Microbial Ecology
https://doi.org/10.1007/s00248-019-01357-z

ENVIRONMENTAL MICROBIOLOGY

Microbial Ecology of Snow Reveals Taxa-Specific Biogeographical Structure

Shawn P. Brown [1] · Ari Jumpponen [2]

Received: 1 October 2018 / Accepted: 6 March 2019

Abstract

Snows that persist late into the growing season become colonized with numerous metabolically active microorganisms, yet underlying mechanisms of community assembly and dispersal remain poorly known. We investigated (Illumina MiSeq) snow-borne bacterial, fungal, and algal communities across a latitudinal gradient in Fennoscandia and inter-continental distribution between northern Europe and North America. Our data indicate that bacterial communities are ubiquitous regionally (across Fennoscandia), whereas fungal communities are regionally heterogeneous. Both fungi and bacteria are biogeographically heterogeneous inter-continentally. Snow algae, generally thought to occur in colorful algae blooms (red, green, or yellow) on the snow surface, are molecularly described here as an important component of snows even in absence of visible algal growth. This suggests that snow algae are a previously underestimated major biological component of visually uncolonized snows. In contrast to fungi and bacteria, algae exhibit no discernible inter-continental or regional community structure and exhibit little endemism. These results indicate that global and regional snow microbial communities and their distributions may be dictated by a combination of size-limited propagule dispersal potential and restrictions (bacteria and fungi) and homogenization of ecologically specialized taxa (snow algae) across the globe. These results are among the first to compare inter-continental snow microbial communities and highlight how poorly understood microbial communities in these threatened ephemeral ecosystems are.

Keywords Snow · Snow algae · Fungi · Bacteria · Biogeography · Nival microbiology

Introduction

Cosmopolitanism of microorganisms is often assumed with environmental conditions selecting for local community membership (*ab origine* Baas Becking [1]; *see* [2]). Despite this apparent ubiquity, the diverse, dynamic, and metabolically active microbial communities inhabiting snow are a surprise to many. In snowflakes, ice nucleators are often viable bacteria [3] exemplified by *Pseudomonas syringae* [4]. Despite this initial "seeding" by snow bacteria, it remains unclear if these nival (snow-borne) bacteria persist as common constituents in communities inhabiting lingering snows (snows that persist late into the growing season), as propagules are continuously allochthonously deposited onto the snow surface. There is a fundamental lack of understanding of snow-borne microbial processes and biogeographical distributions as well as a general paucity of studies into nival microbial communities.

Perhaps, the best-known snow-borne organisms are the microalgae that establish distinct colorful blooms on the snow surface (usually red caused by blooms of *Chlamydomonas* cf. *nivalis* but can also be green or yellow depending on the dominant algal taxa present [5, 6], hereafter referred to as "red snow"). While much is unknown about how these algae subsist, are dispersed, or are distributed, it is generally assumed that they exist as resting structures while in frozen snow and when snows begin to melt, these resting structures produce flagellated vegetative sexual gametes and utilize dissolved nutrients in the liquid water [7]. However, this lifestyle varies widely depending on algal species.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00248-019-01357-z) contains supplementary material, which is available to authorized users.

✉ Shawn P. Brown
spbrown2@memphis.edu

[1] Department of Biological Sciences, The University of Memphis, Memphis, TN 38152, USA

[2] Division of Biology, Kansas State University, Manhattan, KS 66506, USA

Published online: 13 March 2019

Because the "red snows" and associated algae are ephemeral, little is known about their temporal and inter-annual community dynamics or their dispersal and community assembly. Not only are these algae patches dynamic, but they provide a habitat to diverse bacterial [8, 9] and fungal [10, 11] communities. Pliny the Elder in his natural history tome (*Historia Naturalis*) commented on this unusual red snow [12] and the 1818 Ross Arctic expedition collected algae in snows ([13]; originally described as fungi, *see* [14]). Studies on bacteria in the snow, although preceded by studies focusing on eukaryotic organisms, have been conducted for more than a century [15, 16]. While a growing body of literature exists on microbial dynamics associated with snow algae, only few have examined general microbial ecology in visually algae-free snows (hereafter referred as "white snow"; *but see* [17, 18]). Consequently, snow is a vast uncharted and poorly documented ecosystem [19] that may provide a new appreciation for global microbial biodiversity.

Microbial dispersal remains an enigmatic process and is assumed to occur predominantly aerially through single-cell organismal transport or spore dispersal, although there is ample evidence of animal-vectored dispersal in eukaryotic microbes [20–23]. However, very little is known about the dispersal mechanisms and vectors of snow algae specifically. Aerial propagation is unlikely to play a major role in dispersal [24] although some snow algae seem capable of intercontinental aerial transport [25]. However, no appreciable evidence of snow algae has been observed in aerial surveys [26] suggesting perhaps aerial dispersal only coinciding with infrequent and extreme weather events [27]. Snow algae occur on every continent [28] and these discrete communities are compositionally very similar [29] with little documented endemism (*but see* [30–32]).

While bacteria and fungi disperse readily in the air, these groups likely differ in dispersal ranges. A size-dependent passive dispersal is often assumed based on a prediction that smaller propagules are more likely to aerially disperse over vast distances (*but see* [33]). A simulation study demonstrated that when microbes exceed 20 μm diameter, they are less likely to rely on wind dispersal, and microbes larger than 60 μm in diameter have limited dispersal over long distances [34]. This size fractionation in dispersal range suggests that bacteria (most which are smaller than 20 μm) should be globally cosmopolitan. Others have suggested that a 2-mm diameter may be the global/local determinant for free-living eukaryotic microbes and microinvertebrates [35]. This diminutive size limit for global dispersal and distribution has been previously postulated using global recombination rates [36] and directly observed to be generally true for snow-borne bacteria [26]. However, the global distribution of small organisms seems context-dependent with bacterial specialists having more constrained distributions (e.g., cyanobacteria in [26]) than generalist bacteria. Similarly, fungi exhibit this dichotomous distribution: generalists are more likely true cosmopolitans than specialists, e.g., mycorrhizal fungi [37]. Ectomycorrhizal spore dispersal is subject to strong distance-decay patterns, i.e., the number of dispersed spores is negatively correlated with distance [38]. Yet, there remains much stochasticity in Basidiomycete spore dispersal distances [39]. In contrast to these findings, other evidence suggests that the fungal specialists may be more globally dispersed (e.g., fungal pathogens [40]; arbuscular mycorrhizal fungi [41]).

Though bacteria can be globally distributed, neither terrestrial bacterial communities [42, 43] nor bacterial atmospheric abundances are globally homogeneous. Cryospheric ecosystems are much less likely to be a source or sink of propagules to or from other ecosystems based on observed depauperate bacterial abundances [44]. Therefore, air samples collected above ice and snow fields have far lower bacterial abundances than those above other ecosystems [45] suggesting that snow- and ice-inhabiting bacteria may either have a lower propensity to become airborne or have inherently lower biomass. This disconnect suggests the potential for high endemism of snow bacteria, perhaps explained by the distinct adaptive physiology required for a psychrophilic lifestyle.

The biogeography of snow-borne microbes remains inadequately studied. Evidence suggests no isolation by distance for snow algae [9, 46] because algal communities are similar across global sampling sites. In contrast, Brown et al. [47] showed that while algal communities on snow are similar on a species level, their constituents may be genetically distinct in discrete snow algae patches and their populations indeed genetically isolated by distance depending on the propagating cell size. Further, Segawa et al. [32] demonstrated that while snow algal communities are globally ubiquitous, a low but not insignificant number of algal OTUs are endemic to either polar region. Snow-borne fungal communities have been observed to be more geographically distinct than algae, with more similar communities locally but distinct across farther distances [11] indicating strong isolation by distance (*see also* [10]). Bacterial communities are generally similar across different sampling sites, particularly so for those within snow algae patches [9]. These observations corroborate global distributions of some alpine bacteria [17], although some bacterial communities exhibit distinction across space [18].

Here, we present a cross-kingdom/domain (fungi, bacteria, and algae) examination across a latitudinal gradient and intercontinental distances to better understand the biogeography of snow-borne microbes, in the absence of visually observable snow algae. Previous work suggests that nival algae are functionally absent in white snow [7], although algae have been observed in snows without visible blooms both using flow cytometry [11] and clone sequence data [26].

