) greater than 70% bootstrap support. Thus, pseudogenes, oversplitting, or chimeric artifacts appear unlikely to explain the great species richness reported in this study.

The large majority (67%) of the 1002 OTUs had a best BLAST match, based on bit scores, to an uncultured/environmental sequence, rather than a well-identified sequence from a fungal isolate or sporocarp. Only 28% of the OTUs could be considered matched to the species level (score A). Another 20% of the OTUs had a close match, but only to another uncultured/environmental sequence (score B). A further 40% were only moderately related to any known sequence (score C), and 12% were distantly related to any known sequence (score D). Based on the best BLAST matches, we assigned each OTU to its most likely phylum. The Ascomycota and Basidiomycota dominated the data set, and were nearly equal in abundance. The Ascomycota accounted for 55.2% of the OTUs and 48.4% of the clones, while the Basidiomycota accounted for 39.4% of the OTUs and 48.3% of the clones (Table 1). The Glomeromycota and Mortierellomycotina accounted for 2% and <1% of the clones, respectively. The Helotiales, including many putative ericoid mycorrhizal fungi and dark septate endophytes (DSE), were the most abundant and diverse order of Ascomycota, accounting for 180 OTUs and 34.5% of Ascomycota clones. Similarly, among the Basidiomycota, the majority of OTUs were related to putative ectomycorrhizal taxa. Summing

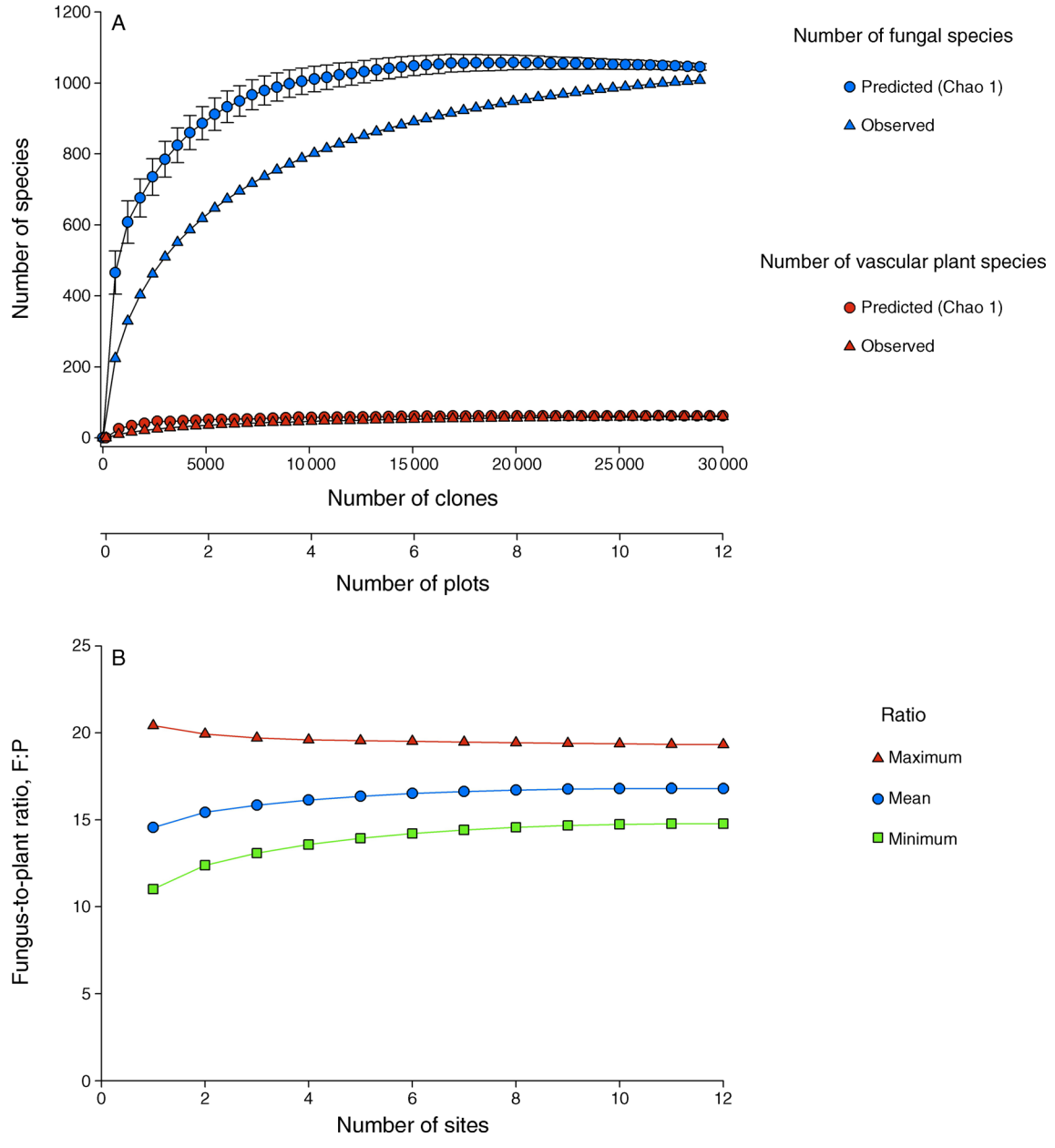

FIG. 1. (A) Saturation of the fungal rarefaction curve in black spruce forests of interior Alaska, USA. The *x*-axis represents subsets of the total number of clones sequenced (28 884) for fungi, or the number of sites sampled (12) for plants. (B) Fungus–plant ratios as a function of the number of sites. Notice that the ratio increases only slightly over the first five sites and is nearly constant when adding sites 9–12.

across both phyla, OTUs with top matches that are suspected to be ectomycorrhizal comprised 12 312 clones, or 42.6% of the data set. Thus, the majority of taxa uncovered in this study appear to be plant associated in some capacity. We note that typical wood and litter decay basidiomycetes that are known from sporocarps in interior Alaska (e.g., *Agaricus*, *Clitocybe*, *Marasmius*, *Mycena*, *Pholiota*, *Pleurotus*, and *Pluteus*) were present at low abundances in the data set.

Structure of fungal communities

The coexistence of so many fungal species in these sites motivates investigation of fungal habitat preferences within and among the study sites. We investigated how the soil fungal communities are structured with respect to the aboveground plant communities and a suite of environmental variables using nonmetric multidimensional scaling (NMS). Ordinations and multiple response permutation procedure (MRPP) analyses failed

FIG. 2. Phylogenetic tree of *Cortinarius*-like OTUs (operational taxonomic units). This maximum-likelihood tree was estimated with RAxML 7.04 (Stamatakis et al. 2005, 2008); bootstrap support values above 70% based on 1000 replicates are shown. Notice that most *Cortinarius*-like OTUs (boldface type) are close matches to sequences reported from other studies, and nearly every OTU falls out in a distinct clade defined by a sporocarp sequence with a different specific epithet (i.e., a different species).

to detect a difference between fungal communities in 2004 compared to 2005. In the MRPP tests, soil horizon had by far the largest effect size with respect to community composition (Table 2), in agreement with our third hypothesis. The strong distinction in community composition between horizons was also seen in the NMS ordination of sites (Fig. 3). Because the fungal communities of these two soil horizons are so distinct, we carried out further analyses to investigate their structure independently.

Considering soil chemistry and site factors, the occurrence and abundance of fungal taxa in the organic horizon were most strongly related to soil pH (Appendix C). A suite of soil and site factors co-vary with pH and were thus strongly related to soil PCA axis 2, including concentrations of Na, Mg, and Ca, the percentages of clay, sand and silt, elevation and topographic position (Appendix C). Vegetation variables that were strongly correlated with organic horizon fungi included tree density, the proportions of seedlings vs. trees, and the percentage of graminoid species. In contrast, mineral-

FIG. 2. Continued.

