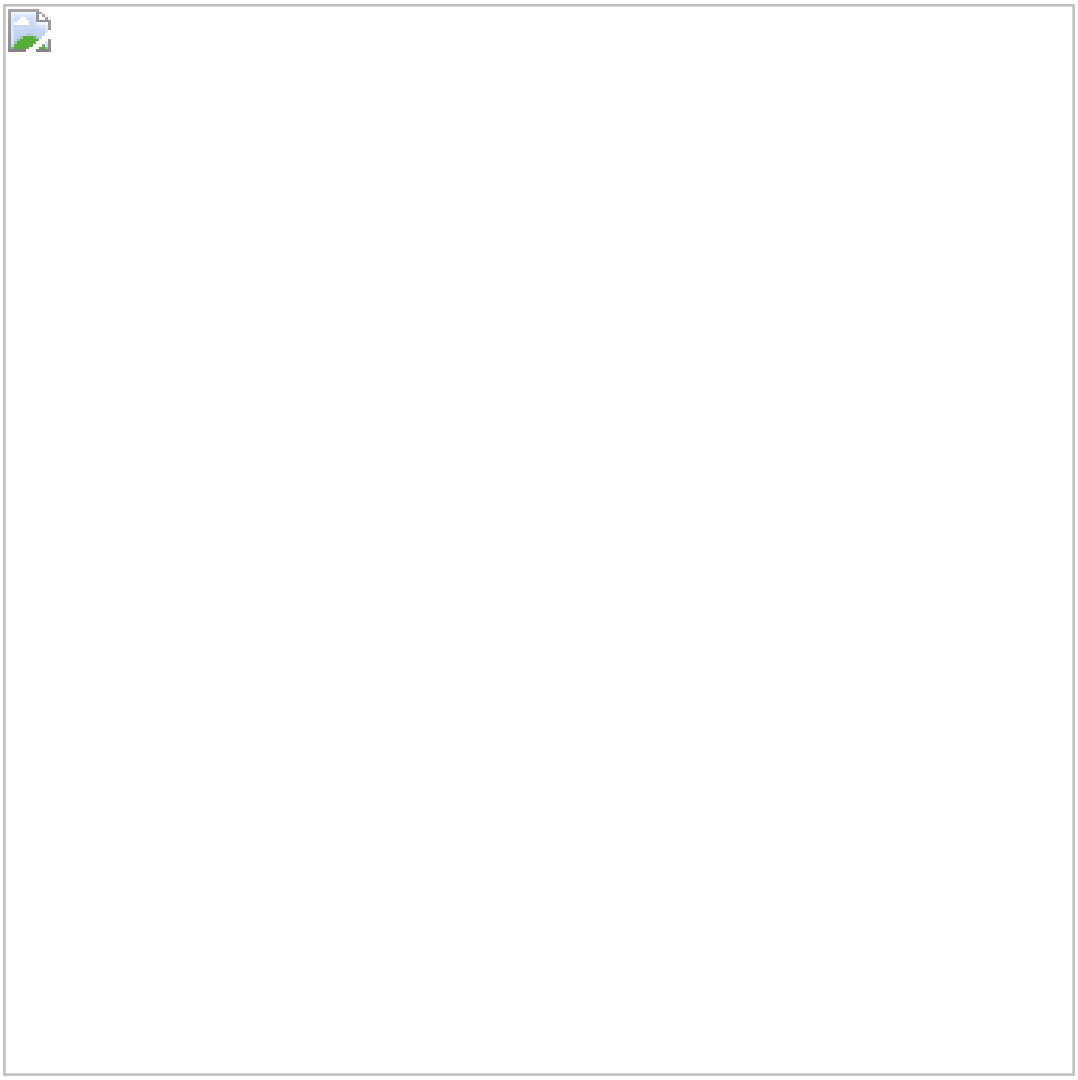

Fungi and Algae Co-Occur in Snow: An Issue of Shared Habitat or Algal Facilitation of Heterotrophs?

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Fungi and algae co-occur in snow: an issue of shared habitat or algal facilitation of heterotrophs?

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Abstract

Late season alpine snows are often colonized by psychrophilic snow algae that may provide a source of nutrients for microbes. Such late season snows are a harsh environment, but support a diverse and complex fungal community. We used culture independent methods (Illumina MiSeq) to test if the presence of snow algae influences fungal communities. We compared algae-colonized snows to adjacent (3 m distant) noncolonized snows in a paired experimental design. Our data indicate that several fungi are locally enriched in algae colonized snows. Although many such fungi were basidiomycetous yeasts, our analyses identified a large number of snow-borne members of phylum Chytridiomycota. While the ecology and function of these Chytridiomycetes remain unclear, we hypothesize that their enrichment in the algal patches suggests that they depend on algae for nutrition. We propose that these chytrids are important components in snow ecosystems, highlighting the underestimation of their diversity and importance. Taken together, our data strongly indicate that fungal communities are heterogeneous in snow even among adjacent samples. Further, fungal and algal communities may be influenced by similar environmental drivers resulting in their co-occurrence in snow.

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Introduction

Earth's cryosphere is composed of solid water persisting for more than one month (Fountain et al*.,* 2012) and includes all snow, permafrost, sea ice, freshwater ice, glaciers, and ice shelves. It plays important roles in global climate (Walsh et al*.,* 2005) and terrestrial energy balance by influencing surface albedo (Prestrud, 2007). With the predicted increase in the mean global annual temperature, the cryosphere is declining and becoming a more endangered ecosystem (Derksen et al*.,* 2012). The decrease in the annual persistence of late season snow has exceeded climate model projections in the Northern Hemisphere (Derksen and Brown, 2012). These changes likely impact local watershed dynamics as well as global biogeochemical cycles (Fountain et al*.,* 2012). In addition to the consequences to local and global hydrological cycles, the cryosphere decline sets constraints on the distribution and diversity of organisms that depend on these unique habitats (Hoham and Duval, 2001). The transience of the late season snow coupled with its changing volume highlights the importance of understanding the endemic biodiversity residing in late season snow packs.

Snowpacks are a harsh environment characterized by low temperatures and intense ultraviolet irradiation that may act as mutagenic stressors. Yet, there is evidence for rich and diverse metabolically active microbial life in snow (Carpenter et al*.,* 2000; Harding et al*.,* 2011; Hell et al*.,* 2013). These metabolically active communities contrast the belief of some that snow is but a passive recipient of aerially dispersed propagules whose metabolism is limited because of minimally available liquid water (Warren and Hudson, 2003). Yet, microbial activity or at least the presence of diverse microbial communities has been suggested for over a century (Hersey, 1913). These dichotomous viewpoints emphasize our present rudimentary understanding of microbial diversity in snow, particularly so for eukaryotic microbes. Consequently, snow can arguably be considered as a vast unexplored and undocumented ecosystem for microbial diversity (Larose et al., 2013).

Alpine and polar snows that persist through or linger late into the growing season often house snow-borne algal communities, frequently dominated by the red-pigmented algae *Chlamydomonas nivalis* or *Chloromonas nivalis,* both of whose taxonomies remain currently unresolved. These algae often produce colonies that are visible to the naked eye as a result of their characteristic red color. The red color of these algae is a result of the secondary carotenoid astaxanthin (Müller et al., 1998) and its fatty acid ester derivatives (Gorton et al*.,* 2001) produced in large quantities during their diploid, zygotic stage that is also characterized by thickened cell walls that are resistant to harsh environmental conditions including repeated freezing temperatures (Hoham, 1980; Remias et al*.,* 2005, 2010). During the warm season in small meltwater pools, the zygotes undergo meiosis, producing haploid offspring that are green, are metabolically active, and multiply asexually. Late in the season, when nitrogen is more limiting, these organisms develop into flagellated sexual gametes that mate producing new zygotes that can survive the next season's cold temperatures and snow (Müller et al*.,* 1998).

Although the red *C. nivalis* colonies may be visually dominant, snow algal communities often consist of many species representing Chlorophyceae (Fujii et al*.,* 2010; Remias et al*.,* 2010). In addition, snow colonized by algae houses a broad range of fungi and bacteria that may be specifically adapted to grow in such environments (Hodson et al*.,* 2008; Naff et al*.,* 2013). Some evidence suggests syntrophic relationships between the snow algae and bacteria or fungi. In the most comprehensive microscopic examination to date, Weiss (1983) described the ultrastructure of the snow alga *C. nivalis* and repeatedly found encapsulated gram-negative bacteria on the surface of the zygotic resting stage. Weiss (1983) posited that the microscopic observations suggested syntrophy, as no similar bacteria were present in the adjacent snow without algal colonization. Similar syntrophisms have been suggested for snow algae and fungi (Kol, 1968; Hoham et al*.,* 1993). In such syntrophic or loose symbiotic relationships, algae-associated microorganisms

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may utilize dissolved organic carbon (DOC) excreted by the algae. This is indirectly supported by recent studies of *Chlamydomonas reinhardtii,* suggesting that algae excrete large amounts of carbon (Kind et al*.,* 2012). Algae, in turn, may benefit from the "shade" provided by the microorganisms, thus buffering the algae against the harsh environmental conditions (Light and Belcher, 1968; Hoham and Duval, 2001; Remias et al*.,* 2005). However, Kol (1968) argued that some of these fungi might simply parasitize the algae rather than form mutually beneficial associations.

To our knowledge, studies of snow-borne fungi and bacteria are rare and limited primarily to select fungi such as "snow molds." Generally, these "snow molds" are filamentous and thrive on organic substrates in the snow-soil interphase, but are not active in the snow itself (Robinson, 2001; Matsumoto, 2009). Recently, Naff et al. (2013) suggested that Chytridiomycota are abundant in snow and significantly influence snow food-web dynamics. Additional broad inquiries of snow-borne microbial communities indicate an abundance of microbes in snow (Harding et al*.,* 2011) and suggest that these snow/ice-inhabiting microbes are physiologically adapted to psychrophilic environments (Gunde-Cimerman et al., 2003). Our current understanding of the general ecology of fungi in snow remains rudimentary and is based primarily on soils liberated by thawing snow (de Garcia et al., 2007) or periglacial soils (Freeman et al., 2009; Schmidt et al., 2012; Brown and Jumpponen, 2014). Studies of fungal communities associated with snow algae are usually motivated by the great abundance of fungi observed in the course of microscopic examination (Stein and Amundsen, 1967; Kol, 1968; Hoham et al*.,* 1993).

Here we present the first high-throughput investigation of fungal communities associated with the snow algae by targeted ITS2 Illumina MiSeq sequencing. The Internal Transcribed Spacer (ITS) regions are the designated fungal barcode of life (see Schoch et al*.,* 2012). The ITS2 region in particular has been more frequently utilized because its diminutive and largely conserved length allows for sequencing the entire region even when using more recent sequencing technologies that provide relatively short reads. We utilized a community-filtering framework (Diamond, 1975; Keddy, 1992) and explicitly test hypotheses on co-occurrence of algae and snow fungi (see Jumpponen and Egerton-Warburton, 2005). We compared paired adjacent samples with and without visible snow algae. We utilized this paired sample design because both snow algae (Müller et al., 1998) and nutrient loads (Fahnestock et al., 2000) in snow are heterogeneous and potentially confound landscape level analyses. Thus, any shifts in community-wide distributions should be detected locally (in all or most of the paired samples). Paired sample designs to interrogate community ecology are not new (Wellington, 1982; Schooley et al., 2000), but are often under-utilized in microbial community analyses (but see Taylor and Bruns, 1999; Hartmann et al., 2014). In this study, we addressed the following three questions: (1) do snow algae enrich the communities with specific fungi; (2) do snow algae shift fungal community composition; and, (3) will such shifts be consistent across larger geographic scales?

Materials and Methods

SAMPLING SITES

We sampled late season snows at six paired locations in September 2011 and August 2012 in the Glacier Peak Wilderness area, Wenatchee National Forest, Washington, U.S.A. (see Table 1 for locations and dates). Samples from each of the locations in Washington State were within 150 m of each other between 2011 and 2012. Additionally, in Colorado we sampled two paired sampling locations within Niwot Ridge Long Term Ecological Research Site (http://culter.colorado.edu/NWT) in August 2011 and in July 2012 at two paired sampling locations within nearby Indian Peaks Wilderness area, Arapahoe and Roosevelt National Forest. Colorado sites at Niwot Ridge LTER could not be sampled in 2012 due to lack of snow in areas where 2011 samples were collected. The sampling locations were still adjacent: the maximum distance between the 2011 and 2012 Colorado sampling locations was 5.7 km. We sampled all the accessible algae colonized snows at each location, resulting in 16 paired samples (32 total). Most snow colonized by algae was inaccessible and could not be sampled (similar to Müller et al., 1998). At each sampling site, snows were collected only if the following conditions were met: (1) there were no signs of foot traffic or other anthropogenic disturbance; (2) there was an adjacent patch of uncolonized snow (based on visual assessment) within 3 m from the boundary of algae-colonized patch but within the same snow pack; and (3) there were no topographical differences between algal and nonalgal colonized snows, thus minimizing potential confounding effects of microsites. We confirmed the absence of algae in the uncolonized snows using flow cytometry.