There is a dearth of latitudinal investigations of microbial communities, and to our knowledge, none have investigated

latitudinal variation of snow-borne microbial communities. The few studies conducted in other substrates besides snow suggest that there is generally a negative correlation between latitude and bacterial richness in marine systems [48] and Fierer and Jackson [49] found a slight quadratic relationship (concave down) between latitude and soil bacterial diversity. Finer-scale investigations suggest that Antarctic soil bacterial and fungal communities may differ across latitudes [50]. Fungal diversity in soil has generally (but not exclusively) been reported to be negatively associated with latitude with the exception of ectomycorrhizal fungi [51, 52], but it is yet unclear if these reported correlations hold true for nival communities.

Here, we present the first of its kind investigation into drivers of snow-borne microbial communities (fungi, bacteria, and algae) across late-season snow packs along a latitudinal gradient across Fennoscandia with the inclusion of inter-continental (Colorado, USA) samples. In doing so, we examine global similarities and differences in snow-borne microbial communities. Specifically, we examined if (1) snow-inhabiting microbial communities change with latitude, both regionally (within Fennoscandia) and globally; (2) fungi, bacteria, and algae exhibit differential distance decay based on propagule size; (3) communities of snow-inhabiting microbes differ across these scales; (4) snow algae regularly occur in "white snow"; and (5) core snow community distribution patterns (community members found consistently and across all snows) are congruent across taxa to better understand ubiquity of snow-inhabiting microbes.

Methods

Sampling

We sampled late-season snow packs across a latitudinal gradient in Fennoscandian Lapland (Finland, Norway, and Sweden; June 13–15, 2013) and Colorado, USA (July 8, 2013). At each latitudinal transect, four snow samples were collected at approximately 5 km (east-west) intervals [only three could be collected in Colorado, USA, due to low snow pack; *see* Table 1]. In all, the Lapland samples represent about a 300-km south-north gradient. A total of five individual and separate 85 cm^3 volumetric surface subsamples were collected by scraping the top 5 cm of snow packs using a sterilized steel cylinder (following [11]); these subsamples were composited into one clean 1-gal zip-top plastic bag to represent each snow pack. Snows were only collected if they were visually undisturbed and free of any apparent anthropogenic or animal disturbances. The composite samples were allowed to melt at ambient temperature. As soon as the snow had melted, melt water was agitated and 100 mL was passed through a 1.0-μm Nuclepore Track-Etch Membrane filter (47 mm; Whatman®, Kent, UK) encased in a 47-mm Swin-Lok Plastic Filter Holder (Whatman®) using a sterile 30 mL syringe (BD 30 mL Syringe; Becton, Dickson and Co., Franklin Lakes, NJ, USA). Filtrate was collected into 500 mL polyethylene plastic bottles (Nalgene) field-sterilized with denatured ethanol. The collected filtrate was filtered through a 0.22-μm filter as described above. This dual filtration approach permitted size fractionation of particles in the melt water to allow

Table 1 Sampling locations of late-season snow packs across Fennoscandia and Colorado including sampling date, geographic coordinates, transect number, and nearest landmark

Sampling date	Latitude	Longitude	Elevation (m ASL)	Transect number	Country	Nearest city/road	Orientating landmark
June 13, 2013	69° 03′ 01″ N	20° 50′ 19″ E	888	1	Finland	Kilpisjärvi, Finland	SE side Mt. Saana
June 13, 2013	69° 03′ 51″ N	20° 33′ 06″ E	492	1	Norway	Kilpisjärvi, Finland	National Boundary Tripoint
June 13, 2013	69° 04′ 23″ N	20° 37′ 16″ E	645	1	Finland	Kilpisjärvi, Finland	W Side Mt. Malla
June 13, 2013	69° 04′ 14″ N	20° 41′ 33″ E	699	1	Finland	Kilpisjärvi, Finland	E Side of Mt. Malla
June 14, 2013	68° 31′ 10″ N	17° 52′ 16″ E	416	2	Norway	Highway E 10 (Narvik, Norway)	Skitdalsvatnet (lake)
June 14, 2013	68° 30′ 45″ N	17° 57′ 56″ E	334	2	Norway	Highway E 10 (Narvik, Norway)	Øvre Jernvatnet (lake)
June 14, 2013	68° 27′ 40″ N	18° 01′ 42″ E	480	2	Norway	Highway E 10 (Narvik, Norway)	Øvre Geitvatnet (lake)
June 14, 2013	68° 26′ 53″ N	18° 06′ 04″ E	536	2	Norway	Highway E 10 (Narvik, Norway)	Near Bjørnfjell
June 15, 2013	66° 43′ 55″ N	16° 01′ 59″ E	770	3	Sweden	SW Highway 95	Near Lake Mierkenisvare
June 15, 2013	66° 42′ 02″ N	15° 56′ 33″ E	781	3	Sweden	SW Highway 95	Near Lake Kuoletisjaure
June 15, 2013	66° 45′ 59″ N	15° 49′ 55″ E	707	3	Norway	SW Highway 95	Near Lake Sraggajaure
June 15, 2013	66° 12′ 10″ N	14° 33′ 57″ E	637	3	Norway	NO Highway E6	Near Hjartasen
July 8, 2013	40° 04′ 07″ N	105° 37′ 26″ W	3340	4	USA	Ward, CO, USA	Lake Isabelle
July 8, 2013	40° 04′ 15″ N	105° 36′ 47″ W	3335	4	USA	Ward, CO, USA	Lake Isabelle
July 8, 2013	40° 04′ 10″ N	105° 36′ 38″ W	3275	4	USA	Ward, CO, USA	Lake Isabelle

prokaryotes to pass through the first filter, thus minimizing bacterial cell capture and enriching target organism's DNA (eukaryotes) in the first filter, whereas the second filter is enriched for prokaryotes (should not contain eukaryotes) allowing for a more efficient capture of bacterial DNA (*see* [11]). After filtration, membrane filters were placed into individual MoBio UltraClean® Soil DNA Isolation Kit bead tubes (Carlsbad, CA, USA) and stored on ice until transported to a laboratory, where frozen at − 20 °C until extractions occurred.

DNA Extraction and Amplicon Library Preparation

Total genomic DNA was extracted following manufacture's protocol using the following procedural modifications: (1) extraction tubes with the filters were sonicated on high setting for 10 min to dislodge cells from the filter (*see* [53]), and (2) samples were homogenized after filters were removed using a FastPrep (FP120, ThermoFisher Scientific, Waltham, MA, USA) after the addition of two 2.4 mm zirconia beads (BioSpec Products, Bartlesville, OK, USA). The samples from Fennoscandia were extracted at the University of Helsinki's Department of Environmental Science (Lahti, Finland) and the Colorado samples were extracted at Kansas State University, where immediately frozen at − 20 °C. Upon extraction, DNA was frozen, and the Fennoscandian samples were shipped frozen to Kansas State University and immediately placed in − 20° upon arrival.

Extracted DNA was quantitated (ND1000 spectrophotometer; NanoDrop Technologies, Wilmington, DE, USA) and standardized to 5 ng/μL concentrations. Eukaryotic Amplicon libraries were generated using a combination of the universal eukaryotic primer ITS4 [54] and the fungal-specific primer fITS7 [55] to target the variable Internal Transcribed Spacer 2 (ITS2) region of the rRNA gene repeat. Although fITS7 primer is reportedly fungus-specific [55], it also has been shown to amplify DNA from snow algae [11]. Bacterial amplicons were generated using primers 515F and 806R [56] to amplify the V4 region of the 16S rRNA. Multiplex identifiers (MIDs; 12-bp) were used from the published list [56] and synthesized with the ITS4 and 806R primers into hybrid primers (see Table S1 for complete list of primer sequences and MID used). Each sample was PCR-amplified in triplicate 50 μL reactions with the following reagent concentrations and volumes: for fungi and algae, 25 ng template DNA, 1 μM primers fITS7 and MID-ITS4, 200 μM of each dNTP, 10 μL 5 x Green GoTaq® Flexi Buffer (Promega, Madison, WI, USA), 14.6 μL molecular biology grade water, and 2 U GoTaq® Hot Start polymerase (Promega, Madison, WI, USA). PCR cycling conditions consisted of a 94 °C initial denaturation for 3 min, 30 cycles of 94 °C denaturation for 1 min, 53° annealing for 1 min, and 72 °C extension for 2 min, followed by a final 72 °C extension step for 8 min. Bacterial PCR conditions were 25 ng DNA template, 1 μM each of primers 505F and MID-806R, 25 μL AmpliTaq Gold® Master Mix (Applied Biosystems, Foster City, CA, USA), and 15 μL molecular biology grade water. The PCR cycling parameters were 95 °C initial denaturation step for 4 min, followed by 30 cycles of 95 °C for 1 min, 50 °C annealing for 1 min, and 72 °C extension for 2 min, followed by a final extension step at 72° for 8 min. All PCRs were performed on Eppendorf MasterCycler thermocyclers (Hamburg, Germany). Negative controls of sterile molecular grade water were included throughout the extraction and PCR protocols and remained free of observable contamination. A total of 10 μl of the PCR products were visualized on 1.5% agarose (*w:v*) gels to confirm successful PCR amplification. The remaining 40 μL of the PCR products were cleaned using the Agencourt® AmPure® cleanup kit using a SPRIplate 96-ring magnet (Beckman Coulter, Beverly, MA, USA) as recommended by the manufacturer except for a 1:1 of bead solution to reaction volume ratio to allow for a more stringent exclusion of short fragments and primer-dimers [57]. Cleaned triplicate PCR products were pooled per sample and quantitated. Experimental units were pooled to equal concentrations and the pooled library was cleaned once more using the AmPure® cleanup kit as above. Illumina MiSeq adaptors were ligated onto the amplicon library using a NEBNext® DNA MasterMix for Illumina Kit (New England BioLabs, Ipswitch, MA, USA) and sequences were generated using a MiSeq Reagent Kit v2 (2 × 250 cycles; Illumina, San Diego, CA, USA) at the Kansas State University Integrated Genomics Facility (Manhattan, KS, USA). Sequence data is archived at Sequence Read Archive (SRA) at NCBI under accessions BioProject (PRJNA518106) and BioSamples (SAMN10853300-SAMN10853314).