TABLE 1. Representation of fungal phyla in this study in black spruce forests of interior Alaska, USA.

Phylum	No. OTUs	No. clones
Ascomycota	551	13 992
Basidiomycota	397	13 962
Glomeromycota	7	587
Mortierellomycotina	24	185
Mucoromycotina	9	29
Chytridiomycota	7	10
Unplaced early diverging fungal lineage (*Olpidium*)	1	2
Blastocladiomycota	1	1
Entomophthoromycota	1	1
Unknown	4	115
Totals	1002	28 884

Note: OTU stands for operational taxonomic unit.

horizon fungal community structure was more strongly correlated with site moisture and its covariates on PCA soil axis 1, including percentage C, organic soil depth, cation exchange capacity, and bulk density (Appendix C). Parameters describing tree size and density (tree height, diameter at breast height, and stand basal area) were the vegetation factors most strongly related to mineral-horizon fungal composition. These aspects of stand structure are highly correlated with site moisture.

We directly compared the composition of plant and fungal communities using Mantel tests of correlations between community Bray-Curtis dissimilarity matrices. The test was significant for organic-horizon fungi ($P = 0.004$), with a correlation of $r = 0.43$ between the plant and fungal matrices, but was weakly correlated and nonsignificant for mineral-horizon fungi ($r = 0.09$, $P = 0.29$). The Mantel tests are consistent with the MRPP tests, in which the four site classes based on vegetation types were a significant predictor only for the organic-horizon fungal community (Table 2). Hence, our results demonstrate horizon-dependent variation in the nature of linkages between structures of aboveground plant and belowground fungal communities.

TABLE 2. Multiple-response permutation procedure tests for differentiation of fungal communities.

Factor	Effect size, *A*	Significance, *P*
All samples ($N = 48$)		
Soil horizon (mineral vs. organic)	**0.0716**	**<0.00000001**
Vegetation type (four site classes)	**0.0367**	**0.000106**
Year (2004 vs. 2005)	0.000469	0.369
Organic horizon ($N = 12$)		
Vegetation type	**0.0901**	**0.0330**
Mineral horizon ($N = 12$)		
Vegetation type	0.0462	0.0621

Notes: Significant test results are in bold. Notice that horizon is the strongest factor, while year is nonsignificant. Vegetation type is highly significant in the complete 48-sample data set, marginally significant for the organic horizon samples with years combined, and marginally nonsignificant for the mineral horizon samples with years combined.

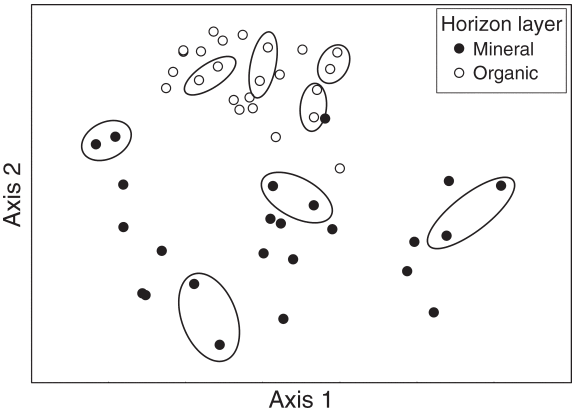

FIG. 3. Strong horizon partitioning of fungal communities illustrated by NMS (nonmetric multidimensional scaling) ordination. This analysis included all 48 samples, representing two collection years and two soil horizons from each of the 12 sites. Several examples of the same site and horizon sampled in successive years are indicated with ovals, illustrating the year-to-year consistency of fungal communities within a site.

More surprisingly, indicator-species analyses showed that it is often species belonging to the same genus and broad functional guild (e.g., ectomycorrhizal, saprotrophic, pathogenic) that have divergent preferences with respect to soil horizon or plant community type (Table 3). The most abundant taxon in the entire data set, OTU221 (*Cortinarius collinitus*, an ectomycorrhizal species), was identified in 1321 clones from 25 of the 48 site–year–horizon combinations, but was found almost exclusively in the organic horizon (indicator value = 92.3, $P = 0.0002$). In contrast, another *Cortinarius* species, OTU442, was a strong indicator for the mineral horizon (indicator value = 25, $P = 0.019$) (Appendix C). Many additional examples of divergent habitat preferences among closely related taxa were apparent, such as *Piloderma* OTU320 in comparison to OTU322 (both ectomycorrhizal; wet nonacidic vs. dry acidic preferences), *Leptodontidium* OTU173 vs. *Leptodontidium* OTU177 (both DSE; mineral vs. organic horizon preferences) and *Cryptococcus* OTU246 in comparison to OTU247 (saprotrophic; organic- vs. mineral-horizon preferences). Note that this pattern of niche differentiation spans major functional guilds, including several types of mycorrhizae, saprotrophs, and parasites. Niche differentiation among closely related species was also seen in each of the three predominant phyla recovered in this study, the Basidiomycota, Ascomycota, and the "basal fungal lineages" (primarily Mortierellomycotina).

DISCUSSION

We report here a much higher fungus-to-plant (F:P) ratio than has been seen in prior nonmolecular studies, and the first molecular saturation of a rarefaction curve for soil fungi. Our data support the idea that F:P ratios may be predictable on a regional scale. To the extent to

which this first saturated F:P ratio can be extrapolated globally, it suggests far higher global numbers of fungal species than other recent estimates. Moreover, our estimate of the regional F:P ratio, though stable, is likely to be low because many taxa will not be represented in a soil census. We also find strong structuring of fungal communities related to environmental variables and understory plant community composition. Perhaps most surprisingly, species that show the strongest and most divergent habitat preferences are often closely related.

Expanding the global estimate of fungal diversity

Next-generation sequencing studies have typically discarded rare sequences and have often failed to distinguish artifacts such as non-fungal sequences and chimeras, and thus have generally not attempted to enumerate total fungal species richness. We have overcome these issues using an alternative molecular approach combined with rigorous bioinformatics steps and large-scale sampling. Instead of relying on next-generation sequencing of short amplicons that are less amenable to phylogenetic analyses, taxon identification (Porter and Brian Golding 2011), and chimera detection, we amplified and Sanger-sequenced a relatively long ~1200 bp region that spans the entire highly variable nuclear ribosomal internal transcribed spacer (ITS) and extends roughly 700 bp into the large subunit (LSU). The ITS fragment is ideal for species discrimination (Schoch et al. 2012), while the LSU portion provides significant resolution for deeper-level phylogenetic analyses. This approach reduces amplification of DNA from dead organisms, since most soil DNA is quickly degraded to short fragments (Willerslev and Cooper 2005), and improves detection and exclusion of chimeras and non-fungal OTUs. Furthermore, our comparisons of OTU clustering using the genome assembly program Cap3 (Huang and Madan 1999) to phylogenetic groupings inferred from tree-building exercises in three diverse genera support the claim that clusters based on 97% sequence identity are reasonable approximations of species-level divisions. Most importantly, these analyses suggest that we are not over-splitting, and thereby overestimating fungal diversity.