SAMPLING PROTOCOL

Snows colonized by algae and paired noncolonized controls 3 m away from the edge of the algal colony were selected (selection criteria detailed above) and a total of five 85 cm^3 volumetric surface subsamples were collected using a steel cylinder. Surface scrapings of the top 5 cm were combined into clean 1-gallon zip-top plastic bags and allowed to melt at ambient temperature. Once melted, one 100 mL sample was drawn with a sterile syringe (BD 30 mL Syringe, Dickinson and Company, Franklin Lakes, New Jersey) and passed through a 1.0 μm Nuclepore Track-Etch Membrane filter (47 mm diameter) encased in a 47 mm Swin-Lok Plastic Filter Holder (Whatman, Kent, U.K.) to collect large cells (mainly fungi and algae). The flow-through was collected into a field-sterilized container (using denatured alcohol) and passed through 0.22 μm Nuclepore Track-Etch Membrane filter (47 mm diameter) to collect bacterial cells (data not shown here). Collection and flow through containers were field sterilized with denatured alcohol between sample collections. Filter holders and syringes were used only once to minimize cross contamination between samples. After filtration, filters were stored in MoBio UltraClean Soil DNA Isolation Kit bead tubes (Carlsbad, California) with reagents S1 and IRS added to aid in sample preservation. In 2012, additional unfiltered samples were collected into sterile 15 mL falcon tubes for flow cytometric cell counts. Samples were shipped to Kansas State University and stored at –20 °C until processed. Total proportions of autofluorescent microbial constituents in snow (cells with chloroplasts relative to all cells) from the 2012 samples were estimated using flow cytometry (Guava Technologies PCA-96, Hayward, California) equipped with a 532 nm (green) excitation laser to confirm the low abundance of algae in noncolonized snows. Triplicate 10 μL samples were diluted in 250 mL 1X PBS. A combination of fluorescence emission at 675 nm (PM2, measure of chloroplast autofluorescence) and Forward Scatter (FCS) directly related to particle size were used to generate dot-plots. Using the program Flowing Software (v.2.5; www.flowingsoftware.com), boundaries of segregating autoflorescent

TABLE 1

Sampling locations of paired algae-colonized and uncolonized snows across consecutive years. WA = Washington State, U.S.A., CO = Colorado, U.S.A. All Washington sampling locations were collected at or near the Lyman Glacier Basin.

State	Site	Landmark	Latitude	Longitude	Date	Elevation (m)
2011						
CO	Niwot 1	Near Soddie Laboratory	40°02′56″N	105°34′51″W	10 Aug.	3368
CO	Niwot 2	Saddle	40°03′30″N	105°35′20″W	10 Aug.	3514
WA	Glacier Peak 1	Cloudy Pass	48°12′09″N	120°55′28″W	13 Sept.	1961
WA	Glacier Peak 2	Terminal moraine	48°10′59″N	120°54′11″W	13 Sept.	1802
WA	Glacier Peak 3	Lyman Glacier	48°10′21″N	120°53′50″W	14 Sept.	1880
WA	Glacier Peak 4	Spider Gap N	48°10′14″N	120°52′55″W	14 Sept.	2135
WA	Glacier Peak 5	Spider Gap S	48°10′10″N	120°52′53″W	14 Sept.	2123
WA	Glacier Peak 6	Lower Spider snowfield	48°09′42″N	120°52′42″W	14 Sept.	1897
2012						
CO	Indian Peaks 1	E of Shoshoni Peak	40°04′02″N	105°37′44″W	15 July	3407
CO	Indian Peaks 2	S shore Lake Isabelle	40°04′01″N	105°04′01″W	15 July	3358
WA	Glacier Peak 1	Cloudy Pass	48°12′10″N	120°55′27″W	2 Aug.	1966
WA	Glacier Peak 2	Terminal moraine	48°10′58″N	120°54′11″W	2 Aug.	1794
WA	Glacier Peak 3	Lyman Glacier	48°10′24″N	120°53′49″W	2 Aug.	1866
WA	Glacier Peak 4	Spider Gap N	48°10′14″N	120°52′55″W	2 Aug.	2173
WA	Glacier Peak 5	Spider Gap S	48°10′10″N	120°52′53″W	2 Aug.	2137
WA	Glacier Peak 6	Lower Spider snowfield	48°09′41″N	120°52′35″W	2 Aug.	1893

cells along the FCS axis were manually generated based on visual clustering. The proportions of snow algae were estimated by the proportion of counts that autofluoresced but were larger than bacteria (to discriminate against cyanobacteria). Pollen grains may autofluoresce using these cytometric filters (gymnosperms have paternal ctDNA transmission and may be included) potentially overestimating the algal counts. However, pollen grain deposition in snow is generally spatially homogenous (Bourgeois et al., 2001) at the local scale and unlikely to impact the efficacy of the analyses of algal abundance. We used a paired t-test to test if the proportion of algae in the visibly colonized and uncolonized snows differed. The autofluorescent particles were on average 35 times more abundant within the algal snows than in the adjacent noncolonized snows ($t = 3.25$, $P = 0.007$). In conclusion, the algae were functionally negligible in the noncolonized snows.

DNA EXTRACTION AND AMPLICON GENERATION

Total genomic DNA was extracted using MoBio extraction kits according to the manufacturer's protocol with the following modifications: (1) filters were sonicated for 10 minutes (FS20; ThermoFisher Scientific, Waltham, Massachusetts) in DNA extraction tubes to dislodge any cells adhering to the filters; (2) the filter was removed and two 2.4 mm zirconia beads (BioSpec Products, Bartlesville, Oklahoma) were added into the bead tubes; and (3) particles were homogenized in a FastPrep instrument (FP120; ThermoFisher Scientific, Waltham, Massachusetts) at setting 4.0 for 60 s. The extracts were quantified (ND1000 spectrophotometer; NanoDrop Technologies, Wilmington, Delaware) and each sample was aliquoted to a 96-well plate at 2 ng μL^{-1} DNA concentration. PCR amplicons for Illumina MiSeq sequencing were generated using fungus specific primers to amplify the Internal Transcribed Spacer 2 (ITS2) region of the fungal rRNA gene repeat with primers fITS7 (Ihrmark et al., 2012) and ITS4 (White et al., 1990). Unique molecular identifier tags (MIDs) were incorporated to the ITS4 primer (MID-ITS4). MIDs were selected from the published Illumina MID list (Caporaso et al., 2012) and each MID-ITS4 combination was tested in silico (OligoAnalyzer 3.1; Integrated DNA Technologies, Coralville, Iowa, http://www.idtdna.com/analyzer/Applications/OligoAnalyzer) for possible hairpins and/or primer dimers at melting temperatures above 40 °C. Primers that passed this rubric were synthesized. PCR amplicons were generated in 50 µL reactions under the following conditions: 1 µM forward and reverse primers, 10 ng template DNA, 200 µM of each deoxynucleotide, 2.5 mM $MgCl_2$, 10 µL 5x Green GoTaq Flexi Buffer (Promega, Madison, Wisconsin), 14.6 µL molecular biology grade water, and 2 U GoTaq Hot Start Polymerase (Promega, Madison, Wisconsin). PCR cycle parameters consisted of 94 °C initial denaturing step for 4 min, followed by 30 cycles of 94 °C for 1 min, 54 °C annealing for 1 min, and 72 °C extension step for 2 min, followed by a final extension step at 72 °C for 10 min. All PCR reactions were done in triplicate to control for PCR stochasticity. Negative PCR controls (sterile molecular grade water in place of DNA template) were included in each run; these controls remained free of observable amplification. Triplicate PCR products were pooled by experimental unit (total of 32) and cleaned with Agencourt AmPure cleanup kit using a SPRIplate 96-ring magnet (Beckman Coulter, Beverly, Massachusetts) following the manufacturer's protocol, ex-

TABLE 2

The most abundant OTUs and sequence abundance for each observed phylum are provided. Nested within phylum, the most abundant order and family (genera where Incertae sedis at the family level are represented parenthetically) are provided in subsequent columns. Taxonomic representations of OTUs based on best BLASTn matches across accessioned fungi deposited in GenBank. Purported ecologies at the family (genus) level are also reported. Number of OTUs representing each taxonomic rank are provided (number of OTUs at phylum level sum to 200, and OTU counts within phylum are a subset of the total OTUs shown for phylum).

Phyla	Orders	Families/(*Genera*)	Ecology	Percentage of sequences	Number of OTUs
Ascomycota				12.6%	67
	Dothideales			5.9%	13
		Dothiorceae	Biotrophic or Necrotrophic	4.8%	8
		(*Celosporium*)	Uncertain (BFM)	1.0%	5
	Chaetothyriales			2.3%	12
		Herpotrichiellaceae		0.9%	8
		(*Sarcinomyces*)	Uncertain (BFM)	1.3%	4
	Pleosporales			1.5%	7
		Pleosporaceae	Necrotrophic or Saprobic	1.4%	5
Basal fungal lineages				1.1%	6
	Mucorales			0.7%	2
		(*Umbelopsis*)		0.7%	2
	Mortierellales			0.4%	4
		Mortierellaceae	Saprobic	0.4%	4
Basidiomycota				77.3%	99
	Incertae sedis			32.3%	15
		(*Rhodotorula*)	Saprobic/(Pathogen?)	32.3%	15
	Kriegariales			20.5%	17
		Kriegeriaceae	Saprobic/(Pathogen?)	20.5%	17
	Leucosporidiales			7.3%	9
		(*Leucosporidiella*)	Uncertain (non-phytoparasitic)[1]	4.1%	4
		(*Leucosporidium*)	Uncertain (non-phytoparasitic)[1]	3.2%	5
Chytridiomycota‡				9.4%	26
	Rhizophydiales			6.6%	10
		Incertae sedis	Uncertain	4.3%	2
		Rhizophydiaceae	Saprobic	2.3%	7
	Polychytriales			1.1%	4
		(*Polychytrium*)	Saprobic	1.1%	4
	Incertae sedis			0.2%	11
Glomeromycota‡				0.5%	2
	Glomerales			0.5%	2
		Glomeraceae		0.5%	2

‡Best BLASTn match to phyla Chytridiomycota and Glomeromycota are extremely dissimilar to any accessioned taxa (query coverage ≤ 25% and BLAST score ≤ 90; see Appendix Table A2). Thriving Glomeromycetes are unlikely in absence of host, thus these likely represent unknown taxa.
BFM = black meristematic fungi, polyphyletic group, primarily anamorphic, known to be resistant to harsh environments.
[1]These genera are defined as nonphytoparasitic (Sampaio et al., 2003) but ecologies remain uncertain.

cept we used a 1:1 bead solution to amplicon ratio to better discriminate against small nontarget DNA. Barcoded samples were equimolarly combined so that each experimental unit was equally represented. This final pool was cleaned with Agencourt AmPure cleanup kit once more as above. Illumina MiSeq sequencing linkers were ligated onto the library and paired-end sequenced on a MiSeq Personal Sequencing System (Illumina, San Diego, California) using a MiSeq Reagent Kit v2 with 500

cycles. Ligation and sequencing were performed at the Integrated Genomics Facility at Kansas State University.