Sequence Processing

All sequences were processed using the program mothur (v.1.32.1; [58]). Bacterial and eukaryotic sequences were analyzed independently. Paired fastq files were contiged and screened such that a minimum 50-bp overlap was required to retain a sequence. Sequences were further filtered to cull any sequence with any ambiguous bases, long homopolymers (maximum size = 8), non-exact match to the MID sequence, or more than one basepair difference to primer sequence. Bacterial sequences were aligned against a mothur-formatted 16S Silva Alignment (www.arb-silva.de). Sequences underwent a pseudo single-linkage clustering (99%; [59]) to control for platform generated errors and screened for potential chimeras (UCHIME; [60]); putative chimeras were culled. The sequence data were assigned to taxon affinities using mothur-embedded Naïve Bayesian Classifier [61]. Bacterial sequences were screened against the Ribosomal Database Project's 16S reference training set (v.9) and sequences without a reliable assignment to Domain Bacteria (less than 100%

bootstrap support) or assigned to Eukaryotes, Archaea, mitochondrial, or plastids were removed. A sequence distance matrix was generated for aligned bacterial sequences (not punishing terminal gaps).

Eukaryotic sequences were quality controlled as above followed by truncation of the ITS2 sequences to 250 bp to facilitate preclustering. Retained eukaryotic sequences (this quality control culled one fungal sample from transect 1) were screened against the fungus-specific UNITE plus INSD ITS database (v.6; *see* [62]). These sequences were screened to remove all sequences that could not be reliably classified. Sequences were pairwise aligned (Needleman-Wunsch, not punishing terminal gaps) to generate a eukaryotic distance matrix.

The resultant distance matrices were clustered into Operational Taxonomic Units (OTUs) using an average-neighbor algorithm (UPGMA) at a 97% similarity threshold. The most representative sequences of an OTU (centroid) were identified and OTUs assigned to taxon affinities. Eukaryotic OTUs were further screened by manually examining the representative sequences for each OTU whose best classification was Kingdom = Fungi, Phylum = unclassified. Representative sequences were queried against the GenBank nucleotide collection—nr/nt (www.ncbi.nlm.nih.gov) using the BLASTn algorithm while excluding uncultured/environmental sample sequences. This step was conducted for two reasons: (1) The UNITE reference set included no non-fungal representatives (subsequent releases contain non-fungal representatives for culling non-fungal sequences) resulting in assignment of all classifiable sequences as Fungi regardless of their true affinity; as a result, confirming that these sequences were fungal is mandatory. (2) Previous examination of snow-borne communities using the fungus-specific primers fITS7-ITS4 included abundant off-target amplification [11, 47] with a propensity for Chlorophyta. We aimed to also analyze this chlorophyte community. All OTUs that were not fungal or algal were culled and the eukaryotic Sample × OTU matrix was split to make separate fungal and algal matrices. All putative algae sequences were queried against BLASTn (nr/nt; excluding environmental sequences) to assign algae OTUs to putative taxonomic identities. Genus level identities were assigned when total bit score was greater than 300 for an alignment. To exclude potentially spurious OTUs (*see* [63, 64]), all OTUs with a global sequence count less than 10 were excluded from downstream analyses.

Community and Statistical Analyses

Observed richness (S_{obs}), complement of Simpson's Diversity (1-D), and Simpson's Evenness (E_D) were estimated iteratively by subsampling (15,000 sequences per sample for bacteria, 9000 per sample for fungi, and 250 per sample for algae) using 1000 iterations and the average of these diversity estimators used for analyses. Subsampling depth was selected to maximize sequence representation while allowing for all samples to be retained. A pairwise Bray-Curtis dissimilarity matrix was calculated for bacterial, fungal, and algal communities with 1000 iterations with the above subsampling depths and the average Bray-Curtis values used for downstream analyses. Diversity estimators and Bray-Curtis dissimilarities were calculated using the program mothur. Obtained Bray-Curtis matrices were inputted into the program PC-ORD (v.5.31; [65]) and community differences were tested using Multi-Response Permutation Procedure (MRPP), and locally abundant OTUs identified using Indicator Species analyses [66] using imputed OTU × Sample matrices.

Both the diversity indices (richness, diversity, and evenness) and Bray-Curtis dissimilarity matrices were analyzed using two different methods. First, regression analyses were used to examine changes in response variables with latitude (across Fennoscandian only) to determine if communities change across a latitudinal gradient. Second, individual transects (each east-west transect as a categorical variable) were compared using one-way analysis of variance (ANOVA) with post hoc Tukey's HSD tests where applicable. The community compositional attributes were tested using Multi-Response Permutation Procedure (MRPP) analyses using the acquired Bray-Curtis distance matrices with weighting option of $n/\Sigma n$ where n equals the number of samples per group. All statistics were conducted using a combination of mothur, R, PC-ORD (v.4; MjM Software, Gleneden, Oregon, USA), and JMP (v.7.0.2; SAS Institute Inc., Cary, North Carolina, USA).

To investigate snow-borne microbial geographic patterning and community isolation by distance, average Bray-Curtis dissimilarities of bacterial, fungal, and algal communities were analyzed against geographic distance using a combination of regression analyses and Mantel tests against pairwise geographic distances for the Northern European samples. Additionally, a series of ANOVAs using categories associated with geographic distance were conducted to investigate intercontinental community distinctions. The geographic distance categorical classifications used were (1) samples collected from within the same transect (pairwise geographic distance of less than 15 km); (2) samples collected between two adjacent latitudinal transects (pairwise geographic distance between 70 and 140 km), (3) samples collected from the farthest Fennoscandian transects (1 vs. 3; 210–400 km), and (4) samples collected from Fennoscandia and the North America (7000–7200 km).

We also sought to examine the presence of a common "core snow microbiome," that is, taxa (OTUs) that are prevalent in snows generally—or alternatively—whether or not community membership is highly location dependent. To quantify the core snow microbiome, we compiled list of OTUs that are present in all or all but one sample. Additionally, to investigate if some taxa are locally abundant, we performed indicator

species analyses in PC-ORD (*following* [66]) based on 9999 Monte Carlo simulations for each algal, bacterial, and fungal OTU with transect identities as the demarcated grouping factor.

Procrustean Association Metric

To investigate if fungal, bacterial, and algal communities respond similarly to latitudinal changes, we implemented a Procrustean Association Metric (PAM) approach (*following* [67]). This PAM approach performs pairwise comparisons for each targeted taxon group's community responses with latitude to elucidate if taxon groups respond similarly or differently across latitudinal gradients. Briefly, the first four Nonmetric Multidimensional Scaling (NMDS) axes for each group were fitted to each other (Bacteria vs. Fungi, Bacteria vs. Algae, and Fungi vs. Algae) using Procrustes analyses (999 permutations) and the residual vectors calculated (PAM; NMDS and PAM conducted in the R (v.3.3.3) package *vegan,* [68]) between matching samples (e.g., Bacteria vs Fungi for sampling location one). These data were used in regression analyses against latitude (log transformed). The resulting regression slopes, if significant, indicate that the pair of tested groups respond differently across the latitudinal gradient.