Methods that have been used to extrapolate the richness of hyperdiverse groups have included body-size frequency distributions, latitudinal gradients, species–area relationships, host-specificity ratios, time–species accumulation curves, and expert opinions (Mora et al. 2011). Body-size relationships are not applicable to fungi due to their fragmented growth patterns and mostly unknown biomasses and sizes. Currently, we lack adequate data to apply latitudinal gradients, species–area relationships or host-specificity ratios to fungi. Combining opinions of taxonomic experts has been attempted for fungi (Hawksworth and Rossman 1997), but is suspect due to the bias toward species with macroscopic sporocarps. Mora et al. (2011) recently proposed a general mathematical method that extrapolates numbers of species from the more stable numbers of taxa at higher ranks, i.e., families through Kingdoms, while simultaneously modeling the accumulation of higher ranks over time (i.e., the asymptotic completeness of higher level taxonomy since Linnaeus). Their method resulted in an estimate of 616 000 species of terrestrial fungi worldwide.

There are compelling reasons why this type of extrapolation may perform poorly for fungi. The "known" species of fungi on which the extrapolation was based included no molecular data, thereby discounting numerous cryptic species. Furthermore, higher taxonomic ranks do not yet appear stable in the Eumycota, as major new classes and even phyla have recently been described (Jones et al. 2011, Rosling et al. 2011). It has been argued that using ratios of diversity between major taxonomic groups (i.e., fungi vs. plants) is a crude and unreliable approach to estimating richness of a target group (May 1991, Mora et al. 2011). However, our results support our hypothesis and contribute to growing evidence that F:P ratios are consistent at regional scales in at least some ecosystems. For example, in a meta-analysis, diversity of tree species and mushroom-forming fungi were found to be strongly correlated (Schmit et al. 2005), as were plant species richness and fungal richness in soil across 24 grassland plots in Japan (Sugiyama et al. 2008).

If F:P ratios are consistent at regional scales, the next key question is whether these ratios vary across habitats or biomes. To arrive at the 1.5 million species estimate, it was argued that the ratio observed in the temperate United Kingdom could be applied globally (Hawksworth 1991). However, there are reasons to suspect that such ratios may not be universal. For example, diversity of ectomycorrhizal fungi appears to peak in temperate and boreal regions and decline in tropical and arctic regions (Tedersoo et al. 2012). On the other hand, some guilds of fungi, especially plant endophytes, have been shown to increase in diversity from high latitudes to the tropics (Arnold and Lutzoni 2007). Whether endophyte : plant ratios vary along this gradient is not yet clear.

When our 17:1 F:P ratio is combined with the current estimate of vascular plant diversity of 352 000 (Paton et al. 2008), it would suggest a global fungal richness of 6 million species, as opposed to the 616 000 recently suggested by Mora et al. (2011). Moreover, our estimate of the fungus-to-plant ratio at our sites is conservative for several reasons. First, single-linkage clustering of fungal ITS sequences at 97% identity often lumps sequences belonging to different, closely related species (Kindermann et al. 1998, Peintner et al. 2004, Geml et al. 2009). Second, our census of soil does not encompass the numerous fungal species that occur only in aboveground habitats, such as the diverse fungal species of the Laboulbeniales (Weir and Hammond 1997) and Entomophthorales (Humber 1989), which may number

TABLE 3. Indicator species analyses, showing habitat preferences for selected taxa, with significant indicator values demonstrating niche differentiation among closely related taxa.

Taxon	Top BLAST match	Phylum†	Guild‡	Observed indicator value§	*P*	Habitat preference¶
OTU585	*Byssocorticium atrovirens* MC01-541	B	SAP	25	0.0252	dry nonacidic
OTU584	*Byssocorticium atrovirens* RT00008	B	SAP	31.2	0.028	wet nonacidic
OTU374	*Cadophora finlandica*	A	DSE	25	0.0268	wet acidic
OTU656	*Cadophora* sp. E 7R29-1	A	DSE	29.8	0.0194	wet nonacidic
OTU196	*Candida bituminiphila*	A	SAP	33.1	0.0038	organic horizon
OTU614	*Candida castrensis* NRRL Y-17329	A	SAP	24.2	0.0406	dry nonacidic
OTU333	*Candida* sp. BCMU BX03	A	SAP	26.6	0.0348	organic horizon
OTU759	*Candida* sp. NRRL Y-27117	A	SAP	20.8	0.048	organic horizon
OTU759	*Candida* sp. NRRL Y-27117	A	SAP	31	0.0274	wet nonacidic
OTU611	*Candida* sp. NRRL YB-2263	A	SAP	35.8	0.0162	wet nonacidic
OTU609	*Candida* sp. NRRL YB-3827	A	SAP	24.7	0.0234	mineral horizon
OTU202	*Candidsa tepae*	A	SAP	47	0.0174	dry acidic
OTU200	*Candida tepae*	A	SAP	29.2	0.035	mineral horizon
OTU205	*Candida tepae*	A	SAP	64	0.0006	mineral horizon
OTU206	*Candida tepae*	A	SAP	54.2	0.0002	mineral horizon
OTU195	*Candida tepae*	A	SAP	59.6	0.0062	organic horizon
OTU198	*Candida tepae*	A	SAP	44.8	0.045	organic horizon
OTU195	*Candida tepae*	A	SAP	57.2	0.0026	wet nonacidic
OTU760	*Candida tepae*	A	SAP	25	0.0286	wet nonacidic
OTU91	*Capronia* sp. UBCTRA 1322.11	A	DSE	43	0.0258	dry nonacidic
OTU635	*Capronia* sp. UBCTRA1522.6	A	DSE	26.9	0.0344	wet nonacidic
OTU96	*Capronia villosa*	A	DSE	43	0.003	wet acidic
OTU803	*Capronia villosa*	A	DSE	33.3	0.012	wet nonacidic
OTU98	*Capronia villosa*	A	DSE	45.7	0.0038	wet nonacidic
OTU778	*Cordyceps crassispora*	A	PATH	25	0.0296	wet nonacidic
OTU399	*Cordyceps cylindrica*	A	PATH	23.9	0.0354	wet acidic
OTU230	*Cortinarius armeniacus*	B	ECM	79.5	0.0002	organic horizon
OTU237	*Cortinarius brunneifolius*	B	ECM	32.4	0.032	dry acidic
OTU237	*Cortinarius brunneifolius*	B	ECM	56.1	0.0002	organic horizon
OTU219	*Cortinarius caperatus*	B	ECM	39.2	0.014	organic horizon
OTU219	*Cortinarius caperatus*	B	ECM	41.4	0.0156	wet acidic
OTU218	*Cortin*arius cf. *vibratilis* F16068	B	ECM	29.2	0.01	organic horizon
OTU221	*Cortinarius collinitus*	B	ECM	92.3	0.0002	organic horizon
OTU714	*Cortinarius cystidiocatenatus*	B	ECM	33.3	0.0078	wet nonacidic
OTU711	*Cortinarius delibutus*	B	ECM	26.8	0.0256	wet nonacidic
OTU223	*Cortinarius glandicolor*	B	ECM	53.3	0.0004	organic horizon
OTU239	*Cortinarius neofurvolaesus*	B	ECM	29.2	0.0108	organic horizon
OTU442	*Cortinarius* sp. HM900724	B	ECM	25	0.0194	mineral horizon
OTU227	*Cortinarius testaceofolius*	B	ECM	36.6	0.008	organic horizon
OTU724	*Cortinarius umbilicatus*	B	ECM	20.8	0.0484	organic horizon
OTU246	*Cryptococcus arrabidensis*	B	SAP	33.3	0.003	organic horizon
OTU248	*Cryptococcus fuscescens*	B	SAP	37.5	0.0012	mineral horizon
OTU247	*Cryptococcus terricola*	B	SAP	67.6	0.002	mineral horizon
SEOTU1502	*Gyoerffyella rotula*	A	SAP	25	0.0258	dry nonacidic
OTU393	*Gyoerffyella* sp. B54J5	A	SAP	25	0.0248	wet acidic
OTU709	*Hebeloma crustuliniforme*	B	ECM	25	0.03	wet nonacidic
OTU251	*Hebeloma mesophaeum*	B	ECM	50.5	0.0076	dry nonacidic
OTU563	*Hebeloma velutipes*	B	ECM	37.5	0.0032	dry nonacidic
OTU117	Helicoon aff. Maioricense	A	SAP	34.6	0.0112	dry acidic
OTU119	Helicoon aff. Maioricense	A	SAP	51.5	0.0008	wet acidic
OTU936	Inocybe cf. lanuginosa	B	ECM	25	0.0288	dry nonacidic
OTU277	*Inocybe lacera*	B	ECM	25	0.0226	mineral horizon
OTU898	Inocybe lacera var. helobia	B	ECM	52.5	0.0002	mineral horizon
OTU281	*Inocybe nitidiuscula*	B	ECM	64.6	0.0004	wet nonacidic
OTU280	*Inocybe tetragonospora*	B	ECM	25	0.0208	dry acidic
OTU308	*Lactarius aurantiosordidus*	B	ECM	43.3	0.0138	dry nonacidic
OTU308	*Lactarius aurantiosordidus*	B	ECM	35.9	0.0454	organic horizon
OTU700	*Lactarius caespitosus*	B	ECM	33.1	0.0168	dry nonacidic
OTU452	*Lactarius glyciosmus*	B	ECM	33.3	0.0084	wet acidic
OTU451	*Lactarius necator*	B	ECM	25	0.0302	wet acidic
OTU309	*Lactarius scrobiculatus*	B	ECM	32.5	0.0134	dry nonacidic
OTU309	*Lactarius scrobiculatus*	B	ECM	27.6	0.0112	organic horizon
OTU145	*Lecythophora mutabilis*	A	DSE	47.7	0.0012	mineral horizon
SEOTU615	*Leptodontidium elatius* A39	A	DSE	25	0.0266	dry nonacidic
OTU177	*Leptodontidium elatius* A39	A	DSE	19.3	0.0472	organic horizon
OTU648	*Leptodontidium elatius olrim*23	A	DSE	28.8	0.0176	dry nonacidic
OTU175	Leptodontidium elatius *olrim*23	A	DSE	43.6	0.0018	wet acidic
OTU173	*Leptodontidium* sp. aurim688	A	DSE	46.3	0.0452	mineral horizon
OTU69	*Mollisia minutella*	A	DSE	37.4	0.0206	organic horizon