SEQUENCE ANALYSIS

Sequence data were processed using MOTHUR (v. 1.29.1; Schloss et al., 2009). The two obtained fastq (bidirectional reads) were contiged and the resultant fasta and qual files used as inputs for further sequence processing that we briefly describe below. We screened contiged sequences and required the following for inclusion: exact match to the MIDs (see Appendix Table A1 for complete list of MID sequences), at most 1 bp difference in match to both forward and reverse primers, and with a quality score of ≥35 over a 50 bp sliding window for the sequence. In other words, a sequence was culled if the average Q-score fell below 35 for any 50 bp window (with a 1 bp slide) or if the sequences did not match both primers or MID. Additionally, sequences were culled if they had homopolymers longer than 8 bp or contained ambiguous nucleotides. This ensured that only high-quality full-length ITS2 reads remained. ITS2 sequences were truncated to 250 bp for further analysis removing any conserved 5.8S regions, sequences shorter than 250 bp culled, and putative chimeras removed (UCHIME, Edgar et al., 2011). Remaining sequences were pair-wise aligned (Needleman-Wunsch) and the resulting distance matrix was clustered at 97% similarity using the average-neighbor algorithm. The rare OTUs (operational taxonomic units) (OTUs not among the 200 most abundant OTUs) were eliminated to focus on only the most abundant and presumably also the metabolically active taxa in these analyses. The 200 most abundant fungal OTUs represented 97.12% of all fungal sequences, therefore likely minimizing the impact of resident dormant propagules in these analyses. Randomly selected sequences representing each of the 200 most abundant OTUs were queried (BLASTn nr/nt with the exclusion of uncultured and environmental samples against GenBank) and best BLAST matches were recorded with full taxonomic string (see Appendix Table A2). Despite the use of fungal specific primers, many common OTUs were classified to nonfungal phyla: Chlorophyta (15 OTUs), Streptophyta (3 OTUs), Ciliphora (1 OTU), and supergroup Rhizaria (1 OTU). We omitted these nonfungal OTUs and appended our analyses to include only the most abundant 200 fungal OTUs. Of note is that the most abundant OTU was algal (best BLASTn match to *Coenochloris* sp.) and seven times more abundant than the next most abundant OTU, suggesting that primer bias was not adequate to discriminate against algal targets in samples highly enriched with phylum Chlorophyta under the reaction conditions that we used. Richness and diversity estimates were calculated iteratively—OTU richness = S_{obs}, Good's coverage = $1 - n_1 N^{-1}$ where n_1 is number of local singletons and N is number of sequences within each sample, complement of Simpson's Diversity = $1 - D = 1 - \sum pi^2$ (where pi is the proportion of individuals in the ith species), and Simpson's Evenness = $Ed = (1\ D^{-1})\ S^{-1}$ (where S is the OTU richness at each sample and D is the Simpson's diversity index)—using 1000 iterations at a subsampling depth of 1500 sequences per experimental unit to control for biases due to unequal sampling (Gihring et al., 2011), the average of each estimator used in our analyses. This iterative approach controls for potential false positives on diversity estimates due to single subsample-based (rarefying) diversity estimates as detailed by McMurdie and Holmes (2014) by diminishing the importance of false positives as one extreme measurement has little impact on community-wide metrics after multiple iterations. Nonmetric Multidimensional Scaling (NMDS), based on a subsampled Bray-Curtis dissimilarity matrix with 1000 iterations (at 1500 subsampling depth) was used to examine fungal community composition for the first three axes (73.3% community variation, 3D stress = 0.198). To determine if the Washington sites possessed different fungal communities than the Colorado sites or if the 2011 and 2012 Washington samples differed, we used Analysis of Molecular Variance (AMOVA; PERMANOVA in Anderson, 2001). Diversity estimates and NMDS generation and analyses based on iterative subsampling were implemented in MOTHUR. Across the resolved three-dimensional NMDS space, linear (Euclidian) distances were calculated between paired algae colonized and nonalgae colonized samples and these values were analyzed using Student's t-test to test if Colorado and Washington paired samples differ in their community similarities. To test for OTU enrichment between paired algae-colonized and uncolonized snows, we used a nonparametric paired test of count data. Because of our paired design, our richness, diversity, NMDS axes scores, and OTU abundance were analyzed using a nonparametric two-tailed Wilcoxon signed-rank test (*H0:* $M_{algae} = M_{non\text{-}algae}$, *H1*: $M_{algae} \neq M_{non\text{-}algae}$) and any significant responses were corrected for multiple comparison effects using a liberal False Discovery Rate (FDR = 0.50). Data were also analyzed using a parametric paired t-test. These analyses were consistently congruent with the nonparametric tests. As a result, we only report the nonparametric test statistics, as those rely on no assumptions on data distribution or variance homogeneity. All statistics were performed using a combination of MOTHUR and JMP (v. 10.0.2, SAS Institute, Cary, North Carolina).

The taxon assignment of OTUs to phylum Chytridiomycota (26—or 13%—of the top 200 OTUs) was challenging due to their low similarity to any vouchered or uncultured/environmental accessions. Since the low coverage and low similarity to any accessioned sequence made these BLASTn assignments to Chytridiomycota tentative, we further explored these data through a confirmatory Maximum Likelihood (PhyML) analysis. The hypervariable ITS gene regions are difficult to align globally at the phylum level and thus considered poor at discerning taxonomic relationships in phylogenetic analyses at more inclusive taxonomic ranks (order/family). Even though we used the designated barcode for identifying fungal taxa, these putative Chytridiomycota consistently failed to be assigned with satisfactory affinities. As a result, we utilized the PhyML approach as a method to confirm the phylum-level placement of the OTUs tentatively assigned within Chytridiomycota. ITS2 reads for representatives of our chytridiomycetous environmental reads (26 OTUs) were aligned with a number of accessioned and vouchered Chytridiomycota and two closely related basal phyla (Blastocladiomycota and Monoblepharidomycota) with full-length ITS2 gene regions in GenBank as well as the ascomycetous yeast *Saccharomyces cerevisiae,* as an outgroup. Reference sequences were selected across phylum Chytridiomycota to include all available orders of Chytridiomycota (orders Cladochytriales and Polychytriales were not included because no full-length ITS2 sequences from vouchered specimens were available in GenBank at the time of analysis). Sequences were aligned using MUSCLE (1000 iterations) as implemented in GENEIOUS (v. 5.3.4, BioMatters, Auckland, New Zealand). The alignment was trimmed manually to only include full-length ITS2 regions and a Maximum Likelihood tree was generated (100 bootstrap iterations, substitution model = HKY85) in GENEIOUS with *Saccharomyces cerevisiae* as an

outgroup. The fasta file of the representative sequences of OTUs used for this confirmatory PhyML analysis along with reference sequences used are available as a Appendix File A1 (available as an open access file accompanying the online version of this article).

Results

SEQUENCE DATA CHARACTERIZATION

We obtained more than 11×10^6 sequences from our MiSeq library (paired-end fastq files are deposited in Sequence Read Archive at NCBI: SRR1104197). Of these, 1,466,702 full-length ITS2 sequences were retained after quality control. After the removal of nontarget, mainly algal OTUs (363,005 sequences) and OTUs that were not among the 200 most abundant, 466,243 sequences remained with unequal sequence counts per experimental unit (range 1971–24,964). Among the total of 7835 OTUs are OTUs that are exceedingly uncommon (3949 global singletons and an additional 3686 nonsingleton rare OTUs) and most likely represent dormant organisms and aerially deposited spores or hyphal fragments that unlikely contribute to ecosystem function, or methodological artifacts of uncertain origin (Brown et al., 2015). For this reason, we limited our analysis to the 200 most abundant OTUs (see Appendix Fig. A1) that represent more than 97% of all fungal sequences in our data set (see Appendix File A2 for OTU × Sample community matrix) (File A2 available as an open access file accompanying the online version of this article).

TAXONOMIC DISTRIBUTION

The fungal communities were dominated by Basidiomycota (see Table A2 for complete taxonomic assignments for the 200 most abundant OTUs and Appendix File A3 [available as an open access file accompanying the online version of this article] for representative sequences of the 200 most abundant OTUs). Sequence and OTU counts of the most abundant orders and families with inferred ecological roles are presented in Table 2. Cosmopolitan polyphyletic yeasts in the genus *Rhodotorula* (46 OTUs—23% of all OTUs and 53.30% of total sequences) dominated among Basidiomycetes, as also observed in other snow and glacier fungi surveys (de Garcia et al., 2007, 2012). The four most abundant OTUs were classified as *Rhodotorula* and the next most abundant OTU was classified as *Cryptococcus saitoi*, a commonly encountered snow-dwelling yeast with uncertain ecology. OTUs assigned to Lyophyllaceae were also common (8 OTUs) and included 2 OTUs with close BLASTn affinities to accessioned sequences of *Asterophora*—a genus with members known to be parasites. The remaining 6 OTUs in the family were marginally similar to genus *Lyophyllum*, members of which are often described as soil-borne saprobic or mycorrhizal macrofungi. Chytridiomycota were surprisingly frequent (26 OTUs—13% and ~10% of total sequences). Of these OTUs, none had a strong similarity to any accessioned sequences based on BLASTn alignments (coverage ranged from 15% to 60% with very low total BLAST scores; see Table A2). Because of the low similarity for known accessioned Chytridiomycota, we reanalyzed these data in queries that also included uncultured/environmental sequences. These BLASTn analyses consistently failed to match closely any accessioned sequences. Despite their low similarity to sequences in the combined global genetic databases, our confirmatory Maximum Likelihood (PhyML) analysis supported placement of the 26 OTUs with 99% bootstrap support within Chytridiomycota. Additionally, our environmental OTUs were most closely related to the soil-borne chytridiomycetous order Lobulomycetales (Appendix Fig. A2). Yet, bootstrap support within the environmental Chytridiomycota clade remains low, a likely result of use of the hypervariable ITS2 region that tends to perform poorly in analyses in more inclusive taxonomic ranks (e.g., order or family). As a result, the placement of these OTUs below phylum cannot be deduced from our PhyML analysis. Yet, most of our snow Chytridiomycota OTUs form a distinct clade suggesting that these taxa may represent a monophyletic group of snow-borne Chytridiomycetes with very little ITS2 sequence similarity to anything known. Unfortunately, our phylogenetic analyses suffered from the poorly populated databases that include no vouchered reference ITS2 sequences similar to the observed environmental chytrids.

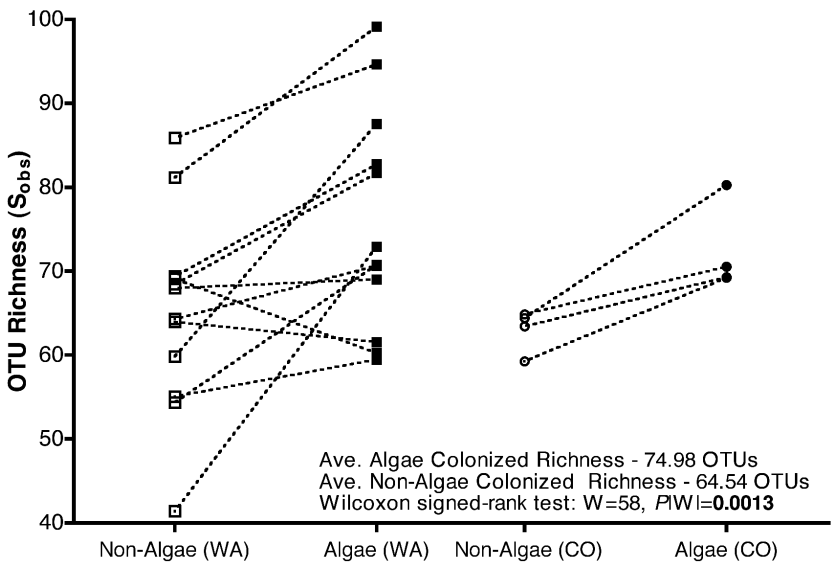

FIGURE 1. Operational taxonomic unit (OTU) richness in the algae-colonized snows are higher than in the adjacent paired uncolonized snows (Wilcoxon Sign-Rank test). This analysis is based on 1000 iterations to generate average richness estimates (1500 sequences subsampled per iteration) based on the 200 most abundant OTUs. Uncolonized fungal OTU richness estimates are solid symbols and algae colonized richness estimates are open symbols. Dashed lines connect paired samples.

DIVERSITY ESTIMATORS

Coverage and rarefaction (see Appendix Fig. A3) estimators indicated that the fungal community was adequately captured (Good's Coverage for colonized and noncolonized snow 0.972 ± 0.003 and 0.986 ± 0.001, respectively) in our sequencing. We find that a greater proportion of the 200 most abundant OTUs occur in the algal-colonized snow than in the snow without algae. Estimated OTU richness in the algae-colonized snow (74.98 ± 11.72; mean ±1 SD) was greater than in the adjacent, noncolonized snow (64.54 ± 11.72) across both sites and years (Fig. 1; paired two-tailed Wilcoxon Sign-Rank test; $W = 58$, $P = 0.0013$). In contrast, neither diversity (complement of Simpson's Diversity, 1 – D: 0.890 ± 0.051 for the algae-colonized and 0.865 ± 0.067 for noncolonized snows; $W = 30$, $P = 0.130$) nor evenness (E_D: 0.146 ± 0.053 for algae-colonized and 0.145 ± 0.073 for noncolonized snows; $W = 10$, $P = 0.632$) differed between the paired colonized and noncolonized snow samples.