Results

Community Composition

Bacteria Snow-borne bacterial communities were diverse and included a total of 2979 OTUs assigned to 22 phyla. Of these 22 phyla, Proteobacteria were the most abundant both in terms of OTU and sequence counts (32.4% of OTUs and 48.3% of all sequences) followed by Actinobacteria (12.7% of OTUs and 7.1% of sequences) and Bacteroidetes (11.2% of OTUs and 27.8% of sequences). Additional phyla that were well represented included Acidobacteria (8.5% of OTUs and 2.7% of sequences), Firmicutes (6.0% of OTUs and 3.1% of sequences), and Planctomycetes (4.4% OTUs but only 0.5% of sequences). Additionally, a large proportion of OTUs remained unclassified at a level below Domain (15.1% of OTUs and 8.3% of sequences). In all, a total of 51 bacterial classes were identified, but just four classes represented nearly 75% of all sequences in the sampled snows: Sphingobacteria (Bacteroidetes), Betaproteobacteria, Gammaproteobacteria, and Alphaproteobacteria made up 26.5%, 17.4%, 17.0%, and 13.4% of the total sequence count, respectively.

Fungi The Ascomycete snow fungi were the most species rich (greatest number of OTUs), whereas the phylum Basidiomycota was assigned the greatest number of sequences. Of the 358 fungal OTUs, 205 OTU were assigned to Ascomycota (57.3%). However, only 15.7% of the sequences were assigned to Ascomycota, whereas Basidiomycota (105 OTUs—29.3%) accounted for 79.4% of all fungal sequences. Other fungi were infrequent: Chytridiomycota (11 OTUs—3.07% and 1.45% of sequences), Monoblepharidomycota (4 OTUs—1.12% and 0.20% of sequences), and other early diverging lineages (Mucorales; 2 OTUs—0.56% and 0.08% of sequences). Further, a small number of OTUs (a total of 31) could not be reliably classified below Kingdom (8.7% of OTUs and 3.2% of sequences). Similar to other snow surveys [11], snow fungi largely consisted of basidiomycetous yeasts (particularly the genus *Rhodotorula* [OTU1] that also represented more than 43% of all sequences in this study).

Algae We were able to delineate 23 algal OTUs (phylum Chlorophyta). Of these 23, a total of 16 OTUs were assigned to the Class Trebouxiophyceae (69.6% of OTUs but only 13.1% of all obtained sequences) and 7 to the Class Chlorophyceae (30.4% of OTUs but 86.9% of sequences). Of the algal OTUs, 17 could be assigned to putative genera (bit score > 300): *Trebouxia* (13 OTUs—56.5% of OTUs and 10.2% of the sequences), *Chlamydomonas* (2 OTUs—8.6%, 85.8% of sequences). Additional genera with only one assigned OTU were *Asterochloris* and *Stichococcus*. *Asterochloris* represented 2.8% of all sequences, whereas *Stichococcus* represented only a few sequences. See Tables S2 and S3 for full breakdown of taxonomic identities of Bacteria, Fungi, and Algae.

Richness, Diversity, and Evenness

Generally speaking, the prokaryote and eukaryote diversity did not change across the latitudinal gradient (regression of Fennoscandian samples) or when the transect was treated as a categorical variable (ANOVA). Observed bacterial richness did not change with latitude in Fennoscandia ($t = -1.14$, $P = 0.283$) in regression analyses but decreased with latitude when transects were treated as a categorical variable (Colorado and Fennoscandian samples), ($F_{3,11} = 4.061$, $P = 0.036$) with Tukey's HSD post hoc test indicating that average Colorado bacterial iterative subsampled richness estimates (391.21) were lower than the northernmost Fennoscandian samples (891.39) (Fig. S1). Other pairwise comparisons indicated no differences. Diversity (1-D) and evenness (E_D) did not change with latitude using either regression analyses (Fennoscandia: $t = -0.43$, $P = 0.678$; Global: $F_{3,11} = 0.169$, $P = 0.915$) or ANOVAs (Fennoscandia: $t = 0.73$, $P = 0.479$; Global: $F_{3,11} = 0.169$, $P = 0.914$).

Similarly to bacteria, observed fungal richness did not change with latitude in either regression analyses ($t = 0.79$, $P = 0.447$) or ANOVAs ($F_{3,10} = 2.787$, $P = 0.096$)—nor did

the complement of Simpson's diversity (Regression—t = 1.61, P = 0.141; ANOVAs—$F_{3,10}$ = 0.436, P = 0.732). Similarly, fungal evenness did not change with latitude (t = 1.61, P = 0.141) or when transects were categorical ($F_{3,10}$ = 0.960, P = 0.449). The results of algal community analyses were consistent with those of fungi. Algal richness did not change with latitude using regression analyses (t = 1.07, P = 3.33) or when transects were categorical ($F_{3,10}$ = 2.679, P = 0.104). Further, algal diversity and evenness were indifferent to latitudinal changes in both regression analyses (t = 0.52, P = 0.618, t = 0.66, P = 0.523, respectively) and ANOVAs ($F_{3,10}$ = 0.993, P = 0.435, $F_{3,10}$ = 1.555, P = 0.261).

Community Shifts and Biogeographic Structure

Regression analyses and Mantel tests of Bray-Curtis community dissimilarities against geographic distance indicated that fungal communities in Fennoscandia are more dissimilar (Fig. 1) among samples that are further apart (t = 2.45, P = 0.017 and r = 0.306, P = 0.033, respectively), whereas bacteria and algae showed no evidence of biogeographic structuring within Fennoscandian snows (see Table 2). Global snow microbial community analyses revealed some interesting patterns. ANOVAs of the pairwise Bray-Curtis dissimilarities using categorical pairwise distances (based on within, adjacent, or distant transect occupancy) and MRPP analyses indicate (Fig. 2) that bacterial and fungal communities do differ between Fennoscandia and Colorado (ANOVAs: $F_{3,104}$ = 18.687, P < 0.001 and $F_{3,90}$ = 4.581, P = 0.005, respectively; MRPP: T = − 3.897, P < 0.001 and T = − 3.033, P = 0.004). In

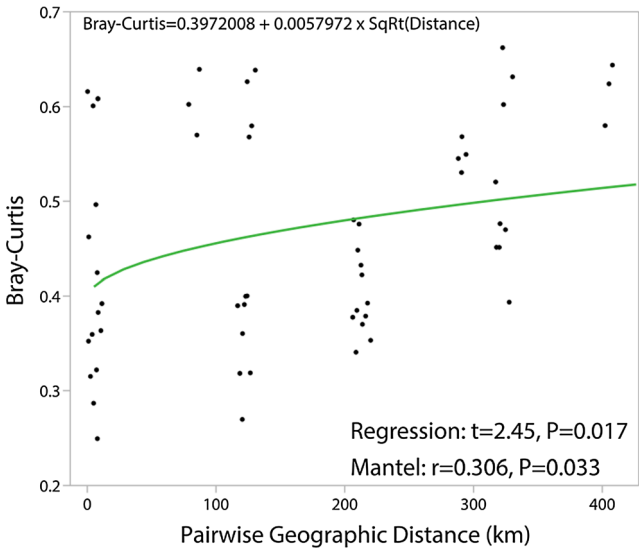

Fig. 1 Regression analysis and Mantel test of pairwise geographic distance (km) against iterative Bray-Curtis dissimilarity values for fungi across Fennoscandia. Both the regression analysis and Mantel test indicate that the further apart two samples are from each other, the more dissimilar the fungal communities are. Geographic distance was square root transformed prior to regression analysis

contrast to bacteria and fungi, algae did not differ globally in either ANOVAs of the pairwise Bray-Curtis dissimilarities or MRPP analyses (see Table 3).

Core Snow Microbiome

Core microbial taxon (defined as an OTU present in all or all but one sample) occurrence differed among the targeted groups of organisms (*see* Table S4 for breakdown of core OTUs). Algae had the greatest proportion of core taxa (13.0% of all algal OTUs) and these core taxa encompassed 92.2% of all algal sequences (the two most abundant algal core OTUs were unsurprisingly the commonly described snow algae—*Chlamydomonas* cf. *nivalis*). This dominance of algal core taxa between Fennoscandia and Colorado reaffirms previous findings that snow algae communities are highly specialized, almost identical, and cosmopolitan [47]. Fungal core taxa made up 12.9% of all fungal OTUs (dominated by genera *Rhodotorula, Cryptococcus, Kabatiella,* and *Sydowia)* whereas bacterial core OTUs only included 3.0% of bacterial OTUs (dominated by genera *Solitalea, Pseudomonas, Collimonas,* and *Ferruginibacter*). Unsurprisingly, most of the core microbial taxa were also the most abundant OTUs.