TABLE 3. Continued.

Taxon	Top BLAST match	Phylum†	Guild‡	Observed indicator value§	*P*	Habitat preference¶
OTU394	*Mollisia* sp. aurim650	A	DSE	33.3	0.0054	mineral horizon
OTU58	*Mollisia* sp. aurim650	A	DSE	20.8	0.0482	mineral horizon
OTU474	*Mortierella* cf. *hyalina* TEA059	M	SAP	30.1	0.0118	mineral horizon
SEOTU1428	*Mortierella gamsii*	M	SAP	25	0.0258	dry nonacidic
OTU476	*Mortierella* sp. Finse 15-07-00	M	SAP	33.3	0.01	wet acidic
OTU526	*Mortierella* sp. Ppf23	M	SAP	23.1	0.0458	wet nonacidic
OTU111	*Nectria mauritiicola*	A	SAP	30.6	0.0366	wet acidic
OTU870	*Nectria vilior*	A	SAP	23.3	0.0496	wet nonacidic
SEOTU1109	*Phialocephala fortinii* K93 395	A	DSE	33.3	0.0084	wet acidic
OTU66	*Phialocephala fortinii* PFO-2	A	DSE	75.6	0.0002	organic horizon
OTU33	*Phialocephala sphaeroides* UAMH 10279	A	DSE	30.4	0.0084	wet acidic
OTU525	*Phialocephala sphaeroides* UAMH 10279	A	DSE	32.5	0.0078	dry nonacidic
OTU75	*Phialocephala virens* CBS 452.92	A	DSE	68.6	0.0004	organic horizon
OTU378	*Phialophora finlandia*	A	DSE	25	0.0294	wet acidic
OTU380	*Phialophora gregata*	A	DSE	41.7	0.0016	wet acidic
OTU113	*Phialophora phaeophora*	A	DSE	52.7	0.0002	organic horizon
OTU57	*Phialophora* sp. *aurim*712	A	DSE	43	0.0034	dry nonacidic
OTU51	*Phialophora* sp. *aurim*712	A	DSE	73.1	0.0012	organic horizon
OTU57	*Phialophora* sp. *aurim*712	A	DSE	25	0.0352	organic horizon
OTU385	*Phialophora* sp. *aurim*712	A	DSE	33.3	0.0108	wet acidic
OTU54	*Phialophora* sp. *aurim*712	A	DSE	24	0.0422	wet acidic
OTU180	*Phialophora* sp. DF33	A	DSE	36.9	0.0132	dry nonacidic
OTU180	*Phialophora* sp. DF33	A	DSE	33.4	0.044	mineral horizon
SEOTU779	*Phialophora* sp. p3847	A	DSE	33.3	0.0112	wet nonacidic
OTU138	*Phialophora* sp. p3901	A	DSE	27.2	0.0216	mineral horizon
OTU531	*Phialophora* sp. WRCF-AW10	A	DSE	25	0.0208	mineral horizon
OTU377	*Phialophora verrucosa*	A	DSE	28.7	0.0304	wet acidic
OTU56	*Phialophora verrucos*a WRCF-AB6	A	DSE	50	0.0366	organic horizon
OTU321	*Piloderma fallax* 13.1	B	ECM	39.9	0.0438	organic horizon
OTU582	*Piloderma fallax* EL202	B	ECM	23.6	0.0426	dry acidic
OTU323	*Piloderma fallax* EL202	B	ECM	72	0.0002	organic horizon
OTU326	*Piloderma fallax* EL202	B	ECM	20.8	0.045	organic horizon
OTU325	*Piloderma fallax* EL316	B	ECM	39.9	0.0136	dry acidic
OTU322	*Piloderma fallax* UP113	B	ECM	29.8	0.0262	dry acidic
OTU583	*Piloderma lanatum* JS 22149 (O)	B	ECM	25	0.0266	dry nonacidic
OTU320	*Piloderma* sp. A18	B	ECM	63.2	0.0002	organic horizon
OTU320	*Piloderma* sp. A18	B	ECM	50.2	0.0064	wet nonacidic
OTU313	*Russula aeruginea*	B	ECM	35.5	0.0238	dry acidic
OTU313	*Russula aeruginea*	B	ECM	28.9	0.0078	mineral horizon
OTU304	*Russula cessans*	B	ECM	53.4	0.0008	dry nonacidic
OTU454	*Russula nitida*	B	ECM	33.3	0.0102	wet acidic
OTU314	*Russula persicina* 499RUS26	B	ECM	38	0.044	mineral horizon
OTU455	*Russula persicina* 499RUS26	B	ECM	48.8	0.0022	dry nonacidic
OTU184	*Scleropezicula alnicola* CBS200.46	A	SAP	20.8	0.0448	mineral horizon
OTU183	*Scleropezicula alnicola* CBS200.46	A	SAP	25	0.0202	organic horizon
OTU204	*Urnula helvelloides* CBS763.85	A	SAP	64.3	0.0172	organic horizon
OTU612	*Urnula helvelloides* CBS763.85	A	SAP	32.4	0.01	mineral horizon
OTU615	*Wilcoxina rehmii* d111	A	ECM	31.2	0.0282	dry acidic
OTU620	*Wilcoxina rehmii* NS211	A	ECM	36.2	0.0084	wet nonacidic
OTU146	*Wilcoxina* sp. Hy16	A	ECM	48.2	0.044	mineral horizon

† The phyla are: B, Basidiomycota; A, Ascomycota, M, Mucoromycotina.