COMMUNITY DIFFERENCES

Fungal communities were resolved optimally on three NMDS axes (stress = 0.198, $r^2 = 0.733$). Our community-wide AMOVA (PERMANOVA in Anderson, 2001) analyses indicated regional (Colorado vs. Washington) but not temporal differences in the snow fungal communities. These analyses failed to distinguish fungal communities in the colonized and noncolonized snow ($F_{1,30} = 1.247$, $P = 0.227$) globally. However, paired analyses of the Axis 2 ordination loading scores (representing 57.16% of community variability) indicated that fungal communities in the algae-colonized snow were distinct from those in the paired uncolonized snows (Fig. 2; $W = 48$, $P = 0.011$). In contrast, neither Axis 1 ($r^2 = 6.38\%$) nor Axis 3 ($r^2 = 9.85\%$) distinguished between the paired algal and nonalgal snows ($W = 21$, $P = 0.298$; $W = 23$, $P = 0.252$, respectively). When the paired Axis 2 values were analyzed separately for Colorado and Washington, an interesting pattern emerged. Our Colorado locations showed no discernable difference between the fungal communities of the paired samples across all three axes (see insert in Fig. 2). The Washington samples show a contrast; a shift in fungal communities in the paired algae colonized/uncolonized snows ($W = 27$, $P = 0.034$ for Axis 2; see insert in Fig. 2). Further, our AMOVA suggested no difference across years ($F_{1,30} = 1.135$, $P = 0.286$) in the Washington sites but clearly distinguished the snow inhabiting fungal communities from Colorado and Washington ($F_{1,30} = 8.654$, $P < 0.001$). These analyses suggest that although our sites located in Colorado and Washington are distinct, the communities remain fairly stable over time—likely as a result of local and/or regional fungal propagule inputs.

We also analyzed Euclidian distances between the paired samples from algae colonized and uncolonized snows to test if they differed in the magnitude of fungal community-wide shifts between Colorado and Washington. The Euclidian distances across the three resolved axes between paired algae-colonized and uncolonized snow-fungal communities was greater in the Washington snows than in Colorado ($t = 2.47$, $P = 0.0267$; Appendix Fig. A4). This difference may be the result of a constrained shift in the fungal community associated with sampling locations or dates.

In all, 13 of the 200 most abundant OTUs were more common in algae-colonized snow (see Table 3), whereas none were enriched in the uncolonized snow as determined by paired Wilcoxon Sign-Rank analyses of OTU abundance. An additional 3 OTUs differed between paired algal-colonized and uncolonized snows but were no longer significant after controlling for multiple comparisons. The colonized snow was enriched for saprobic and putatively pathogenic OTUs. Several *Rhodotorula* OTUs were enriched in the algal-colonized snows, suggesting the opportunistic utilization

TABLE 3

Fungal operational taxonomic units that are enriched in algal colonized snow compared to paired nonalgal colonized snow based on Wilcoxon Sign-Rank test after correction for multiple comparisons (P |W| is the two-tailed P-value between colonized and paired uncolonized snow). Best BLASTn matches and putative ecologies are also reported (EcM = ectomycorrhizal). The symbol '‡' represents taxa whose best BLASTn match is extremely dissimilar to any accessioned taxa (query coverage ≤ 25% and BLAST score ≤ 90; see Appendix Table A2) that are likely novel fungal taxa whose ecologies remain uncertain.

OTU number	Wilcoxon Sign-Rank test statistic (*W*)	*P*-value (*P* |*W*|)	Ecology	Best BLASTn match
2	47	0.0126	Saprobic	*Rhodotorula* sp.
4	41.5	0.03	Saprobic	*Rhodotorula psychrophenolica*
9	43	0.0248	Unknown	Rhizophydiales sp. ‡
37	30	0.0059	Unknown	Chytridiomycota sp. ‡
40	14	0.0156	Unknown	Chytridiomycota sp. ‡
43	23.5	0.0156	Saprobic	*Rhodotorula* sp.
45	32	0.0103	Saprobic	*Rhodotorula* sp.
48	10.5	0.0313	EcM (‡Unknown)	*Tylopilus formosus* ‡
59	47	0.0122	Pathogenic	*Ilyonectria macrodidyma*
119	22.5	0.0234	Saprobic	*Rhodotorula* sp.
163	14	0.0156	Saprobic (‡Unknown)	*Lyophyllum* sp. ‡
195	14	0.0156	Saprobic	*Rhodotorula* sp.
199	10.5	0.0313	Saprobic	*Leucosporidium scottii*

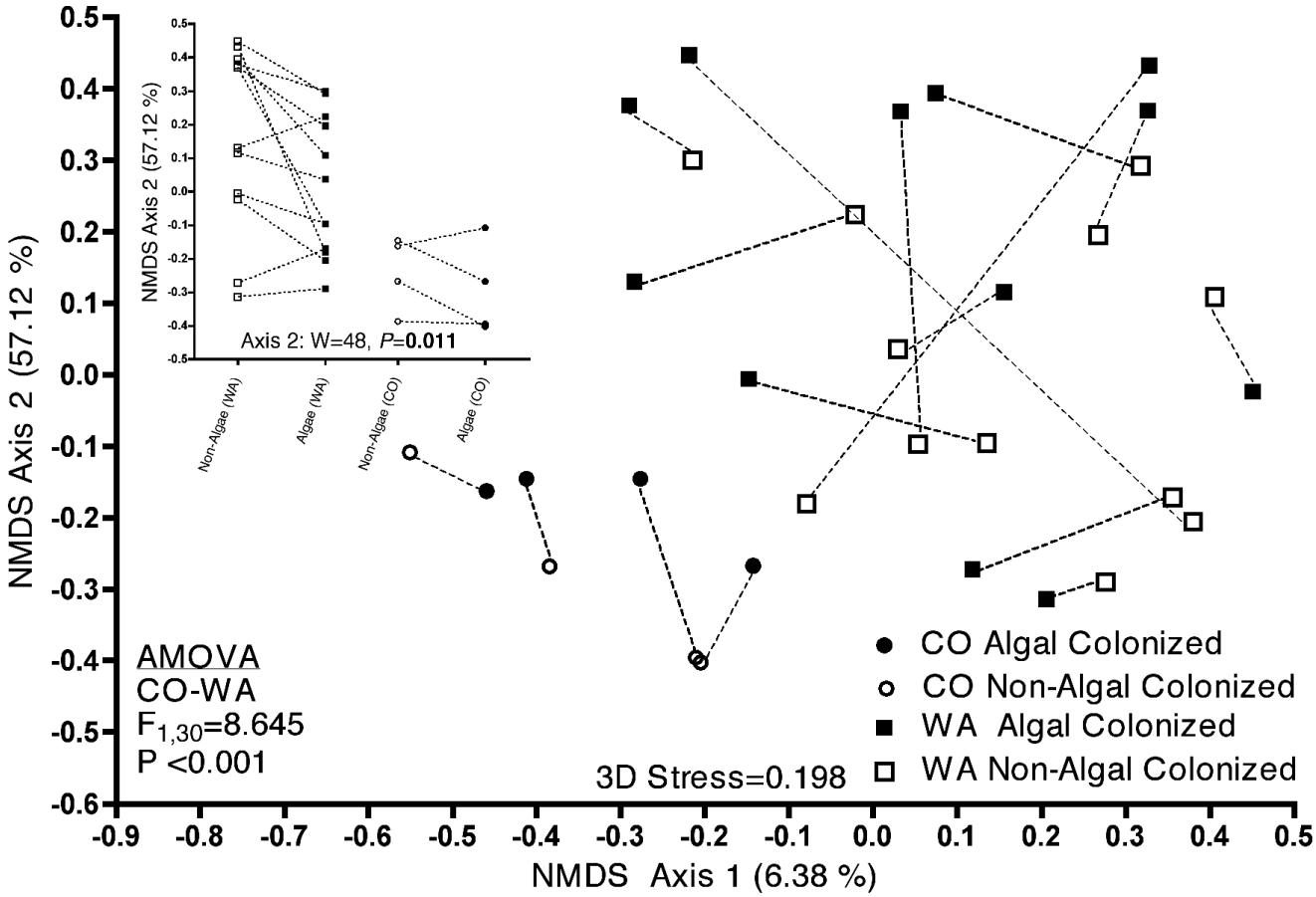

FIGURE 2. Non-Metric Multi-Dimensional Scaling (NMDS) plot of snow-borne fungi in algal colonized snow (solid symbols) and uncolonized snow (open symbols). Dashed lines connect paired algal colonized and adjacent uncolonized samples. Analysis of Molecular Variance (AMOVA) indicates that Colorado (circles) and Washington (squares) fungal communities are distinct. Insert represents paired Wilcoxon Signed-Rank test across Axis 2 (57.12% of community variability) and indicates that snow algae colonized snows possess different fungal communities than paired uncolonized snows (W = 48, *P* = 0.011); dashed lines connect paired samples.

of increased organic matter associated with these snow algae. Particularly interesting are OTUs 9, 37, and 40 that—based on our PhyML analyses—represent novel chytrids whose functions remain unknown (Fig. A2). OTUs 48 and 163 were more common in algal-colonized snow but are highly dissimilar to any known accessioned fungi (see Table A2 for full BLAST scores). Best BLASTn matches identify these OTUs within the genera *Tylopilus* (ectomycorrhizal) and *Lyophyllum* (saprobic or parasitic). Given the great dissimilarity to any accessioned taxa, these OTUs most likely represent novel taxa and/or taxa that are underrepresented in the global nucleotide repositories. Thus, further and more detailed investigation is needed to better understand the fungal communities in this environment.

Discussion

We present one of the very first deep-sequencing studies of snow-borne fungi. The late season alpine snow-packs are a declining ecosystem; climate change predictions suggest that the earth's cryosphere will dramatically decline in volume (Derksen and Brown, 2012). As a result, assessment of the biodiversity in these "endangered" ecosystems is timely and critical. There is a dearth of knowledge about snow-borne life or microbial communities and their function in snow, particularly so for fungi. Our analyses suggest strongly coupled snow-borne fungi/algae co-occurrence patterns.

Our data indicate that the presence of snow algae may act as an environmental filter (Jumpponen and Egerton-Warburton, 2005) that structures the snow-borne fungal community. Alternatively, the snow algae and co-occurring fungal communities share some yet unidentified environmental variable that determines their greater frequency in those samples. This co-occurrence is best evidenced by the shift in the Axis 2 loading scores between the paired colonized and uncolonized samples (Fig. 2). Interestingly, this shift only was significant in the Washington samples in our conservative nonparametric tests. There are two primary reasons that may underlie the lack of significance in Colorado samples. (1) Our sampling in Colorado was more superficial than that in Washington and included only two algae patches that we were able to locate within the Niwot Ridge LTER or Indian Peaks Wilderness. With so few samples, we may lack the statistical power to resolve those trends. (2) Alternatively, for logistical reasons, the Colorado snows were sampled about one month before the Washington snows in both years. This temporal difference may explain this observation; perhaps the shifts in communities are not as strong in Colorado

because the communities have not had as much time to diverge early in the growing season. We speculate that the establishment of greater algal abundance later in the growing season or similar environmental tolerances may facilitate a component of the extant snow fungal community. However, our Colorado sampling preceded this community shift. Our ordination analyses partially support this explanation: the Euclidian distance between the paired samples in ordination space is smaller in Colorado than in Washington (Fig. A4), suggesting that sampling later in the season may permit a further divergence among the compared snow communities. Naturally, we cannot distinguish between the temporal effects of sampling and the confounding spatial effects in this case.

Alpine snow supports diverse fungal communities dominated by basidiomycetous yeasts whose ecologies and taxonomies are poorly understood. Yeast-dominated systems have been reported on glacier surfaces (de Garcia et al., 2012) and in periglacial soils (Schmidt et al., 2012; Brown and Jumpponen, 2014). In our analyses, the most common fungal OTU was assigned to genus *Rhodotorula* (46 OTUs in all; 6 of which are enriched in algal snows). These yeasts are polyphyletic, understudied, and their generic delineation is historically morphological, not phylogenetic (Toome et al., 2013). Additionally, many OTUs were placed into taxa grouped as black meristematic fungi (BMF), another polyphyletic grouping based on anamorph phenotypes. Although BMFs are overall poorly understood, they are suspected to play a large role in mineral transformations and often are resilient in harsh environments (Onofri et al., 2007). These cosmopolitan BMFs most likely utilize allochthonous organic matter such as wind-blown particulate matter common on the snow surface. These results reiterate that there is a dearth of information on psychrophilic/tolerant fungi and highlights the importance for future studies into these systems.