Locally Abundant Taxa

Indicator taxon analyses identified numerous OTUs that were enriched or locally abundant in a particular sampling location (transects). Similar to the core taxon analyses, the indicator analyses revealed differences among the analyzed target groups. In all, 8.7% of algal OTUs (two OTUs in total) were indicators for a sampling location compared to 6.4% of bacterial and 20.1% of fungal OTUs. The algae indicators were rare, only representing ~3% total algal sequence counts, supporting high OTU cosmopolitanism but does indicate low levels of endemism. In contrast, the bacterial and fungal indicators contained many abundant taxa (Table S5). Interestingly, the majority of fungal indicator taxa and all algal indicator taxa were indicative of the North American transect, whereas—although there were many bacterial indicators for the North American transect—the majority of bacterial indicators were sampled from the northernmost Fennoscandian transect (*see* Table 4 and Table S5 for complete indicator taxon test statistics).

Procrustean Association Metric

Results of the Procrustean Association Metric (PAM) regressions against latitude indicated that snow bacterial communities follow fundamentally distinct latitudinal dynamics from those of fungi (t = − 2.24, P = 0.044) and algae (t = − 3.13, P = 0.008). In a nutshell, northern bacteria behaved more

Table 2 Snow-borne microbial communities within Fennoscandian samples demonstrate fungal communities exhibit strong geographic structuring based on regression analyses and Mantel tests of Bray-Curtis dissimilarity values against pairwise geographic distances. Regression analyses based on square root transformation of geographic distance and Mantel tests are passed on Pearson's correlation coefficients. Significant tests are in italics

	Algae	Bacteria	Fungi
Regression	$t = 0.59$, $P = 0.557$	$t = 0.04$, $P = 0.969$	*$t = 2.45$, $P = 0.017$*
Mantel	$r = 0.024$, $P = 0.281$	$r = -0.013$, $P = 0.438$	*$r = 0.306$, $P = 0.033$*

similarly to northern fungi and algae, but this similarity diverged with decreasing latitude leading to significantly positive slopes (Fig. S2). In contrast, fungal and algal communities responded similarly with latitude ($P = 0.108$).

Discussion

Here, we present the first broad cross-kingdom/domain examination of snow-borne microbial communities across a latitudinal gradient. By doing so, we have demonstrated interesting ecological patterns and expanded our understanding of global microbial distributions highlighting contrasts between microbial groups. One of the most striking results is the apparent size dependency of microbial distributional patterns in snow between fungi and bacteria. Within Fennoscandia, there is a disconnect between bacterial and fungal community distributions where bacterial communities are indistinguishable across this latitudinal gradient, whereas fungal communities change with latitude (Table 2). This fungus-bacteria disconnect follows the expected distance-decay relationship dichotomy based on dispersal propagule size: bacteria—that are much smaller than fungi on average—are less likely to have dispersal barriers and therefore their communities have minimal regional community heterogeneity. Fungi, in contrast, have larger propagules, are more dispersal limited, and their communities thus more heterogeneous across the Fennoscandian snows. Interestingly, this size dependency of community composition within Fennoscandia whereby smaller organisms (propagules) are cosmopolitan poorly applies to the algal communities. Algae, with propagules on average much larger than fungi, were nevertheless cosmopolitan (Fig. 2). Further, in contrast to fungi and bacteria, we observed no indicator species of

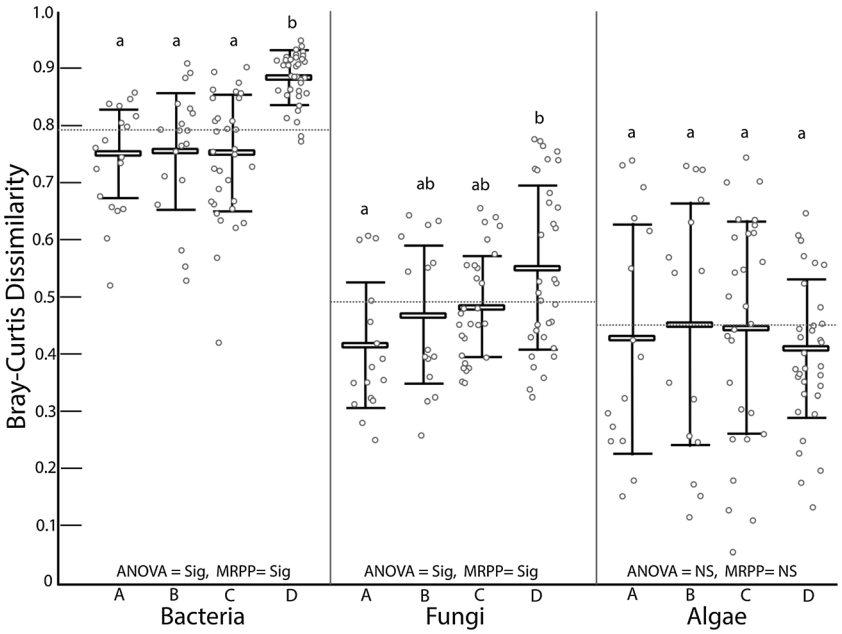

Fig. 2 Pairwise Bray-Curtis dissimilarity values for snow-borne bacterial, fungal, and algal communities across geographic transect categories [A: samples within the same transect (range 0–15 km); B: comparing samples in adjacent samples within Fennoscandia (1 vs. 2; 2 vs. 3; range 70–140 km); C: comparing samples between the farthest samples within Fennoscandia (1 vs 3; range 200–410 km); D: comparing inter-continental samples (Colorado vs. Fennoscandia; range 7000–7200 km)]. Both ANOVA and MRPP analyses (Table 3) indicate that the further away two sample are, the more dissimilar the communities are for Bacteria and Fungi, but Algal communities are consistently similar across all samples. Horizontal dashed lines represent mean Bray-Curtis dissimilarity values for each group

Table 3 Global snow microbial analyses show bacterial and fungal communities differ globally whereas algal communities are strikingly consistent between Fennoscandian and North American samples. ANOVA and MRPP tests were conducted on pairwise Bray-Curtis dissimilarities across transects (three in Scandinavian and one in North America). Significant tests are in italics

	Algae	Bacteria	Fungi
ANOVA	$F_{3,90} = 0.428$, $P = 0.733$	*$F_{3,104} = 18.687$, $P < 0.001$*	*$F_{3,90} = 4.581$, $P = 0.005$*
MRPP	$T = 0.227$, $P = 0.546$	*$T = -3.897$, $P < 0.001$*	*$T = -3.033$, $P = 0.004$*

algae within any of the Fennoscandian transects (Table 4) although some existed when the North American and Fennoscandian samples were compared. We cannot discount other factors that may be contributing to Fennoscandian microbial distributions, including average wind velocity or differential nutrient deposition rates, which were not measured. However, our Fennoscandian samples were all collected from similar landscapes with similar topological attributes. As a result, we find that across this relatively short latitudinal gradient, inherent organismal dispersal limitations are the most likely drivers of these patterns for fungi and bacteria. However, given algal deviations from this expected propagule size-distance decay, it seem that this is ill-suited to explain dispersal patterns for snow algae or very specialized organisms within snow.

When inter-continental communities were considered (Fennoscandia vs western USA), algae were cosmopolitan (communities were indistinguishable [Table 3]) and were remarkably consistent wherever snows were collected. In contrast, both fungal and bacterial communities differed compositionally between these Northern Hemisphere snows. This is most likely due to the vast distance between Fennoscandia and Colorado but elevational differences between these two regions and sun exposure angle may also be important: the Colorado sites are ~ 3000 m higher than Fennoscandian samples. Although our initial work hints toward global patterning, additional research is needed to confirm inter-continental microbial distributional patterns.

The observed algal OTU cosmopolitism is interesting as it suggests that snow-inhabiting algae are extreme specialists with little evidence for endemism. What endemism we did observe (two OTUs or ~ 8% of the 23 OTUs that we observed were indicative of North American snows) was from OTUs that had low sequence abundances; this rare endemism rate is similar to those previously described in "red snow" [32]. Previous studies have proposed that highly specialized taxa may be more likely to be cosmopolitan [40, 41]. However, it has also been suggested that generalist microbes in highly specialized environments (e.g., Porifera mutualists) show global cosmopolitanism [69]. It is uncertain if snow algae are cosmopolitan because they are specialists, or because their habitat is specialized, nonetheless they appear cosmopolitan in our survey. Despite ubiquitous snow algae distributions, finer-scale genetic (strain) or ecological variability may yet to be determined. Some recent studies suggest that distinct snow algal haplotypes or oligotypes may constitute undiscovered functional attributes [47, 70]. Further research is necessary to better understand underlying population ecology and community functionality.