‡ Guilds are: SAP, saprotroph; DSE, dark septate endophyte; PATH, Pathogen; ECM, ectomycorrhiza.

§ "Observed indicator value" is a product of abundance (proportion of individuals falling into a site class) and frequency (proportion of sites in that class in which the species is found) of a species, indicating degree of specificity to that site class.

¶ The habitat preferences refer to soil horizon or four previously defined plant community types (Hollingsworth et al. 2006).

as many species as certain of their host groups among the Insecta, the ascomycetous yeasts that live primarily within the digestive tracts of insects (Blackwell and Jones 1997, Suh et al. 2005) and other animal hosts, and the hyperdiverse lichens and associated lichenicolous fungi (Esslinger and Egan 1995). Even among soil fungi, our census overlooked some guilds; we did not sample coarse woody debris, fresh litter, or decaying moss, each of which is known to host specialist fungi (Gilbert and Sousa 2002, Lindahl et al. 2007, Davey et al. 2012), nor deep soil. Third, although we utilized primers and barcodes that we have previously shown to have minimal taxonomic biases (Taylor et al. 2008), there are no fungal-selective primers that do not miss some lineages (Taylor and McCormick 2008). Regardless of taxa we may have missed, our findings clearly imply that the estimate of Mora et al. (2011) is likely to be far too low.

A key issue in interpreting the high F:P ratio we report concerns the origins and identities of the fungal species we observed. Over 70% of our OTUs could not be identified to species by way of BLAST matches to known species. Our unidentified species span a wide range of precision of identification. Many appear to represent species that have not been previously sequenced, but that belong to well known genera (some class B and C identifications). However, many also appear to belong to families and even deeper taxonomic divisions that lack previously sequenced representatives. There are few known families without a sequenced representative, implying that some of our novel OTUs may be members of previously unknown higher taxa. Some of the poorly identified class D OTUs were also rare, which might suggest a higher likelihood that they represent artifacts rather than real taxa. However, this may not necessarily be the case. We studied in considerable detail a highly novel sequence type for which we have only found two clones out of tens of thousands sequenced at the same sites. Our analyses of functional attributes of these ribosomal sequences suggest that they represent a legitimate taxon (Glass et al. 2013). Moreover, many of our class B and C sequences are now known to belong to the new class Archaeorhizomyces (Rosling et al. 2013). Lastly, some of the class C and D identifications were derived for high abundance OTUs, making an artifactual origin unlikely.

As expected, well-studied macroscopic basidiomycete genera such as *Cortinarius* (Fig. 2), *Tricholoma*, *Russula*, *Leccinum*, and *Alnicola* had high proportions of species matches, as did frequently isolated "weedy" fungi in the genera *Neurospora*, *Penicillium*, *Trichoderma*, and *Verticillium*. Counter to these trends, however, were several OTUs that appear to belong to the Agaricomycotina, yet were distantly related to any previously sequenced fungi. The top matches for some of these OTUs belonged to the genera *Clavulina*, *Exidiopsis*, *Sebacina*, *Sistotrema*, *Piloderma*, *Mycena*, *Hydnopolyporus*, *Peniophora*, *Inocybe*, *Hydnum*, *Lentinula*, *Hygrophorus*, and *Hebeloma*. Some of these taxa have microscopic and/or resupinate fruitbodies—groups that are noted for incomplete taxonomic knowledge (Gardes and Bruns 1996, Kõljalg et al. 2003, Smith et al. 2007). On the other hand, the latter four genera are surprising members of the list, given that they produce relatively large and well-known sporocarps. *Inocybe* is similar in this regard, but has previously been shown to have extremely high species diversity at high latitudes and a rapidly evolving ITS locus (Ryberg et al. 2008, Geml et al. 2012, Timling and Taylor 2012).

The immense fungal diversity reported here appears to be due to novel (unsequenced) diversity at all taxonomic levels, but is unlikely to be due principally to a "rare biosphere" or sequence artifacts. Many taxonomically described fungi have no corresponding molecular barcode sequence available (Hibbett et al. 2011). Furthermore, new species descriptions continue to accelerate for fungi (Hawksworth 2012), despite a dearth of trained taxonomists. These observations call for increasing allocation of resources to taxonomic work on fungi, as well as support for standardized barcode submission to go along with all new species descriptions.

Structure of fungal communities

All our sites were completely dominated by a *Picea mariana* overstory, yet we found strong structuring of fungal communities in relation to nearly every abiotic and biotic variable examined. In our view, the consistency of community composition from year to year in the same site, juxtaposed with strong distinctions among sites, principally in accord with site pH and moisture status, argues against either technical issues (e.g., barcode biases) or stochastic spore dispersal processes in driving the patterns we found. Instead, our results strongly support our hypothesis that soil horizon would be an important environmental variable driving fungal community composition. The partitioning by soil horizon is so strong that the arrays of fungi present in two samples collected over 100 km apart, but from the same horizon, are more similar than are the arrays of fungi in different horizons collected only centimeters apart, well within the distance that can be spanned by mycelium from a single fungal individual. Thus, these strong species horizon preferences demonstrate partitioning of the alpha niche (Silvertown 2004), i.e., well within the spatial scale of single populations.

Other environmental variables were measured at the site level, providing contrasts among sites, which is of more relevance to beta niches. Our findings of strong community correlations with pH and site moisture agree with other studies of fungal communities in soil (O'Dell et al. 2000, Toljander et al. 2006). Furthermore, these factors are also strongly correlated with the composition of the understory plant communities at these sites (Hollingsworth et al. 2006). Some of these factors likely also influence fungal community composition and niche partitioning at much finer spatial scales, but this cannot be assessed with the present data set due to our within-site pooling protocol, which was required to achieve the sampling intensity and resultant species saturation that were the overriding objectives.

Our data suggest strong yet complex aboveground–belowground linkages. Studies that enumerate the F:P ratio at a particular site implicitly average across plant species that host many fungi and those that host few fungi, as well as fungi that are narrow host specialists and those that are broad host generalists. We found an overwhelming dominance of fungal OTUs that are likely to interact strongly with plants as mycorrhizae, endophytes, or pathogens. Yet the patterns of linkage differed between the two soil horizons. Because these elements are confounded, we cannot clearly infer direct or indirect effects of plants on fungi or vice versa. It is interesting, however, that organic-horizon fungi were

more closely correlated with understory vegetation than were mineral-horizon fungi. Fine-root density is orders of magnitude greater in the organic horizon of *Picea mariana* forests in interior Alaska (Ruess et al. 1996, 2006). One plausible scenario, then, is that the roots or litter from the understory vegetation exert a stronger effect on fungi in the organic horizon. These understories are dominated by ericaceous shrubs (*Ledum* and *Vacinnium* spp.), and a significant fraction of the Helotialean ascomycete diversity we uncovered may form ericoid mycorrhizas and/or dark septate endophytes. On the other hand, it is likely that strong plant–fungal interactions continue into the mineral horizon, despite the lack of Mantel correlations in community matrices, as some of the mineral-horizon indicator species are ectomycorrhizal or ericoid mycorrhizal species. Ectomycorrhizal *Picea mariana* roots obviously occur at all sites, so this factor may not structure *differences* among sites, even while exerting strong influences on fungal ecology. Upland sites that lack *Picea mariana* host yet a different constellation of fungal communities (Taylor et al. 2010); it is important to recognize that conclusions about the relative importance of various structuring agents are scale dependent.