We expected that OTUs enriched in algae-associated snows would provide the most valuable clues on the ecology of these fungi. We initially hypothesized that the algal cells and/or their nutrient-rich exudates provide substrates that potentially facilitate syntrophy or presence of opportunistic saprobes and algal pathogens. Many of the observed enriched OTUs are suspected to have saprobic/parasitic ecologies and thus support our latter hypotheses. Additionally, several taxa that were more abundant in algae-colonized snow were either macrofungi or form ectomycorrhizal associations. The presence of an active community of macrofungi in snow is unlikely but may represent snowbank fungi that fruit at the periphery of snowpacks (Cooke, 1955; Cripps, 2009). Additionally, there may be an abundance of fungal spores representing an allochthonous introduction from surrounding areas. Unfortunately, our analyses do not permit assessing whether these yeasts or other observed fungi are metabolically active in this substrate.

The abundance of Chytridiomycota in our study was surprising. This suggests that these enigmatic fungi may be more abundant and diverse than previously thought. We have just recently begun to appreciate the hidden diversity of Chytridiomycota. Freeman et al. (2009) demonstrated that Chytridiomycota dominate high altitude periglacial soils. The snow chytrids in our study were abundant and dissimilar to any sequences accessioned to the nucleotide repositories. Our confirmatory phylogenetic analyses suggested that these fungi might represent a novel clade of snow Chytridiomycota (Fig. A2). Placement of these novel chytrids from this study at levels below phylum remains uncertain but they may belong to the early divergent snow Chytridiomycetes identified as 'Snow Clades' from North American and European snows (Naff et al., 2013). However, this cannot be determined because our study and that of Naff et al. (2013) targeted different rRNA gene regions, making direct comparisons impossible. Naff et al. (2013) posited that these snow Chytridiomycetes parasitize snow algae because they were common in clone libraries from algal snows. However, these hypotheses were not explicitly tested, nor is parasitism the only reasonable nutritional hypothesis. The present study also differs from Naff et al. (2013) in a very important way, Naff and coauthors only collected snow that was colonized by snow algae and sequenced shallowly, whereas we utilized paired samples with and without algae. Given that Chytridiomycota also were abundant in the snows free of algae, these Chytridiomycota may be saprobes or facultative syntrophs. Nevertheless, to capture a high abundance of Chytridiomycota is striking; Chytridiomycota tend to be infrequent in most locus-targeted community sequencing studies. This may be a result of the highly divergent and difficult to amplify ITS regions of these basal fungi (Schoch et al., 2012), and analyses of environmental DNA may get overwhelmed by more easily amplified templates (Anderson and Cairney, 2004; but see Taylor et al., 2008). Thus, even the relatively high estimates of the chytrid abundance observed here might be an underestimation. Of note, solely relying on OTU abundance as a proxy for organismal abundance may be ill advised as there is no 1:1 relationship between copy numbers and organism abundance (Amend et al., 2010). Also, different fungal lineages may be differentially abundant in environmental sequences due to a myriad of factors including primer bias and differential ITS copy number (Pukkila and Skrzynia, 1993; Porter and Golding, 2012). Yet, despite the potential poor amplification of Chytridiomycota, we found that chytrids were abundant and diverse, highlighting that they both are common and likely important in snows.

It is tempting to speculate on whether these chytrids parasitize or prey on snow algae (Naff et al., 2013) as such associations are common in algae-dominated freshwater systems (Hoffman et al., 2008; Gutman et al., 2009; Rasconi et al., 2011). Snow chytrids may also act as facultative mutualists or have obligate syntrophic relationships; there is a precedence of such relationships in other aquatic systems (Picard et al., 2013). Although our data suggest the enrichment of these communities with such fungi, they do not allow specific statements about their ecology or life strategies. However, it is most likely that these novel chytrids are major players in snow-borne fungal communities. It is also clear that snow fungi are a product of establishment from local propagule pools as the snow-borne fungal communities were compositionally distinct in Washington and Colorado. In contrast, both locations had stable fungal communities over two sampling years. It is probable that snow fungi initially establish from local propagules and the presence of snow algae facilitate their growth and metabolic activity. Many small-scale biotic and/or abiotic factors that vary spatially can select for cryotolerant communities differing in their ecological and functional attributes. Further and more detailed investigations of the snow fungal metabolic activity and community dynamics are needed to better understand them in the cryosphere.

Overall, our results indicate that snow algae and snow fungi co-occur and either share similar environmental tolerances or algae may act as an environmental filter in fungal community assembly. In the latter case, this community filtering is potentially facilitated by enrichment of saprobic and pathogenic fungi that are able to utilize snow algae directly or indirectly through their exudates. Alternatively, the enrichment of specific fungal community constituents may be an outcome of facultative syntrophic associations between algae and fungi that are engaged in loose symbioses. Further in-depth studies on the life history strategies and ecology of snow-inhabiting fungi are required to shed light into these unresolved

questions. Interestingly and congruently with other studies (Naff et al., 2013), our data identified potentially novel groups of Chytridiomycota, some of which were enriched in algae-colonized snows and of undetermined functions. From these studies, it is clear that we are barely scratching the surface of the nearly unexplored cryosphere. To put it simply, snow is an ecosystem that maintains unique communities that may vanish with the declining cryosphere before we have an opportunity to understand them.

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APPENDIX

TABLE A1

Primer sequences (upper) and multiplex Molecular Identifier sequence tags (lower) used in this study.

Gene Primers	
ITS7f	5′-GTGARTCATCGAATCTTTG-3’
ITS4	5′-TCCTCCGCTTATTGATATGC-3’
Tags	Samples
TCCCTTGTCTCC	Lyman-5-2011-Algae
ACGAGACTGATT	Lyman-1-2011-Algae
TACCGCTTCTTC	Niwot-3-2011-Algae
ATCACCAGGTGT	Lyman-3-2011-Algae
TGGTCAACGATA	Lyman-4-2011-Algae
ATCGCACAGTAA	Niwot-1-2011-Algae
GTCGTGTAGCCT	Lyman-6-2011-Algae
GATTATCGACGA	Niwot-2-2011-Algae
ATCCTTTGGTTC	Lyman-2-2011-Algae
GCCTAGCCCAAT	Lyman-6-2011-Non_Algae
ACCGGTATGTAC	Lyman-2-2011-Non_Algae
GATGTATGTGGT	Lyman-4-2011-Non_Algae
TGCATACACTGG	Lyman-5-2011-Non_Algae
AGTCGAACGAGG	Niwot-1-2011-Non_Algae
ACCAGTGACTCA	Niwot-2-2011-Non_Algae
GAATACCAAGTC	Lyman-3-2011-Non_Algae
GTAGATCGTGTA	Niwot-3-2011-Non_Algae
TAACGTGTGTGC	Lyman-1-2011-Non_Algae
ACTCCTTGTGTT	IndianPeaks-2-2012-Algae
CCAATACGCCTG	IndianPeaks-1-2012-Algae
ACTTGGTGTAAG	Lyman-3-2012-Algae
TCACCTCCTTGT	Lyman-4-2012-Algae
CAAACAACAGCT	Lyman-6-2012-Algae
GCAACACCATCC	Lyman-5-2012-Algae
GCACACCTGATA	Lyman-2-2012-Algae
CGAGCAATCCTA	Lyman-1-2012-Algae
AGTCGTGCACAT	IndianPeaks-1-2012-Non_Algae
GCGACAATTACA	IndianPeaks-2-2012-Non_Algae
CGAGGGAAAGTC	Lyman-2-2012-Non_Algae
TCATGCTCCATT	Lyman-6-2012-Non_Algae
AGATTGACCAAC	Lyman-3-2012-Non_Algae
AGTTACGAGCTA	Lyman-4-2012-Non_Algae
GCATATGCACTG	Lyman-1-2012-Non_Algae
CAACTCCCGTGA	Lyman-5-2012-Non_Algae

TABLE A2

Full taxonomic descriptions of the 200 most abundant fungal OTUs including sequence count, best BLASTn match, Max Score, Total Score, Query Coverage, E-Value, Max Identity and Accession numbers for the closet match.

OUT number	Seq count	Species	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Max Score	Total Score	Query Coverage	E value	Max Identity	Accession
2	49594	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		315	315	100%	1.00E-82	89%	JF805370.1
3	45579	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		407	407	100%	3.00E-110	96%	AB474394.1
4	41353	*Rhodotorula psychrophenolica*	Fungi	Basidiomycota			Kriegariales	Kriegeriaceae	*Rhodotorula*	*psychrophenolica*	446	446	100%	3.00E-122	99%	EF151247.1
5	39847	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		297	297	100%	4.00E-77	87%	JF805370.1
6	32545	*Cryptococcus saitoi*	Fungi	Basidiomycota	Agaricomycotina	Tremellomycetes	Filobasidiales		*Cryptococcus*	*saitoi*	125	125	96%	1.00E-25	74%	JX976323.1
7	29737	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina		Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	302	302	100%	9.00E-79	88%	JQ857032.1
8	21673	*Asterophora* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Lyophyllaceae	*Asterophora*		374	374	100%	2.00E-100	93%	HM036644.1
9	20636	*Rhizophydiales* sp.	Fungi	Chytridiomycota		Chytridiomycetes	Rhizophydiales				87.8	87.8	25%	3.00E-14	91%	EF634250.1
10	16371	*Leucosporidiella fragaria*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Leucosporidiales		*Leucosporidiella*		356	356	100%	4.00E-95	92%	JN400812.1
11	13141	*Sydowia polyspora*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales	Dothiorceae	*Sydowia*	*polyspora*	452	452	100%	8.00E-124	100%	JQ780656.1
12	12874	*Leucosporidium* sp.	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Leucosporidiales		*Leucosporidium*		273	273	100%	4.00E-70	85%	JX014242.1
15	7325	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina		Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	340	340	100%	3.00E-90	90%	JN400812.1
16	6726	*Asterophora* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Lyophyllaceae	*Asterophora*		266	266	100%	6.00E-68	85%	HM036644.1
17	6460	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		385	385	100%	9.00E-104	94%	AB474394.1
18	6264	*Rhodotorula psychrophenolica*	Fungi	Basidiomycota			Kriegariales	Kriegeriaceae	*Rhodotorula*	*psychrophenolica*	210	210	95%	4.00E-51	82%	EF151247.1
19	5958	*Sarcinomyces crustaceus*	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales		*Sarcinomyces*	*crustaceus*	452	452	100%	8.00E-124	100%	JN040515.1
20	5880	*Stemphylium* sp.	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Pleosporaceae	*Stemphylium*		446	446	100%	3.00E-122	99%	HQ622100.1
21	5839	*Polyporoletus sublividus*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Albatrellaceae	*Polyporoletus*	*sublividus*	64.4	64.4	18%	4.00E-07	91%	DQ389663.1
22	5764	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina		Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	298	298	100%	1.00E-77	88%	JQ857032.1
23	5057	*Coniozyma leucospermi*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales	Dothiorceae	*Coniozyma*	*leucospermi*	443	443	100%	4.00E-121	99%	EU552113.1
24	4644	*Polychytrium aggregatum*	Fungi	Chytridiomycota		Chytridiomycetes	Polychytriales		*Polychytrium*	*aggregatum*	64.4	64.4	15%	4.00E-07	97%	NG_027613.1
25	4054	*Rhizophydium* sp.	Fungi	Chytridiomycota		Chytridiomycetes	Rhizophydiales	Rhizophydiaceae	*Rhizophydium*		89.7	89.7	21%	1.00E-14	96%	DQ485621.1
26	3238	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		385	385	100%	9.00E-104	94%	AB474394.1
27	2914	*Umbelopsis ramanniana*	Fungi	(Zygomycota)	Mucoromycotina		Mucorales		*Umbelopsis*	*ramanniana*	66.2	66.2	28%	1.00E-07	83%	EU715662.1
28	2877	*Rhizophydium chlorogonii*	Fungi	Chytridiomycota		Chytridiomycetes	Rhizophydiales	Rhizophydiaceae	*Rhizophydium*	*chlorogonii*	64.4	64.4	15%	4.00E-07	97%	JN943815.1
29	2591	*Aureobasidium pullulans*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales	Dothiorceae	*Aureobasidium*	*pullulans*	421	421	100%	1.00E-114	98%	FJ744598.1
30	2523	*Celosporium larixicola*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales		*Celosporium*	*larixicola*	426	426	98%	3.00E-116	98%	FJ997287.1
31	2436	*Glomus diaphanum*	Fungi	Glomeromycota		Glomeromycetes	Glomerales	Glomeraceae	*Glomus*	*diaphanum*	66.2	66.2	20%	1.00E-07	90%	AJ972462.1
32	2339	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		347	347	100%	2.00E-92	91%	AB474394.1
33	2115	*Rhizophydium* sp.	Fungi	Chytridiomycota		Chytridiomycetes	Rhizophydiales	Rhizophydiaceae	*Rhizophydium*		80.5	80.6	60%	5.00E-12	74%	DQ485665.1
35	2040	*cf.Sistotrema* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Corticiales	Corticiaceae	*sistotrems*		260	260	100%	3.00E-66	84%	FR838002.1
36	1908	*Phaeococcomyces nigricans*	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Phaeococcomyces*	*nigricans*	389	389	87%	8.00E-105	99%	AY843154.1
37	1739	*Chytridiomycota* sp.	Fungi	Chytridiomycota							68	68	18%	3.00E-08	93%	EU873018.1
38	1646	*Leucosporidium* sp.	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Leucosporidiales		*Leucosporidium*		316	316	100%	4.00E-83	89%	JQ272411.1
39	1627	*Leucosporidiella* sp.	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Leucosporidiales		*Leucosporidiella*		215	215	89%	4.00E-51	83%	JN197600.1
40	1621	*Chytridiomycota* sp.	Fungi	Chytridiomycota							68	68	18%	3.00E-08	93%	EU873018.1
41	1532	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		307	307	100%	2.00E-80	88%	AB474394.1