One of the most striking results of this research is that snow algae are seemingly omnipresent in "white snows" that are void of visible algal blooms. These algae likely are present in low abundances and when environmental or nutritional conditions become favorable, algae bloom. Previous work has hinted that algae may be present in "white snows" but were assumed to be "functionally absent" [11, 26]. However, given our abundant sequence representation of algae in white snows, it seems unlikely that these algae are metabolically inactive and nonfunctional. Instead, our data suggest that algae may be a much larger and more important component in "white snow" than previously realized. It is crucial to note that we present no evidence that these observed algae are metabolically active; measures of primary productivity and/or transcript load of algae would do much to address this issue but remained beyond the scope of this current study. It is important to stress that there is a multitude of reasons why snow algae might be abundant in sequencing data but may not be so in the environment. For example, copy number

Table 4 Percentage of OTUs identified as indicator taxa with total number of OTUs in square brackets. Indicator taxa analyses show strong taxon differences with fungal indicator taxa consisting of a much greater proportion of total fungal OTUs than either algae and bacteria

	Transect 1	Transect 2	Transect 3	Transect 4	Total indicator taxa
Algae	0.00% [0]	0.00% [0]	0.00% [0]	8.69% [2]	8.69% [2]
Bacteria	3.59% [107]	0.54% [16]	0.50% [15]	1.64% [49]	6.39% [187]
Fungi	1.39% [5]	4.47% [16]	0.84% [3]	13.42% [48]	20.11% [72]

considerations in the rRNA gene repeat of snow algae or extreme primer biases may lead to overestimation of algal abundances. However, given the dearth of information about snow algae genomic architecture, we cannot currently assign these numerous sequences as spurious or inflated. Currently, we have no reason to doubt the veracity of the claim that snow algae are a previously unappreciated major component of late-season snows that are apparently "uncolonized." Nevertheless, more research is required to verify that algae are an abundant component in "uncolonized" snows.

Another striking result is the global distribution of common taxa. It is perhaps unsurprising that many taxa found in snow might be adapted to such an environment, but the high proportions of OTUs and sequence reads that belong to core taxa for fungi, bacteria, and algae are surprising. Overall, algae had the greatest proportion of core taxa (OTUs that were found in all or all but one sample) with ~13% of the total OTUs (3 OTUs), but these OTUs represented greater than 92% or all algal sequences obtained. These core algae (representative sequences had best BLASTn matches to *Chlamydomonas nivalis* [NCBI accession GU117577.1], originally sampled from the Austrian Alps [two core algae OTUs matched to this accession with 100% and 96% sequence similarities] and to *Trebouxia jamesii* [NCBI accession GQ375322.1], isolated from Antarctica with 100% query coverage and sequence similarity) highlight two main points of discussion: (1) *common snow algae are extremely conserved globally with nearly identical ITS2 sequences found across vast distances and across years*, and (2) *we know very little about the global genetic diversity or dispersal patterns of these snow algae*. It should be emphasized, however, that there is a dearth of representatives for snow algae in the combined global sequence data repositories, so identifications based on BLASTn affinities should be met with reasonable skepticism, unless sequences are near identical. Future work should focus on genetic and geographic structure of snow algae. Fungal core taxa represent 12.9% of all fungal OTUs but represents 89.3% of all fungal sequences. While most core fungi could not be identified reliably at taxonomic levels below Family, many were identified to Genus, including *Rhodotorula* and *Kabatiella* among others. *Rhodoturula* is a Basidiomycetous yeast and previously described as a major fungal component of algae-colonized snows [11]. *Kabatiella* is a microfungus whose con-generics are often endophytic or pathogenic in a variety of plants [52]. These taxa suggest that snow-borne fungi may be more likely to have diminutive organismal vegetative states (yeast-like or micro-conidial states) presumably due to the substrate (snow) being nutrient poor [71]. These nival fungi may exist wholly in the snow matrix and are not to be confused with "snow molds" that have extensive mycelial networks at the snow-soil interface [72]. It is unknown at this point what are the primary nutritional requirements for these nival fungi. It may be that they opportunistically utilize allochthonous deposits of organic matter such as pollen or invertebrate carcasses; or parasitize/utilize other snow-inhabiting organisms such as algae or are decomposers of bacteria. While random deposition of allochthonous resources has been suggested to drive early successional community assembly [57, 73], it seems unlikely that these nival organisms would have adapted to this unique nutrient poor environment if subsisting solely on random deposits. Rather, they may exhibit a nutrient syntropy feedback dynamic [11] or have a yet unknown organotrophic nutritional capabilities (as has been documented for some algae [74] and intimated as a potential for snow algae [75, 76]). It is conceivable that random deposition is important to kick-start a positive feedback loop facilitating future autochthonous inputs (a declining allochthonous/autochthonous input ratio over time). Further investigation is required to elucidate what adaptations these snow fungi have that allows for not just survival but thriving in these harsh environments. Common psychrophilic strategies to maintain metabolic activity include a responsive increase of cellular pools of ATP and ADP with colder temperatures [77] to compensate for slower enzymatic kinetics. Additionally, these organisms must survive repeated freeze-thaw cycles, often aided by anti-freeze protein (AFP) generation [78]. How these snow fungi survive, "make a living," and reproduce is not understood, but they likely possess similar adaptive mechanisms.

Bacteria had the fewest numbers of core taxa (3.0%) but these few OTUs represented over 70% all obtained sequences. While the core bacterial taxa are taxonomically diverse, several of the most abundant core taxa are best placed into ecologically interesting groups including the Sphingobacteriaceae (potential autotrophs and degraders of aromatic compounds [79]), the Burkholderiales (including may member of Oxalobacteraceae which may utilize oxalic acid as a carbon source [80]), the Chitinophagaceae which potentially hydrolyze chitin and cellulose [81], the Rhizobiaceae (which with the Burkholderiales likely fix atmospheric nitrogen [82]), and *Albidiferax* which may reduce iron [83]. While these ecological roles cannot be substantiated using only amplicon sequence data, they hint a complex network of autotrophic, chemoheterotrophic, and diazotrophic lifestyles which may facilitate community persistence. Further, one wonders if the underlying cause for fewer bacterial taxa being core OTUs than either fungi or algae is due to the apparent ease at which bacterial disperse which allows more bacterial taxa to be found on distinct snow patches. Alternatively, it may be that bacteria are more generalists and less adapted for in these cryospheric ecosystems or this is a direct result of a more diverse prokaryotic community which directly lowers the probability of capturing the same community member using sequencing technologies compared to the less-diverse Eukaryotes given the same sequencing effort. Given the limited scope of our experimental design, more work is needed to fully disentangle causes of bacterial distributional ecology.

Previous work has suggested that fungi belonging to the phylum Chytridiomycota constitute a remarkable but lesser component of the fungal community [10, 11] in algae-colonized snow. It has been suggested that these snow chytrids, some of which are enriched in snow algae-colonized patches, may opportunistically or deterministically utilize algae or their exudates, perhaps in loose syntrophic or more defined parasitic associations. Here, we demonstrate that these snow chytrids are seen in appreciable but lesser abundances in "white snows" than in "red snow" (4.2% OTU representation in this study compared to 13% OTU representation in [11]) suggesting that snow chytrids are not fully reliant on algal blooms. Interestingly, these snow chytrids (*similar to* [11]) are *extremely* dissimilar in their ITS2 sequences to any accession in the global nucleotide repositories making elucidation of detailed taxonomic placement or ecologies difficult. Previously, using 18S data, Naff et al. [10] placed snow-inhabiting chytrids associated with algae to a sister clade to the Lobulomycetales, later placed into the new order Mesochytriales by Karpov et al. [84]. It is uncertain if our chytrids fall into the Mesochytriales, but it is very likely. Nevertheless, the paucity of information about these novel chytrids is unfortunate and additional investigations into the life histories and ecological roles of these chytrids are warranted.