Our data reveal a novel pattern in the striking degree to which co-occurring, closely related taxa belonging to the same broad guild differ in preference for soil horizon or plant community type (e.g., acidic vs. nonacidic, wet vs. dry). This pattern is seen across trophic guilds, including various root associates, parasites, and saprotrophs (Table 2). Silvertown (2004) proposes four criteria to evaluate the role of niche partitioning in species coexistence: first, establishing that within-species competition is stronger than among-species competition; second, establishing with tests against a null model that species segregate along environmental gradients; third, demonstration of trade-offs in performance related to proposed niche specialization; and, fourth, demonstration of a change in niche occupancy when putative competitors are added or removed. Our evidence simply hints that the second criterion may be fulfilled, i.e., that species are segregated with respect to resources and other environmental variables. While there are challenges with respect to feasibility of field studies, efforts to test these criteria might return rich rewards in the study of fungal community ecology. Another productive avenue might be phylogenetic analyses of community assembly. For example, documentation of phylogenetic clustering of habitat preferences can be interpreted as resulting from the influence of habitat filters, whereas phylogenetic overdispersion of habitat preferences can be interpreted as resulting from the influence of competitive exclusion (Webb et al. 2002). However, other interpretations have also been suggested (Mayfield and Levine 2010). While much additional work is warranted, our findings strongly suggest that individual fungal species often carry out different functional roles in the ecosystem, despite their enormous diversity. Furthermore, ours is a minimal estimate of niche partitioning, since only soil horizon and plant community type were considered here. Because fungi must acquire food from their environment by growing into or onto their preferred substrate (e.g., wood), the distinction between environmental requirements and functional roles becomes clouded in fungi. For example, by growing into dead wood of a preferred host species, fungi are able to decompose the wood and radically alter the ecosystem. Our results point to fine-scale variation in fungal-niche axes that relate primarily to environmental requirements (e.g., pH, moisture), but several that also imply functional roles, particularly the axes of soil horizon and vegetation community type.

Conclusions

With accelerating extinction and reorganization of biodiversity on the planet, our ignorance of even its approximate magnitude is cause for concern. Fungal biodiversity hotspots are currently unknown, and we cannot predict either patterns of extinction or new epidemics. In this work, we demonstrate a consistent F:P ratio in the boreal forest that is much higher than prior non-molecular estimates. A critical need is to carry out comparable, thorough, and rigorous studies in other biomes to quantify patterns of variation in fungus-to-plant ratios and to better understand global drivers of fungal distribution and community assembly. Our results suggest that $<2\%$ of fungal species have been described, implying that the Fungi are equaled only by the Insecta with respect to Eukaryote diversity, and that closely related fungi differ in niche axes related to their roles in the environment.

Acknowledgments

We thank József Geml, Ian Herriott, and Ina Timling for technical assistance, and Sarah Youssof, Meredith Blackwell, and John Taylor for helpful comments on the manuscript. Lisa Green and Scott Anderson assisted with sequencing at Broad. Shawn Houston, and the UAF Life Sciences Informatics core provided critical computational support. This study was supported by the National Science Foundation through awards EF-0333308, ARC-0632332, and DEB-0620579 and by the USDA Forest Service, Pacific Northwest Research Station, through agreement number RJVA-PNW-01-JV-11261952-231. This paper is dedicated to the memory of Michael G. Booth, an inspiring scientist and friend.

Literature Cited

Altshul, S., T. Madden, A. Schäffer, Z. Zhang, Z. Zheng, W. Miller, and D. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25:3389–3402.

Arnold, A. E., and F. Lutzoni. 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? Ecology 88:541–549.

Barnosky, A. D., et al. 2011. Has the earth's sixth mass extinction already arrived? Nature 471:51–57.

Berry, K. J., K. L. Kvamme, and P. W. Mielke, Jr. 1983. Improvements in the permutation test for the spatial analysis

of the distribution of artifacts into classes. American Antiquity 48(3):547–553.
Bever, J. D. 1994. Feeback between plants and their soil communities in an old field community. Ecology 75:1965–1977.
Blackwell, M., and K. Jones. 1997. Taxonomic diversity and interactions of insect-associated ascomycetes. Biodiversity and Conservation 6:689–699.
Buée, M., M. Reich, C. Murat, E. Morin, R. Nilsson, S. Uroz, and F. Martin. 2009. 454 pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytologist 184:449–456.
Chapin, F. S., III, et al. 2010. Resilience of Alaska's boreal forest to climatic change. Canadian Journal of Forest Research 40:1360–1370.
Colwell, R. K. 1997. EstimateS: statistical estimation of species richness and shared species from samples. University of Connecticut, Storrs, Connecticut, USA.
Davey, M. L., E. Heegaard, R. Halvorsen, M. Ohlson, and H. Kauserud. 2012. Seasonal trends in the biomass and structure of bryophyte-associated fungal communities explored by 454 pyrosequencing. New Phytologist 195(4):844–856.
Diamond, J. M. 1978. Niche shifts and the rediscovery of interspecific competition: Why did field biologists so long overlook the widespread evidence for interspecific competition that had already impressed Darwin? American Scientist 66:322–331.
Dickie, I. A., B. Xu, and R. T. Koide. 2002. Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. New Phytologist 156:527–535.
Dufrene, M., and P. Legendre. 1997. Species assemblages and indicator species: The need for a flexible asymmetrical approach. Ecological Monographs 67:345–366.
Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797.
Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200.
Elton, C. 1927. Animal ecology. Sidgwick and Jackson, London, UK.
Esslinger, T. L., and R. S. Egan. 1995. A sixth checklist of the lichen-forming, lichenicolous, and allied fungi of the continental United States and Canada. Bryologist 98(4):467–549.
Ewing, B., L. D. Hillier, M. C. Wendt, and P Green. 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Research 8:175–185.
Gardes, M., and T. Bruns. 1996. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. Canadian Journal of Botany 74:1572–1583.
Gardes, M., T. White, J. Fortin, T. Bruns, and J. Taylor. 1991. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. Canadian Journal of Botany 69: 180–190.
Gause, G. 1932. Experimental studies on the struggle for existence. Journal of Experimental Biology 9:389–402.
Geml, J., G. A. Laursen, I. C. Herriott, J. M. McFarland, M. G. Booth, N. Lennon, H. Chad Nusbaum, and D. L. Taylor. 2010. Phylogenetic and ecological analyses of soil and sporocarp DNA sequences reveal high diversity and strong habitat partitioning in the boreal ectomycorrhizal genus *Russula* (Russulales; Basidiomycota). New Phytologist 187: 494–507.
Geml, J., G. A. Laursen, K. O'Neill, H. C. Nusbaum, and D. L. Taylor. 2006. Beringian origins and cryptic speciation events in the fly agaric (*Amanita muscaria*). Molecular Ecology 15: 225–239.
Geml, J., G. A. Laursen, and D. L. Taylor. 2008. Molecular diversity assessment of arctic and boreal *Agaricus* taxa. Mycologia 100:577–589.
Geml, J., G. A. Laursen, I. Timling, J. W. McFarland, M. G. Booth, N. J. Lennon, C. Nusbaum, and D. L. Taylor. 2009. Molecular phylogenetic biodiversity assessment of arctic and boreal ectomycorrhizal *Lactarius* Pers.(Russulales; Basidiomycota) in Alaska, based on soil and sporocarp DNA. Molecular Ecology 18:2213–2227.
Geml, J., I. Timling, C. H. Robinson, N. Lennon, H. C. Nusbaum, C. Brochmann, M. E. Noordeloos, and D. L. Taylor. 2012. An arctic community of symbiotic fungi assembled by long-distance dispersers: phylogenetic diversity of ectomycorrhizal basidiomycetes in Svalbard based on soil and sporocarp DNA. Journal of Biogeography 39:74–88.
Gilbert, G. S., and W. P. Sousa. 2002. Host specialization among wood-decay polypore fungi in a Caribbean mangrove forest. Biotropica 34:396–404.
Glass, D. J., N. Takebayashi, L. Olson, and D. L. Taylor. 2013. Ribosomal RNA secondary structure modeling and its potential role in environmental DNA gene-authenticity validation: Insights from a highly novel fungal sequence in an Alaskan boreal forest. Molecular Phylogenetics and Evolution 67:234–245.
Grinnell, J. 1917. The niche-relationships of the California Thrasher. Auk 34:427–433.
Hawksworth, D. L. 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycological Research 95:641–655.
Hawksworth, D. L. 2012. Global species numbers of fungi: Are tropical studies and molecular approaches contributing to a more robust estimate? Biodiversity and Conservation (21):2425–2433.
Hawksworth, D. L., and A. Y. Rossman. 1997. Where are all the undescribed fungi? Phytopathology 87:888–891.
Hibbett, D. S., A. Ohman, D. Glotzer, M. Nuhn, P. Kirk, and R. H. Nilsson. 2011. Progress in molecular and morphological taxon discovery in fungi and options for formal classification of environmental sequences. Fungal Biology Reviews 25:38–47.
Hollingsworth, T. N., E. A. Schuur, F. S. Chapin, III, and M. D. Walker. 2008. Plant community composition as a predictor of regional soil carbon storage in Alaskan boreal black spruce ecosystems. Ecosystems 11:629–642.
Hollingsworth, T. N., M. D. Walker, F. S. Chapin, III, and A. L. Parsons. 2006. Scale-dependent environmental controls over species composition in Alaskan black spruce communities. Canadian Journal of Forest Research 36:1781–1796.
Hooper, D. U., et al. 2000. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms, and feedbacks. BioScience 50:1049–1061.
Horton, T. R. 2002. Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale. Plant and Soil 244:29–39.
Huang, X., and A. Madan. 1999. Cap3: a DNA Sequence Assembly Program. Genome Research 9:868–877.
Hubbell, S. P. 2001. The unified neutral theory of biodiversity and biogeography. Princeton Unversity Press, Princeton, New Jersey, USA.
Humber, R. A. 1989. Synopsis of a revised classification for the Entomophthorales (Zygomycotina). Mycotaxon 34:441–460.
Hutchinson, G. E. 1957. Concluding remarks. Cold Spring Harbor Symposia on Quantitative Biology 22:415–427.
Jones, M. D., I. Forn, C. Gadelha, M. J. Egan, D. Bass, R. Massana, and T. A. Richards. 2011. Discovery of novel intermediate forms redefines the fungal tree of life. Nature 474:200–203.
Jumpponen, A., and K. L. Jones. 2009. Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. New Phytologist 184:438–448.
Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence

alignment based on fast Fourier transform. Nucleic Acids Research 30:3059–3066.

Kindermann, J., Y. El-Ayouti, G. J. Samuels, and C. P. Kubicek. 1998. Phylogeny of the genus *Trichoderma* based on sequence analysis of the internal transcribed spacer region 1 of the rDNA cluster. Fungal Genetics and Biology 24:298–309.

Kirk, P., P. Cannon, D. Minter, and J. Stalpers. 2008. Ainsworth and Bisby's Dictionary of the Fungi, 10th edition. CABI, Wallingford, UK.

Kõljalg, U., A. Dahlberg, A. Taylor, E. Larsson, N. Hallenberg, J. Stenlid, K. H. Larsson, P. Fransson, O. Kårén, and L. Jonsson. 2003. Diversity and abundance of resupinate thelephoroid fungi as ectomycorrhizal symbionts in Swedish boreal forests. Molecular Ecology 9:1985–1996.

Kruskal, J. B. 1964. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika 29: 1–27.

Kunin, V., A. Engelbrektson, H. Ochman, and P. Hugenholtz. 2010. Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. Environmental Microbiology 12:118–123.

Leibold, M. A. 1995. The niche concept revisited: mechanistic models and community context. Ecology 76:1371–1382.

Lindahl, B. D., K. Ihrmark, J. Boberg, S. E. Trumbore, P. Hogberg, J. Stenlid, and R. D. Finlay. 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytologist 173:611–620.

Losos, J. B. 1994. Historical contingency and lizard community ecology. Pages 319–333 *in* J. L. Vitt and E. R. Pianka, editors. Lizard ecology: historical and experimental perspectives. Princeton University Press, Princeton, New Jersey USA.

MacArthur, R., and R. Levins. 1967. The limiting similarity, convergence, and divergence of coexisting species. American Naturalist 101:377–385.

May, R. M. 1988. How many species are there on Earth? Science 241:1441–1449.

May, R. M. 1991. A fondness for fungi. Nature 352:475–476.

Mayfield, M. M., and J. M. Levine. 2010. Opposing effects of competitive exclusion on the phylogenetic structure of communities. Ecology Letters 13:1085–1093.

McCune, B., J. B. Grace, and D. L. Urban. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, Oregon, USA.

McCune, B., and M. J. Mefford. 1999. PC-ord. Multivariate analysis of ecological data, version 4. MjM Software Design, Gleneden Beach, Oregon, USA.

Mora, C., D. P. Tittensor, S. Adl, A. G. B. Simpson, and B. Worm. 2011. How many species are there on Earth and in the ocean? PLoS Biology 9:e1001127.

Nilsson, R., G. Bok, M. Ryberg, E. Kristiansson, and N. Hallenberg. 2009. A software pipeline for processing and identification of fungal ITS sequences. Source Code for Biology and Medicine 4:1.

Nowacki, G., P. Spencer, M. Fleming, T. Brock, and T. Jorgenson. 2001. Ecoregions of Alaska: 2001. USGS open-file report 02–297 (map). U.S. Geological Survey, Reston, Virginia, USA

O'Brien, H., J. Parrent, J. Jackson, J. Moncalvo, and R. Vilgalys. 2005. Fungal community analysis by large-scale sequencing of environmental samples. Applied and Environmental Microbiology 71:5544–5550.

O'Dell, T. E., J. F. Ammirati, and E. G. Schreiner. 2000. Species richness and abundance of ectomycorrhizal basidiomycete sporocarps on a moisture gradient in the *Tsuga heterophylla* zone. Canadian Journal of Botany 77:1699–1711.

Osterkamp, T. E., and V. E. Romanovsky. 1999. Evidence for warming and thawing of discontinuous permafrost in Alaska. Permafrost and Periglacial Processes 10:17–37.

Paton, A. J., N. Brummitt, R. Govaerts, K. Harman, S. Hinchcliffe, B. Allkin, and E. N. Lughadha. 2008. Towards target 1 of the global strategy for plant conservation: a working list of all known plant species—progress and prospects. Taxon 57:602–611.

Peintner, U., J. M. Moncalvo, and R. Vilgalys. 2004. Toward a better understanding of the infrageneric relationships in *Cortinarius* (Agaricales, Basidiomycota). Mycologia 96:1042–1058.

Péwé, T. L., D. M. Hopkins, and J. L. Giddings, Jr. 1965. Quaternary geology and archaeology of Alaska. Pages 355–374 *in* H. E. Wright, Jr., and D. G. Frey, editors. The Quaternary of the United States: a review volume for the VII Congress of the International Association for Quaternary Research. Princeton, University Press, Princeton, New Jersey, USA.