TABLE A2
Continued

OUT number	Seq count	Species	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Max Score	Total Score	Query Coverage	E value	Max Identity	Accession
42	1464	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	288	288	100%	2.00E-74	87%	JQ857032.1
43	1457	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		311	311	100%	2.00E-81	88%	JF805370.1
44	1394	*Rhizophydium chlorogonii*	Fungi	Chytridiomycota		Chytridiomycetes	Rhizophydiales	Rhizophydiaceae	*Rhizophydium*	*chlorogonii*	66.2	66.2	16%	1.00E-07	95%	JN943815.1
45	1338	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		302	302	100%	9.00E-79	87%	AY474394.1
46	1212	*Celosporium larixicola*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales		*Celosporium*	*larixicola*	407	407	98%	3.00E-110	97%	FJ997287.1
47	1142	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	425	425	100%	1.00E-115	98%	JQ857037.1
48	1105	*Tylopilus formosus*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Boletales	Boletaceae	*Tylopilus*	*formosus*	69.8	69.8	20%	9.00E-09	90%	HM060320.1
49	1066	*Aureobasidium pullulans*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales	Dothiorceae	*Aureobasidium*	*pullulans*	444	444	100%	1.00E-121	99%	JN400825.1
50	1036	*Leucosporidiella* sp.	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Leucosporidiales		*Leucosporidiella*		232	232	89%	1.00E-57	85%	JN197600.1
52	996	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		324	324	100%	3.00E-85	90%	AB474394.1
53	976	*Cryptococcus podzollcus*	Fungi	Basidiomycota	Agaricomycotina	Tremellomycetes	Filobasidiales		*Cryptococcus*	*podzolicus*	452	452	100%	8.00E-124	100%	HF558652.1
54	967	*Trichosporon gamsii*	Fungi	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales		*Trichosporon*	*gamsii*	452	452	100%	8.00E-124	100%	NR_073247.1
55	934	*Leucosporidiella muscorum*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Leucosporidiales		*Leucosporidiella*	*muscorum*	302	302	100%	9.00E-79	87%	FR717869.1
57	902	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		437	437	100%	2.00E-119	99%	AB474394.1
58	898	*Cladophialophora minutissima*	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	*minutissima*	336	336	97%	4.00E-89	91%	EF016382.1
59	862	*Ilyonectria macrodidyma*	Fungi	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	*Ilyonectria*	*macrodudyma*	452	452	100%	8.00E-124	100%	KC311505.1
60	858	*Mycocentrospora cantuariensis*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Mycocentrospora*	*cantuariensis*	398	398	100%	1.00E-107	95%	EU326864.1
61	840	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	266	266	98%	6.00E-68	86%	JQ857032.1
62	834	*Capronia* sp.	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Capronia*		349	349	99%	7.00E-93	92%	AF284129.1
63	802	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	293	293	100%	4.00E-76	87%	JQ857032.1
65	769	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	260	260	100%	3.00E-66	85%	JQ857032.1
67	709	*Polychytrium aggregatum*	Fungi	Chytridiomycota					*Polychytrium*	*aggregatum*	66.2	66.2	15%	1.00E-07	97%	NG_027613.1
68	692	*Chytridiomycota* sp.	Fungi	Chytridiomycota							68	68	18%	3.00E-08	93%	EU873018.1
70	682	*Mortierella kuhlmanii*	Fungi	(Zygomycota)	Mortierellomycotina		Mortierellales	Mortierellaceae	*Mortierella*	*kuhlmanii*	389	389	97%	8.00E-105	96%	HQ630294.1
71	661	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	232	232	100%	1.00E-57	81%	JQ857032.1
72	643	*Polychytrium aggregatum*	Fungi	Chytridiomycota					*Polychytrium*	*aggregatum*	66.2	66.2	15%	1.00E-07	97%	NG_027613.1
73	612	*Mortierella verticillata*	Fungi	(Zygomycota)	Mortierellomycotina		Mortierellales	Mortierellaceae	*Mortierella*	*verticillata*	452	452	100%	8.00E-124	100%	JN943798.1
74	608	*Trichosporon coprophilum*	Fungi	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales		*Trichosporon*	*coprophilum*	452	452	100%	8.00E-124	100%	AB180199.1
75	600	*Chytridiomycota* sp.	Fungi	Chytridiomycota							64.4	64.4	18%	4.00E-07	91%	EU873018.1
76	593	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	242	242	90%	7.00E-61	86%	JQ857032.1
77	593	*Leucosporidium fellii*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Leucosporidiales		*Leucosporidium*	*fellii*	235	235	99%	1.00E-58	82%	NR_073276.1
78	577	*Umbelopsis nana*	Fungi	(Zygomycota)	Mucoromycotina		Mucorales		*Umbelopsis*	*nana*	452	452	100%	8.00E-124	100%	KC489506.1
79	576	*Capronia* sp.	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Capronia*		446	446	100%	3.00E-122	99%	JQ354915.1
80	567	*Dothidea berberidis*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales	Dothiorceae	*Dothidea*	*berberidis*	437	437	100%	2.00E-119	99%	EU167601.1
81	566	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		289	289	100%	5.00E-75	87%	JF805370.1
82	556	*Chytridiomycota* sp.	Fungi	Chytridiomycota							64.4	64.4	18%	4.00E-07	91%	EU873018.1
83	551	*Polychytrium aggregatum*	Fungi	Chytridiomycota					*Polychytrium*	*aggregatum*	66.2	66.2	15%	1.00E-07	97%	NG_027613.1

TABLE A2

Continued

OUT number	Seq count	Species	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Max Score	Total Score	Query Coverage	E value	Max Identity	Accession
86	509	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		398	398	100%	1.00E-107	95%	AB474394.1
87	497	*Aureobasidium* sp.	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales	Dothiorceae	*Aureobasidium*		432	432	100%	7.00E-118	98%	HQ829153.1
89	492	*Celosporium larixicola*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales		*Celosporium*	*larixicola*	387	387	98%	3.00E-104	95%	FJ997287.1
91	486	*Typhula variabilis*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Thelephorales	Typhulaceae	*Typhula*	*variabilis*	430	430	100%	2.00E-117	98%	AB267395.1
93	470	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	223	223	90%	7.00E-55	84%	JQ857032.1
94	453	*Ulocladium chartarum*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Pleosporaceae	*Urocladium*	*chartarum*	452	452	100%	8.00E-124	100%	KC180717.1
95	450	*Mortierella* sp.	Fungi	(Zygomycota)	Mortierellomycotina		Mortierellales	Mortierellaceae	*Mortierella*		446	446	100%	3.00E-122	99%	HQ608097.1
96	438	*Malassezia* sp.	Fungi	Basidiomycota	Ustilaginomycotina	Exobasidiomycetes	Malasseziales	Malasseziceae	*Malassezia*		439	439	100%	5.00E-120	99%	DQ347480.1
97	437	*Trichoderma spirale*	Fungi	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*spirale*	452	452	100%	8.00E-124	100%	JZ076964.1
98	417	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		293	293	100%	4.00E-76	86%	AB474394.1
99	408	*Lactarius longisporus*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*longisporus*	208	208	100%	1.00E-50	80%	DQ421971.1
100	390	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	302	302	100%	9.00E-79	88%	JQ857032.1
101	380	*Fibulobasidium murrhardtense*	Fungi	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Sirobasidiaceae	*Fibulobasidium*	*murrhardtense*	194	194	100%	3.00E-46	78%	GU327540.1
102	373	*Celosporium larixicola*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales		*Celosporium*	*larixicola*	360	360	98%	4.00E-96	92%	FJ997287.1
103	370	*Ramariopsis laeticolor*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Clavariaceae	*Ramariopsis*	*laeticolor*	293	293	100%	4.00E-76	88%	EU1186181
104	367	*Celosporium larixicola*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales		*Celosporium*	*larixicola*	340	340	98%	3.00E-90	91%	FJ997287.1
105	362	*Rhodotorula* sp.	Fungi	Basidiomycota					*Rhodotorula*		360	360	100%	4.00E-96	93%	AB474394.1
106	358	*Clavulinopsis miyabeana*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Clavariaceae	*Clavulinopsis*	*miyabeana*	168	168	100%	1.00E-38	77%	AB509666.1
107	350	*Alternaria tenuissima*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria*	*tenuissima*	452	452	100%	8.00E-124	100%	KC460834.1
108	344	*Ganoderma* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales	Ganodermataceae	*Ganoderma*		452	452	100%	8.00E-124	100%	HM192933.1
109	319	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	443	443	100%	4.00E-121	99%	JQ857032.1
110	314	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	423	423	100%	4.00E-115	98%	JQ857032.1
111	314	*Lyophyllum* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Lyophyllaceae	*Lyophyllum*		75.2	75.2	22%	2.00E-10	89%	DQ182502.1
112	314	*Aureobasidium* sp.	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales	Dothiorceae	*Aureobasidium*		235	235	100%	1.00E-58	82%	JX462675.1
113	308	*Hyaloraphidium curvatum*	Fungi	Chytridiomycota	Monoblepharidomycetes	Monoblepharidales			*Hyaloraphidium*	*curvatum*	66.2	66.2	39%	1.00E-07	78%	AY997055.1
114	308	*Polychytrium aggregatum*	Fungi	Chytridiomycota					*Polychytrium*	*aggregatum*	66.2	66.2	15%	1.00E-07	97%	NG_027613.1
115	305	*Fusarium acuminatum*	Fungi	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*acuminatum*	452	452	100%	8.00E-124	100%	KF181242.1
116	299	*Tricholoma portentosum*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	*Tricholoma*	*portentosum*	80.6	80.6	20%	5.00E-12	94%	AB699672.1
117	296	*Phaeosclera dermatioides*	Fungi	Ascomycota					*Phaeosclera*	*dematioides*	233	233	87%	4.00E-58	86%	AJ244254.1
118	294	*Chytridiomycota* sp.	Fungi	Chytridiomycota							68	68	18%	3.00E-08	93%	EU873018.1
119	287	*Rhodotorula* sp.	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*		232	232	88%	1.00E-57	84%	KC455921.1
120	284	*Dothideomycetes* sp.	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes					430	430	100%	3.00E-117	98%	GQ153222.1
121	282	*Rhodotorula* sp.	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*		372	372	100%	6.00E-100	94%	KC455921.1
122	274	*Polychytrium aggregatum*	Fungi	Chytridiomycota		Chytridiomycetes	Polychytriales		*Polychytrium*		64.4	64.4	15%	4.00E-07	97%	NG_027613.1
123	268	*Amandinea punctata*	Fungi	Ascomycota	Pezizomycotina	Lecanoromycetes	Caliciales	Caliciaceae	*Amandinea*	*punctata*	408	408	100%	8.00E-111	96%	HQ650627.1
124	267	*Endosporium aviarium*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Myriangiales		*Endosporium*		232	232	90%	1.00E-57	85%	EU304350.1
125	267	*Curvularia spicifera*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Pleosporaceae	*Curvularia*	*spicifera*	452	452	100%	8.00E-124	100%	KC897667.1