The spatial bacterial community dynamics differ from either fungi or algae across latitude. This is evidenced by the differences in community shifts (or lack thereof) across Fennoscandia (Table 2) and when compared to Colorado samples (Table 3), as well as the observation that a majority of indicator bacterial OTUs were locally enriched in the northernmost samples (Table 4). In contrast, indicator taxon numbers increase in more southern samples for both fungal and algae communities. The Procrustean Association Metric (PAM) analysis best exemplifies the difference between bacteria and their Eukaryotic microbial co-inhabitants (Fig. S2). The PAM measures the similarity of two separate ordination loading scores from the same sample (here bacteria vs. fungi, bacteria vs. algae, and fungi vs. algae) after Procrustes fitting and allows a powerful way to test if two datasets behave similarly across an experimental framework. Here, the average PAM estimators correlated positively with decreasing latitude when comparing bacteria to either fungi or algae, whereas for fungi and algae, the regression slope did not differ from zero. This increase in PAM (more dissimilar) with decreasing latitude suggests that far north locations may constrain bacterial communities more, either by a reduced propagule pool or more stringent environmental filtering, which may partially explain the numerous indicator taxa in the northernmost transect. It is uncertain, however, why bacteria respond in fundamentally differently from eukaryotes, but distributional differences have been observed between bacteria and snow algae previously [9]. It may be that bacteria follow different distributional rules due to their diminutive size whereby smaller organisms are more cosmopolitan and our bacterial community analysis is corroborating this ubiquity; further size fractionation of bacteria prior to sequencing and analyses may provide support for this size-dependency hypothesis if larger bacteria are less cosmopolitan. Whatever drives this domain-specific patterning across global snows, one thing remains clear: snow microbiology remains a vast, interesting, but largely undiscovered territory for understanding biodiversity.

Acknowledgements We express great thanks to Francesco Gentili (SLU Umeå) for assistance in sample processing, and to Mary Brown, Vera Brown, and Lyndon Brown for assistance in collection of North American samples. We also greatly thank Unto Jumpponen for providing transportation logistical support, and Rauni Strömmer and Martin Romanchuk (University of Helsinki-Lahti) for use of laboratory space. This work was partially funded through an American-Scandinavian Fellowship (SPB), a Kansas Academy of Sciences grant (SPB), and a US Department of Education Graduate Assistance in Areas of National Need (GAANN) training program.

References

1. Baas Becking LGM (1934) Geobiologie of inleading tot de milieukunde. WP Van Stockum & Zoon, The Hague
2. De Wit R, Bouvier T (2006) 'Everything is everywhere, but, the environment selects'; what did Bass Becking and Beijerinck really say? Environ Microbiol 8:755–758
3. Christner BC, Morris CE, Foreman CM, Dai R, Sands DC (2008) Ubiquity of biological ices nucleators in snowfall. Science 319:1214
4. Maki LR, Galyan EL, Chang-Chien MM, Caldwell DR (1974) Ice nucleation induced by *Pheudomonas syringae*. Appl Environ Microbiol 28:456–459
5. Fujii M, Takano Y, Kojima H, Hoshino T, Tanaka R, Fukui M (2010) Microbial community structure, pigment composition, and nitrogen source of red snow in Antarctica. Microb Ecol 59:466–475
6. Remias D, Karsten U, Lütz C, Leya T (2010) Physiological and morphological processes in the alpine snow alga *Chloromonas nivalis* (Chlorophyceae) during cyst formation. Protoplasma 243: 73–86
7. Müller T, Bleiß W, Martin CD, Rogaschewski S, Fuhr G (1998) Snow algae from northwest Svalbard: their identification, distribution, pigment and nutrient content. Polar Biol 20:14–32
8. Hodson A, Anesio AM, Tranter M, Fountain A, Osborn M, Priscu J, Laybourn-Parry J, Sattler B (2008) Glacier ecosystems. Ecol Monogr 78:41–67
9. Lutz S, Anesio AM, Raiswell R, Edwards A, Newton RJ, Gill F, Benning LG (2016) The biogeography of red snow microbiome and their role in melting arctic glaciers. Nat Commun 7:119687. https://doi.org/10.1038/ncomms11968
10. Naff CS, Darcy JL, Schmidt SK (2013) Phylogeny and biogeography of an uncultured clade of snow chytrids. Environ Microbiol 15: 2672–2680
11. Brown SP, Olson BJSC, Jumpponen A (2015) Fungi and algae co-occur in snow: an issue of shared habitat or algal facilitation of heterotrophs? Arct Antarct Alp Res 47:729–749
12. Whalley JI, Pliny (1982) Pliny the Elder, Historia naturales, Book XI. Victoria and Albert Museum, London, UK.

13. Bauer F (1820) Some experiments on the fungi which constitute the colouring matter of the red snow discovered in Baffin's bay. Phil Trans R Soc Lond 110:165–173
14. Clark FC (1875) Red snow. Am Nat 9:129–135
15. Hersey H (1913) Our friends and our foes of the invisible world: microbes, good and bad. Thomas Y. Crowell Company, New York
16. McLean AL (1918) Bacteria of ice and snow in Antarctica. Nature 102:35–39
17. Darcy JL, Lyncy RC, King AJ, Robeson MS, Schmidt SK (2011) Global distribution of *Polaromonas* phylotypes—evidence for a highly successful dispersal capacity. PLoS One 6: e23742. https://doi.org/10.1371/journal.pone.0023742
18. Yan P, Hou S, Chen T, Ma X, Zhang S (2012) Culturable bacterial isolated from snow cores along the 1300 km traverse from Zhongshan Station to Dome A, East Antarctica. Extremophiles 16:345–354
19. Larose C, Dommergue A, Vogel TM (2013) The dynamic Arctic snow pack: an unexplored environment for microbial diversity and activity. Biology 2:317–330
20. Proctor VW (1959) Dispersal of fresh-water algae by migratory water birds. Science 130:623–624
21. Schlichting HE (1960) The role of waterfowl in the dispersal of algae. Trans Am Microsc Soc 79:160–166
22. Revill DL, Stewart KW, Schlichting Jr HE (1967) Passive dispersal of viable algae and protozoa by certain craneflies and midges. Ecology 48:1023–1027
23. Kristiansen J (1996) 16. Dispersal of freshwater algae—a review. Hydrobiologia 336:151–157
24. Novis PM (2001) Ecology and taxonomy of alpine algae, Mt. Philistine, Arthur's Pass National Park, New Zealand. Dissertation, University of Canterbury, Christchurch, NZ
25. Marshall WA, Chalmers MO (2006) Airborne dispersal of Antarctic terrestrial algae and cyanobacteria. Ecography 20:585–594
26. Harding T, Jungblut AS, Lovejoy C, Vincent WF (2011) Microbes in High Arctic snow and implications for the cold biosphere. Appl Environ Microbiol 77:3234–3243
27. Schneider RW, Hollier CA, Whitam HK et al (2005) First report of soybean rust caused by *Phakopsora pachyrhizi* in the continental United States. Plant Dis 89(774336):151–157
28. Duval B, Duval E, Hoham RW (1999) Snow algae of the Sierra Nevada, Spain and High Atlas Mountains of Morocco. Int Microbiol 2:39–42
29. Thomas WH, Broady PA (1997) Distribution of coloured snow and associated algal genera in New Zealand. N Z J Bot 35:113–117
30. Novis PM, Beer T, Vallance (2008) New records of microalgae from the New Zealand alpine zone, and their distribution and dispersal. N Z J Bot 46:347–366
31. Novis PM, Visnovsky G (2012) Novel alpine algae from New Zealand: Chlorophyta. Phytotaxa 39:1–30
32. Segawa T, Matsuzaki R, Takeuchi N, Akiyoshi A, Navarro F, Sugiyama S, Yonezawa T, Mori H (2018) Bipolar dispersal of red-snow algae. Nat Commun 9:3094
33. Jenkins DG, Brescacin CR, Duxbury CV, Elliott JA, Evans JA, Grablow KR, Hillegass M, Lyon BN, Metzger GA, Olandese ML, Pepe D, Silvers GA, Suresch HN, Thompson TN, Trexler CM, Williams GE, Williams NC, Williams SE (2007) Does size matter for dispersal distance? Glob Ecol Biogeogr 16:415–425
34. Wilkinson DM, Koumoutsaris S, Mitchell EAD, Bey I (2012) Modeling the effect of size on the aerial dispersal of microorganisms. J Biogeogr 39:89–97
35. Finlay BJ (2002) Global dispersal of free-living microbial eukaryote species. Science 296:1061–1063
36. Roberts MS, Cohan FM (1995) Recombination and migration rates in natural populations of *Bacillus subtilis* and *Bacillus mojavensis*. Evolution 49:1981–1094
37. Sato K, Tsujino R, Kurita K, Yokoyama K, Agata K (2012) Modeling the global distribution of fungal species: new insights into microbial cosmopolitanism. Mol Ecol 21:5599–1612
38. Peay KG, Schubert MG, Nguyen NH, Bruns TD (2012) Measuring ectomycorrhizal dispersal: maroecological patterns driven by microscopic propagules. Mol Ecol 21:4122–4136
39. Peay KG, Bruns TD (2014) Spore dispersal of basidiomycetes fungi at the landscape scale is driven my stochastic and deterministic processes and generates variability in plant-fungal interactions. New Phytol 204:180–191
40. Brown JKM, Hovmeller MS (2002) Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science 287:537–541
41. Davison J, Moora M, Öpik M et al (2015) Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. Science 349:970–973
42. Nemergut DR, Costello EK, Hamandy M et al (2010) Global patterns in the biogeography of bacterial taxa. Environ Microbiol 13: 135–144
43. Sul WJ, Oliver TA, Ducklow HW, Amaral-Zettler LA, Sogin ML (2013) Marine bacteria exhibit a bipolar distribution. Proc Natl Acad Sci USA 110:2342–2347
44. Burrows SM, Butler T, Jöckel P, Tost H, Kerkweg A, Pöschl U, Lawrence MG (2009) Bacteria in the global atmosphere—part 2: modeling of emissions and transport between different ecosystems. Atmos Chem Phys 9:9281–9297
45. Burrows SM, Elbert W, Lawrence MG, Pöschl U (2009) Bacteria in the global atmosphere—part 1: review and synthesis of literature data for different ecosystems. Atmos Chem Phys 9:9263–9280
46. Schmidt SK, Lynch RC, King AJ, Karki D, Robeson MS, Nagy L, Williams MW, Mitter MS, Freeman KR (2011) Phylogeography of microbial phototrophs in the dry valleys of the high Himalayas and Antarctica. Proc R Soc B 278:702–708
47. Brown SP, Ungerer MC, Jumpponen A (2016) A community of clones: snow algae are diverse communities of spatially structured clones. Int J Plant Sci 177:432–439
48. Fuhrman JA, Steele JA, Hewson I, Schwalbach MS, Brown MV, Green JL, Brown JH (2008) A latitudinal diversity gradient in planktonic marine bacteria. Proc Natl Acad Sci USA 105:7774–7778
49. Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci USA 103:626–631
50. Yergeau E, Bokhorst S, Huiskes AHL et al (2007) Size and structure of bacterial, fungal and nematode communities along an Antarctic environmental gradient. FEMS Microbiol Ecol 59:436–451
51. Tedersoo L, Nara K (2010) General latitudinal gradient of biodiversity is reversed in ectomycorrhizal fungi. New Phytol 185:351–354
52. Tedersoo L, Bahram M, Põlme S et al (2014) Global diversity and geography of soil fungi. Science 346:1256688
53. Richter-Heitmann T, Eickhorst T, Knauth S, Friedrich MW, Schmidt H (2016) Evaluation of strategies to separate root-associated microbial communities: a crucial choice in rhizobiome research. Front Microbiol 7:773. https://doi.org/10.3389/fmicb.2016.00773
54. White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand NA, Sininsky JJ, White FR (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, pp 315–322
55. Ihrmark K, Bödeker ITM, Cruz-Martinez K (2012) New primers to amplify the fungal ITS2 region—evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol Ecol 82:666–677
56. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R (2012) Ultra-high-throughput

microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621–1624
57. Brown SP, Jumpponen A (2014) Contrasting primary successional trajectories of fungi and bacteria in retreating glacier soils. Mol Ecol 23:481–497
58. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541
59. Huse SM, Welch DM, Morrison HG, Sogin ML (2010) Ironing out the wrinkles in the rare biosphere through improved OTU clustering. Environ Microbiol 12:1889–1898
60. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200
61. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267
62. Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiss M, Larsson KH (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22:5271–5277
63. Brown SP, Veach AM, Rigdon-Huss AR et al (2015) Scraping the bottom of the barrel: are rare high throughput sequences artifacts? Funct Ecol 13:221–225
64. Oliver AK, Brown SP, Callaham MA, Jumpponen A (2015) Polymerase matters: non-proofreading enzymes inflate fungal community richness estimates by up to 15%. Funct Ecol 15:86–89
65. McCune B, Mefford JM (1999) PC-ORD: multivariate analysis of ecological data. Version 4. MjM Software, Gleneden Beach
66. Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol Monogr 67:345–366
67. Lisboa FJG, Peres-Nets PR, Chaer GM et al (2014) Much beyond Mantel: bringing Procrustes association metric to the plant and soil ecologist's toolbox. PLoS One 9:e101238. https://doi.org/10.1371/journal.pone.0101238
68. Oksanen J, Blanchet FG, Friendly M et al (2017) Vegan: community ecology package. R Package version 2.4–2. https://CRAN.R-project.org/package=vegan
69. Thomas T, Moitinho-Silva L, Lurgi M, Björk JR, Easson C, Astudillo-García C, Olson JB, Erwin PM, López-Legentil S, Luter H, Chaves-Fonnegra A, Costa R, Schupp PJ, Steindler L, Erpenbeck D, Gilbert J, Knight R, Ackermann G, Victor Lopez J, Taylor MW, Thacker RW, Montoya JM, Hentschel U, Webster NS (2016) Diversity, structure, and convergent evolution of the global sponge microbiome. Nat Commun 7:11870
70. Lutz S, McCutcheon J, McQuaid JB, Benning LG (2018) The diversity of ice algae communities on the Greenland Ice Sheet as revealed by oligotyping. Microb Genom 4: e000159. https://doi.org/10.1099/mgen.0.000159
71. Hamilton TL, Havig J (2017) Primary productivity of snow algae communities on stratovolcanoes of the Pacific Northwest. Geobiology 15:280–295
72. Schmidt SK, Wilson KL, Meyer AF, Gebauer MM, King AJ (2008) Phylogeny and ecophysiology of opportunistic "snow molds" from a subalpine forest ecosystem. Microb Ecol 56:681–687
73. Hodkinson ID, Coulson SJ, Harrison J, Webb NR (2001) What a wonderful web they weave: spiders, nutrient capture and early ecosystem development in the high Arctic—some counter-intuitive ideas on community assembly. Oikos 95:349–352
74. Casselton PJ (1966) Chemo-organotrophic growth of xanthophycean algae. New Phytol 65:134–140
75. Telling J, Boyd ES, Jones EL, Tranter M, MacFarlane JW, Martin PG, Wadham JL, Lamarche-Gagnon G, Skidmore ML, Hamilton TL, Hill E, Jackson M, Hodgson DA (2015) Rock communities as a source of hydrogen for subglacial ecosystems. Nat Geosci 8:851–855
76. Havig JR, Hamilton TL (2019) Snow algae drive productivity and weathering at volcanic rock-hosted glaciers. Geochim Cosmochim Acta 247:220–242
77. Amato P, Christner BC (2009) Energy metabolism response to low-temperature and frozen conditions in *Psychrobacter cryohalolentis*. Appl Environ Microbiol 75:711–718
78. Hoshino T, Kiriaki M, Ohgiya S, Fujiwara M, Kondo H, Nishimiya Y, Yumoto I, Tsuda S (2003) Antifreeze proteins from snow mold fungi. Can J Bot 81:1175–1181
79. Lambiase A (2014) The family *Sphingobacteriaceae*. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The prokaryotes. Springer, Berlin, pp 907–914
80. Baldani JI, Rouws L, Cruz LM, Olivares FL, Schmid M, Hartmann A (2014) The family *Oxalobacteraceae*. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The prokaryotes. Springer, Berlin, pp 919–974
81. Rosenberg E (2014) The family Chitinophagaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The prokaryotes. Springer, Berlin, pp 493–495
82. Sawada H, Kuykendall LD, Young JM (2003) Changing concepts in the systematics of bacterial nitrogen-fixing legume symbionts. J Gen Appl Microbiol 49:155–179
83. Lu S, Chourey K, Reiche M, Nietzsche S, Shah MB, Neu TR, Hettich RL, Kusel K (2013) Insights into the structure and metabolic function of microbes that shape pelagic iron-rich aggregates (iron snow). Apply Environ Microbiol 79:4272–4281
84. Karpov SA, Kobseva A, Mamkaeva MA et al (2014) Gromochytriales and Mesochytriales (Chytridomycetes). Persoonia 32:115–126