Porter, T. M., and G. Brian Golding. 2011. Are similarity- or phylogeny-based methods more appropriate for classifying internal transcribed spacer (ITS) metagenomic amplicons? New Phytologist 192:775–782.

Quince, C., A. Lanzén, T. P. Curtis, R. J. Davenport, N. Hall, I. M. Head, L. F. Read, and W. T. Sloan. 2009. Accurate determination of microbial diversity from 454 pyrosequencing data. Nature Methods 6:639–641.

Rice, P., I. Longden, and A. Bleasby. 2000. EMBOSS: the European molecular biology open software suite. Trends in Genetics 16:276–277.

Rieger, S., J. A. Dement, and D. Sanders. 1963. Soil survey, Fairbanks area, Alaska. US Soil Conservation Society, Fairbanks, Alaska, USA.

Rosling, A., F. Cox, K. Cruz-Martinez, K. Ihrmark, G. A. Grelet, B. D. Lindahl, A. Menkis, and T. Y. James. 2011. Archaeorhizomycetes: unearthing an ancient class of ubiquitous soil fungi. Science 333:876–879.

Rosling, A., I. Timling, and D. L. Taylor. 2013. Distribution and abundance of Archaeorhizomycetes. Pages 333–349 *in* B. A. Horwitz, P. K. Mukherjee, M. Mukherjee, and C. P. Kubicek, editors. Genomics of soil- and plant-associated fungi. Springer, Heidelberg, Germany.

Ruess, R. W., R. L. Hendrick, J. C. Vogel, and B. Sveinbjornsson. 2006. The role of fine roots in the functioning of Alaskan boreal forests. Pages 189–210 *in* F. S. Chapin, III, M. W. Oswood, K. Van Cleve, L. Viereck, and D. Verbyla, editors. Alaska's changing boreal forest, Oxford University Press, New York, New York, USA.

Ruess, R. W., K. Van Cleve, J. Yarie, and L. A. Viereck. 1996. Contributions of fine root production and turnover to the carbon and nitrogen cycling in taiga forests of the Alaskan interior. Canadian Journal of Forest Research 26:1326–1336.

Ryberg, M., R. H. Nilsson, E. Kristiansson, M. Töpel, S. Jacobsson, and E. Larsson. 2008. Mining metadata from unidentified ITS sequences in GenBank: a case study in *Inocybe* (Basidiomycota). BMC Evolutionary Biology 8:50.

Sale, P. F. 1977. Maintenance of high diversity in coral reef fish communities. American Naturalist 111:337–359.

Schmit, J. P., G. M. Mueller, P. R. Leacock, J. L. Mata, Q. Wu, and Y. Huang. 2005. Assessment of tree species richness as a surrogate for macrofungal species richness. Biological Conservation 121:99–110.

Schoch, C. L., K. A. Seifert, S. Huhndorf, V. Robert, J. L. Spouge, C. A. Levesque, W. Chen, and Fungal Barcoding Consortium. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. Proceedings of the National Academy of Sciences USA 109:6241–6246.

Schuur, E. A. G., J. G. Vogel, K. G. Crummer, H. Lee, J. O. Sickman, and T. E. Osterkamp. 2009. The effect of permafrost thaw on old carbon release and net carbon exchange from tundra. Nature 459:556–559.

Silvertown, J. 2004. Plant coexistence and the niche. Trends in Ecology and Evolution 19:605–611.

Skellam, J. 1951. Random dispersal in theoretical populations. Biometrika 38(1–2):196–218.
Smith, M. E., G. W. Douhan, and D. M. Rizzo. 2007. Ectomycorrhizal community structure in a xeric *Quercus* woodland based on rDNA sequence analysis of sporocarps and pooled roots. New Phytologist 174:847–863.
Soil Survey Division Staff. 1993. Soil survey manual. USDA Handbook 18. Soil Conservation Service. U.S. Department of Agriculture, Lincoln, Nebraska, USA.
Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57:758–771.
Stamatakis, A., T. Ludwig, and H. Meier. 2005. RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatics 21:456.
Sugiyama, S., H. M. Zabed, and A. Okubo. 2008. Relationships between soil microbial diversity and plant community structure in seminatural grasslands. Grassland Science 54: 117–124.
Suh, S., J. McHugh, D. Pollock, and M. Blackwell. 2005. The beetle gut: a hyperdiverse source of novel yeasts. Mycological Research 109:261–265.
Taylor, D. L., M. G. Booth, J. W. McFarland, I. C. Herriott, N. J. Lennon, C. Nusbaum, and T. G. Marr. 2008. Increasing ecological inference from high throughput sequencing of fungi in the environment through a tagging approach. Molecular Ecology Resources 8:742–752.
Taylor, D. L., I. C. Herriott, J. Long, and K. O'Neill. 2007. TOPO TA is A-OK: a test of phylogenetic bias in fungal environmental clone library construction. Environmental Microbiology 9:1329–1334.
Taylor, D. L., I. C. Herriott, K. E. Stone, J. W. McFarland, M. G. Booth, and M. B. Leigh. 2010. Structure and resilience of fungal communities in Alaskan boreal forest soils. Canadian Journal of Forest Research 40:1288–1301.
Taylor, D. L., and S. Houston. 2011. A bioinformatics pipeline for sequence-based analyses of fungal biodiversity. Methods in Molecular Biology (Clifton, N.J.) 722:141–155.
Taylor, D. L., and M. K. McCormick. 2008. Internal transcribed spacer primers and sequences for improved characterization of basidiomycetous orchid mycorrhizas. New Phytologist 177:1020–1033.
Tedersoo, L., M. Bahram, M. Toots, A. Diédhiou, T. Henkel, R. Kjøller, M. Morris, K. Nara, E. Nouhra, and Peay, K. and others. 2012. Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. Molecular Ecology 21:4160–4170.
Thomson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673–4680.
Tilman, D. 1982. Resource competition and community structure. Princeton University Press, Princeton, New Jersey, USA.
Timling, I., and D. L. Taylor. 2012. Peeking through a frosty window: molecular insights into the ecology of Arctic soil fungi. Fungal Ecology 5:419–429.
Toljander, J. F., U. Eberhardt, Y. K. Toljander, L. R. Paul, and A. F. Taylor. 2006. Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. New Phytologist 170:873–884.
Van Cleve, K., and C. T. Dyrness. 1983. Introduction and overview of a multidisciplinary research project: the structure and function of a black spruce (*Picea mariana*) forest in relation to other fire-affected taiga ecosystems. Canadian Journal of Forest Research 13:695–702.
Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics 33:475–505.
Weir, A., and P. M. Hammond. 1997. Laboulbeniales on beetles: host utilization patterns and species richness of the parasites. Biodiversity and Conservation 6:701–719.
White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 42:315–322.
Whittaker, R. H., and W. A. Niering. 1965. Vegetation of the Santa Catalina Mountains, Arizona: a gradient analysis of the south slope. Ecology 46:429–452.
Willerslev, E., and A. Cooper. 2005. Review Paper. Ancient DNA. Proceedings of the Royal Society B 272:3–16.

Supplemental Material

Appendix A

Site coordinates, sampling information, and F:P ratios across sites (*Ecological Archives* M084-001-A1).

Appendix B

Phylogenetic trees of *Leptodontidium* and *Mortierella* OTUs (*Ecological Archives* M084-001-A2).

Appendix C

Graphical and tabular views of fungal habitat preferences as revealed by ordinations and environmental correlations (*Ecological Archives* M084-001-A3).

Supplement

Three files containing BLAST identifications for fungal OTUs and abundances across samples (*Ecological Archives* M084-001-S1).