TABLE A2

Continued

OUT number	Seq count	Species	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Max Score	Total Score	Query Coverage	E value	Max Identity	Accession
126	260	*Lophium mytiilnum*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Mytilinidales	Mytilinidiaceae	*Lophium*	*mytilinum*	242	242	100%	7.00E-61	82%	EF596819.1
127	258	*Verrucaria* sp.	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Verrucariales	Verrucariaceae	*Verrucaria*		206	206	100%	5.00E-50	80%	FJ664851.1
128	257	*Alphamyces chaetifer*	Fungi	Chytridiomycota		Chytridiomycetes	Rhizophydiales	Alphamycetaceae	*Alphamyces*		82.4	82.4	18%	2.00E-12	100%	EF585633.1
129	251	*Leucosporidium fellii*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Leucosporidiales		*Leucosporidium*	*fellii*	185	185	100%	2.00E-43	77%	NR_073276.1
130	244	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Kriegariales	Kriegeriaceae	*Rhodotorula*	*glacialis*	248	248	89%	2.00E-62	86%	KC455919.1
131	240	*Lyophyllum* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Lyophyllaceae	*Lyophyllum*		68	68	24%	3.00E-08	84%	JX966308.1
132	234	*Clavulinopsis miyabeana*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Clavariaceae	*Clavulinopsis*	*miyabeana*	125	125	72%	1.00E-25	76%	AB509796.1
133	233	*Zalerion arboricola*	Fungi	Ascomycota	Pezizomycotina	Sordariomycetes	Lulworthiales		*Zalerion*	*arboricola*	434	434	100%	2.00E-118	98%	FR837917.1
134	230	*Helicosporium gracile*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes		Tubeufiaceae	*Helicosproium*	*gracile*	141	141	100%	2.00E-30	74%	AY916485.1
136	222	*Phaeococcomyces eucalypti*	Fungi	Ascomycota	Pezizomycotina	Arthoniomycetes	Lichenostigmatales	Phaeococcomycetaceae	*Phaeococcomyces*	*eucalypti*	246	246	98%	6.00E-62	84%	KC005769.1
137	222	*Presuuia* sp.	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Sporormiaceae	*Preussia*		448	448	100%	1.00E-122	99%	KC333160.1
138	221	*Dothichiza pityophili*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales	Dothiorceae	*Dothichiza*	*pityophila*	389	389	87%	8.00E-105	99%	AJ244242.1
139	221	*Venturiaceae* sp.	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Venturiales	Venturiaceae			134	134	100%	3.00E-28	73%	JQ272465.1
140	219	*Rhodotorula* sp.	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*		379	349	100%	4.00E-102	94%	AB474394.1
141	217	*Rhizophydium sp*	Fungi	Chytridiomycota		Chytridiomycetes	Rhizophydiales	Rhizophydiaceae	*Rhizophydium*		84.2	84.2	18%	4.00E-13	100%	EF585662.1
142	216	*Paecilomyces* sp.	Fungi	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae	*Paecilomyces*		347	347	96%	2.00E-92	93%	GU108582.1
143	213	*Botrysphaeriacese* sp.	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae			235	235	10%	1.00E-58	82%	HM176528.1
144	210	*Clavulinopsis* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Clavariaceae	*Clavulinopsis*		134	134	100%	3.00E-28	71%	JN569120.1
145	207	*Phialocephala fluminis*	Fungi	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales		*Phialocephala*	*fluminus*	383	383	100%	3.00E-103	93%	NR_103569.1
146	206	*Rhizophydium sp*	Fungi	Chytridiomycota		Chytridiomycetes	Rhizophydiales	Rhizophydiaceae	*Rhizophydium*		86	86	22%	1.00E-13	95%	EF585662.1
147	205	*Rhizophydium chlorogonii*	Fungi	Chytridiomycota		Chytridiomycetes	Rhizophydiales	Rhizophydiaceae	*Rhizophydium*	*chlorogonii*	64.4	64.4	15%	4.00E-07	97%	JN943815.1
148	205	*Tricholoma dulciolens*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	*Tricholoma*	*dulciolens*	141	141	100%	2.00E-30	74%	AB738883.1
150	203	*Rhodotorula* sp.	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*		230	230	96%	5.00E-57	82%	KC455921.1
151	198	*Trechispora confinis*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Trechisporales		*Trechispora*	*confinis*	143	143	92%	5.00E-31	75%	AF347081.1
152	191	*Cryptococcus magnus*	Fungi	Basidiomycota	Agaricomycotina	Tremellomycetes	Filobasidiales		*Cryptococcus*	*magnus*	446	446	100%	3.00E-122	99%	KC455883.1
153	191	*Polychytrium aggregatum*	Fungi	Chytridiomycota		Chytridiomycetes	Polychytriales		*Polychytrium*	*aggregatum*	66.2	66.2	15%	1.00E-07	97%	NG_027613.1
154	188	*Sarcinomyces crustaceus*	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales		*Sarcinomyces*	*crustaceus*	372	372	100%	6.00E-100	94%	JN040515.1
155	188	*Dothideomycetes* sp.	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes					425	425	100%	1.00E-115	98%	GQ153222.1
156	185	*Trichoderma hamatum*	Fungi	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*hamatum*	452	452	100%	8.00E-124	100%	KC884769.1
157	184	*Mrakiella* sp.	Fungi	Basidiomycota	Agaricomycotina	Tremellomycetes	Cystofilobasidiales		*Mrakiella*		452	452	100%	8.00E-124	100%	JN400824.1
158	183	*Entoloma minutum*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Entolomataceae	*Entoloma*	*minutum*	199	199	100%	8.00E-48	80%	JX454829.1
159	179	*Rhodotorula* sp.	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*		381	381	100%	1.00E-102	94%	KC455921.1
160	175	*Tomentella* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*		68	68	20%	3.00E-08	90%	JN129414.1
161	172	*Geminibasidium hirsutum*	Fungi	Basidiomycota		Walleminomycetes	Geminibasidiales		*Geminibasidium*	*hirsutum*	334	334	88%	2.00E-88	94%	JX242880.1
163	170	*Lyophyllum* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Lyophyllaceae	*Lyophyllum*		75.2	75.2	22%	2.00E-10	89%	DQ182502.1
164	169	*Mortierella* sp.	Fungi	(Zygomycota)	Mortierellomycotina		Mortierellales	Mortierellaceae	*Mortierella*		443	443	100%	4.00E-121	99%	GQ302682.1
165	168	*Donadinia nigrella*	Fungi	Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales	Sarcosomataceae	*Donadinia*	*nigrella*	325	325	92%	8.00E-86	91%	JX669836.1

TABLE A2

Continued

OUT number	Seq count	Species	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Max Score	Total Score	Query Coverage	E value	Max Identity	Accession
166	168	*Thysanophora penicillioides*	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Aspergillaceae	*Thysanophora*	*penicillioides*	203	203	100%	6.00E-49	79%	AB213266.1
167	167	*Epicoccum nigrum*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes			*Epicoccum*	*nigrum*	452	452	100%	8.00E-124	100%	KC164754.1
168	166	*Helicoma isiola*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes		Tubeufiaceae	*Helicoma*	*isiola*	271	271	100%	2.00E-69	85%	DQ341099.1
169	160	*Trechispora subsphaerospora*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Trechisporales		*Trechispora*	*subsphaerospora*	253	253	100%	4.00E-64	84%	AF347080.1
170	160	*Tuber mexiusanum*	Fungi	Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales	Tuberaceae	*Tuber*	*mexiusanum*	446	446	100%	3.00E-122	99%	JX030294.1
171	159	*Polychytrium aggregatum*	Fungi	Chytridiomycota		Chytridiomycetes	Polychytriales		*Polychytrium*	*aggregatum*	66.2	66.2	15%	1.00E-07	97%	NG_027613.1
172	159	*Phialocephala fluminis*	Fungi	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales		*Phialocephala*	*fluminus*	374	374	100%	2.00E-100	93%	NR_103569.1
173	158	*Trichosporon dermatis*	Fungi	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales		*Trichosporon*	*dermatis*	68	68	24%	3.00E-08	85%	KC254108.1
174	158	*Rhodotorula* sp.	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*		334	334	100%	2.00E-88	91%	KC333170.1
175	155	*Postia alni*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales		*Postia*	*alni*	82.4	82.4	70%	2.00E-12	74%	KC595931.1
176	154	*Sarcinomyces crustaceus*	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales		*Sarcinomyces*	*crustaceus*	307	307	100%	2.00E-80	89%	JN040515.1
177	152	*Lyophyllum* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Lyophyllaceae	*Lyophyllum*		71.6	71.6	26%	3.00E-09	85%	DQ182502.1
178	150	*Lyophyllum* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Lyophyllaceae	*Lyophyllum*		75.2	75.2	22%	2.00E-10	89%	DQ182502.1
179	150	*Ascomycota* sp.	Fungi	Ascomycota							452	452	100%	8.00E-124	100%	JQ775574.1
180	149	*Glomus* sp.	Fungi	Glomeromycota		Glomeromycetes	Glomerales	Glomeraceae	*Glomus*		66.2	66.2	18%	1.00E-07	91%	AJ504633.1
181	147	*Metarhizium anisopliae*	Fungi	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metarhizium*	*anisopliae*	446	446	100%	3.00E-122	99%	JN256671.1
182	143	*Cryptococcus* sp.	Fungi	Basidiomycota	Agaricomycotina	Tremellomycetes			*Cryptococcus*		389	389	100%	8.00E-105	95%	FJ873574.1
183	143	*Mrakia gelida*	Fungi	Basidiomycota	Agaricomycotina	Tremellomycetes	Cystofilobasidiales	Cystofilobasidiaceae	*Mrakia*	*gelida*	120	120	77%	6.00E-24	75%	JQ857036.1
184	143	*Paecilomyces carneus*	Fungi	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae	*Paecilomyces*	*carneus*	304	304	100%	3.00E-79	89%	KC180711.1
185	143	*Lyophyllum* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Lyophyllaceae	*Lyophyllum*		75.2	75.2	22%	2.00E-10	89%	DQ182502.1
186	139	*Rhizophydiales sp*	Fungi	Chytridiomycota		Chytridiomycetes	Rhizophydiales		*Rhizophydiales*		82.4	82.4	23%	2.00E-12	92%	FR670788.1
187	139	*Hygrocybe irrigata*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Hygrophoraceae	*Hygrocybe*	*irrigata*	116	116	54%	7.00E-23	81%	FM208881.1
188	138	*Sistotrema* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Corticiales	Corticiaceae	*Sistotrema*		233	233	100%	4.00E-58	82%	FR838002.1
189	138	*Rhodotorula* sp.	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*		300	300	98%	3.00E-78	89%	KC455921.1
190	137	*Rhodotorula* sp.	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*		309	309	100%	6.00E-81	88%	AB474394.1
191	137	*Rhodotorula* sp.	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*		273	273	100%	4.00E-70	85%	AB474394.1
192	136	*Penicillium* sp.	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*		452	452	100%	8.00E-124	100%	KF305753.1
193	131	*Exophiala* sp.	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala*		452	452	100%	8.00E-124	100%	JX243973.1
195	129	*Rhodotorula* sp.	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*		309	309	100%	6.00E-81	88%	KC333170.1
196	129	*Lophiostoma* sp.	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Lophiostomataceae	*Lophiostoma*		396	396	100%	5.00E-107	96%	HQ914838.1
197	128	*Rhodotorula glacialis*	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*	*glacialis*	269	269	100%	5.00E-69	96%	KC455919.1
198	127	*Saccharomyces cerevisiae*	Fungi	Ascomycota	Saccharomycotina	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Saccharomyces*	*cerevisiae*	466	466	100%	3.00E-122	99%	KC183729.1
199	125	*Leucosporidium scottii*	Fungi	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Leucosporidiales		*Leucosporidium*	*scottii*	347	347	100%	2.00E-92	91%	JX014242.1
200	124	*Chytridiomycota* sp.	Fungi	Chytridiomycota							69.8	69.8	22%	1.00E-08	88%	EU873018.1
201	124	*Clavulinopsis miyabeana*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Clavariaceae	*Clavulinopsis*	*miyabeana*	163	163	77%	6.00E-37	80%	AB509666.1
202	123	*Clavulinopsis* sp.	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Clavariaceae	*Clavulinopsis*		109	109	82%	1.00E-20	71%	JN569120.1
204	122	*Capronia pulcherrima*	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Capronia*	*pulcherrima*	298	298	100%	1.00E-77	86%	AF050256.1

TABLE A2
Continued

OUT number	Seq count	Species	Kingdom	Phylum	Subphylum	Class	Order	Family	Genus	Species	Max Score	Total Score	Query Coverage	E value	Max Identity	Accession
205	120	*Phaeosclera* sp.	Fungi	Ascomycota					*Phaeosclera*		96.9	96.9	40%	7.00E-17	82%	AY843195.1
206	119	*Rhodotorula* sp.	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*		311	311	100%	2.00E-81	88%	AB474394.1
207	117	*Clavulinopsis miyabeana*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Clavariaceae	*Clavulinopsis*	*miyabeana*	199	199	100%	8.00E-48	78%	AB509666.1
208	114	*Capronia* sp.	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Capronia*		360	360	99%	4.00E-96	93%	AF284129.1
209	112	*Alternaria infectoria*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria*	*infectoria*	452	452	100%	8.00E-124	100%	HG324079.1
210	112	*Mycena oregonenis*	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae	*Mycena*	*oregonensis*	255	255	100%	1.00E-64	82%	JF908409.1
211	111	*Capronia villosa*	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Capronia*	*villosa*	210	210	100%	4.00E-51	81%	AF050261.1
213	110	*Saccharata* sp.	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Saccharata*		239	239	100%	9.00E-60	82%	JN225922.1
214	109	*Sarcinomyces crustaceus*	Fungi	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales		*Sarcinomyces*	*crustaceus*	289	289	100%	6.00E-75	86%	JN040515.1
215	108	*Trichoderma strigosellum*	Fungi	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*strigosellum*	446	446	100%	3.00E-122	99%	EU280139.1
216	108	*Phaeomoniella prunicola*	Fungi	Ascomycota					*Phaeomoniella*	*prunicola*	342	342	100%	1.00E-90	91%	GQ154588.1
217	107	*Lophium mytilnum*	Fungi	Ascomycota	Pezizomycotina	Dothideomycetes	Mytilinidales	Mytilinidiaceae	*Lophium*	*mytilinum*	452	452	100%	8.00E-124	100%	EF596819.1
218	105	*Holtermanniella watticus*	Fungi	Basidiomycota	Agaricomycotina	Tremellomycetes	Holtermanniales		*Holtermanniella*	*watticus*	452	452	100%	8.00E-124	100%	JQ857031.1
219	105	*Rhodotorula* sp.	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*		385	385	100%	1.00E-103	94%	KC455921.1
220	103	*Rhodotorula* sp.	Fungi	Basidiomycota	Pucciniomycotina	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Rhodotorula*		262	262	98%	8.00E-67	85%	KC455921.1

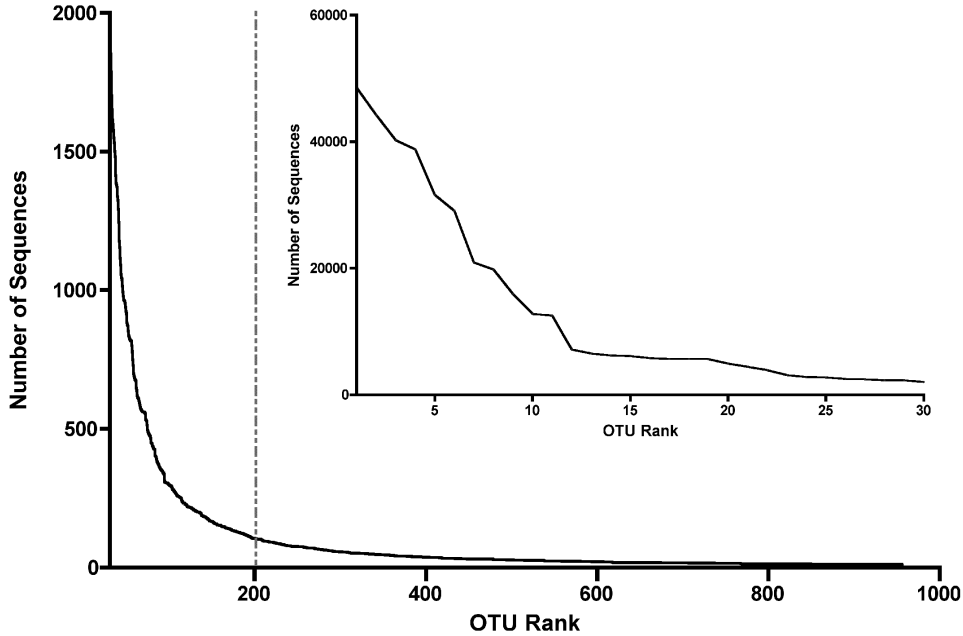

FIGURE A1. Ranked operational taxonomic unit (OTU) abundance distribution plot of all OTUs. The dashed line represents our cutoff of 200 OTUs for analyses and represents greater than 97% of all fungal sequences. Insert represents the 30 most abundant OTUs (notice the change in scale) demonstrating that few OTUs make up most of the fungal community members.

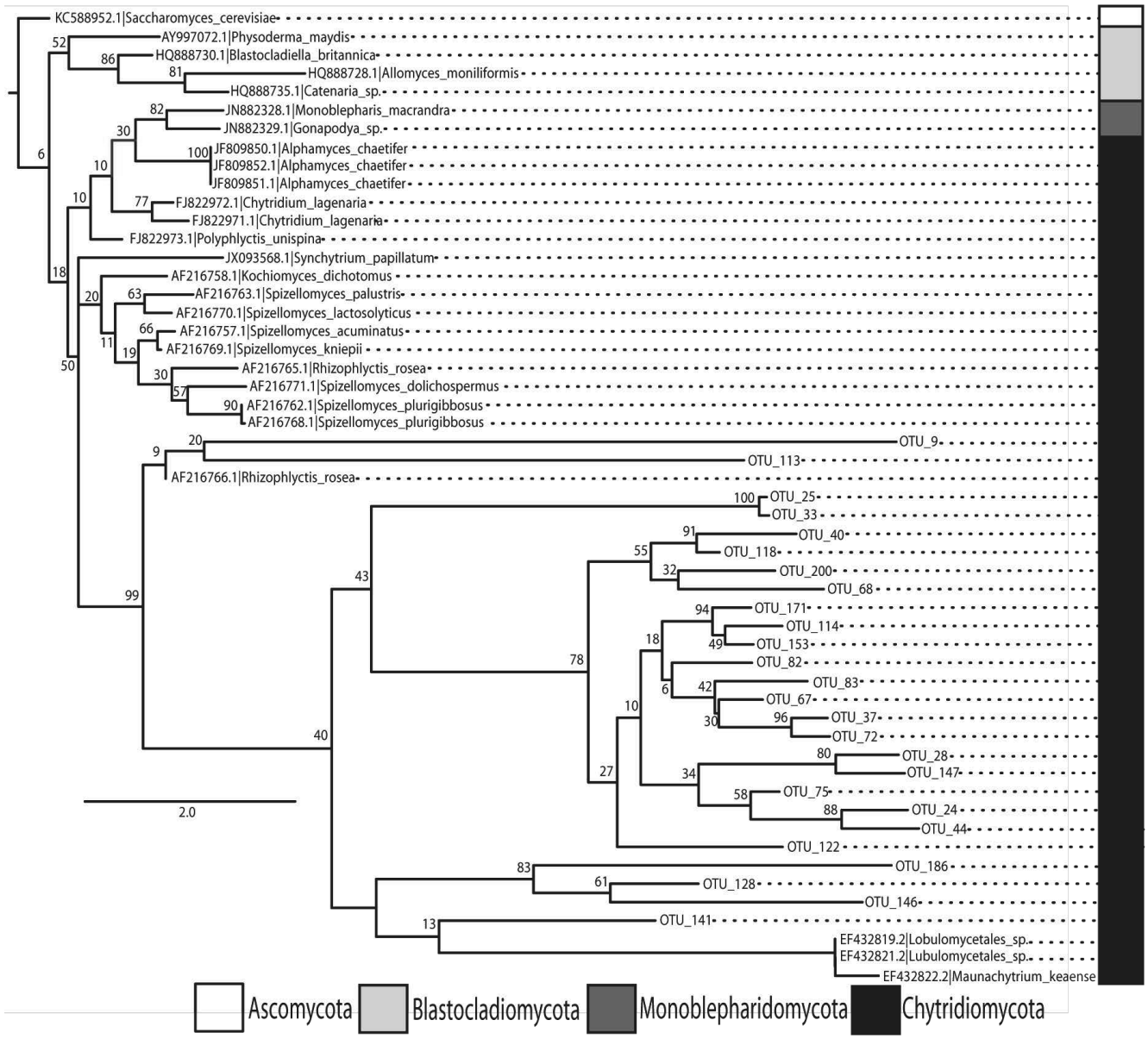

FIGURE A2. Phylogenetic analysis (Maximum Likelihood) of putative chytrid OTUs with vouchered representative ITS2 sequences within phyla Chytridiomycota, Blastocladiomycota, and Monoblepharidomycota indicates that observed novel OTUs are nested within phylum Chytridiomycota with 99% bootstrap support.

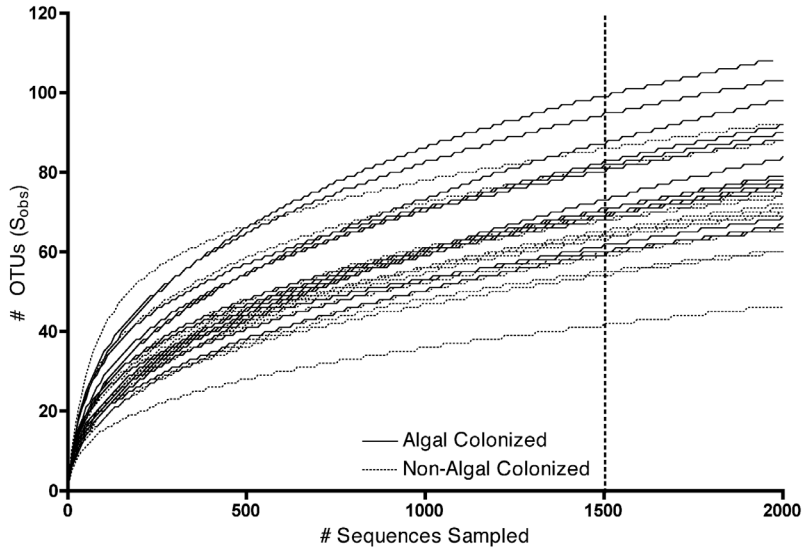

FIGURE A3. Rarefaction analysis of observed OTUs for algal colonized and non-algal colonized snow fungi indicate that at the 1500 sequence subsampling point (dashed line), the majority of community members have been observed as this subsample value is well past the inflection point of the curves.

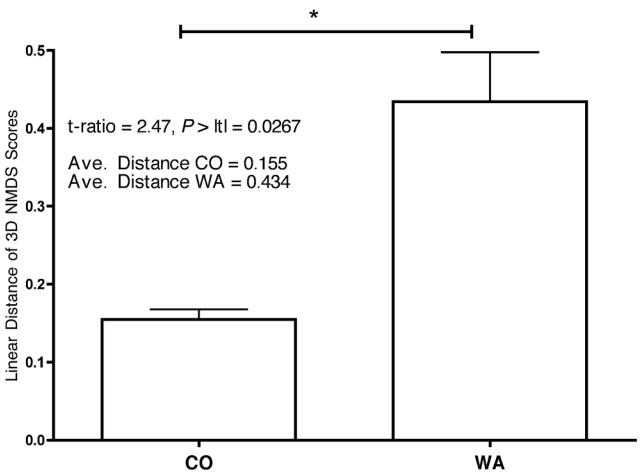

FIGURE A4. Distances of paired algal colonized and non-algal colonized axes loading score across three-dimensional space indicate that Colorado (CO) fungal communities are more similar between paired samples than Washington (WA) paired fungal communities (Euclidian distance between paired samples based on *t*-test).