When snows bloom: snow algae associated microbial communities are underpinned by trophic partitioning

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Abstract

Snow microbial communities (algae, bacteria, and fungi) play major roles in snow ecosystem processes and are linked to snowmelt dynamics, but patterns and mechanisms underpinning their spatial distributions, community assembly, and maintenance dynamics are poorly understood. Here we examine nival microbial communities and physicochemical measures across a semi-continental scale and across categorical snow algae bloom zones to elucidate interrelation between communities and their environment. Evidence suggests that trophic partitioning may be a major driver of snow community sub-networks. Samples from snows from the Cascade Mountains (USA) and the Rocky Mountains (USA) were collected from active red snow algae blooms from the center of the bloom (medial), from the edge of the bloom (peripheral), and in adjacent 'white' snow. Medial sections of snow algae blooms show increased levels of anemophilous bisaccate pollen, lower oxidation-reduction potential, decreased algal and increased bacterial richness, and increased levels of potassium. Fungal communities between the Cascade and Rocky Mountains are distinct but bacterial and algal communities show little intracontinental differentiation. Ecological modules were identified using a weighted gene co-expression analysis (WGCNA), which shows that dominant microbial consortia correlate differentially to environmental parameters, suggesting complex subcommunities drive observed ecological patterns. Individual OTU networks (fungi and bacteria) show high levels of network connectivity compared to networks based on the snow algae *Sanguina nivaloides*, which underscores associative differences between algal dominated networks and other OTU networks, indicative of trophic partitioning.

Introduction

Chlorophycean snow algae often form colorful and vivid blooms on the surface of late-season or perineal snows during summer snowfield ablation. Perhaps the most well-known snow algae is *Sanguina nivaloides* (previously assigned to *Chlamydomonas* cf. *nivalis*) (Procházková et al., 2019), which forms striking red blooms near the end of the growing season as the UV-protectant astaxanthin and fatty acid ester derivatives accumulate (Müller et al., 1998; Gorton & Vogelmann, 2003). While *Sanguina* life cycles are unresolved, leading hypotheses include a motile haploid vegetative cell stage that is active during spring or summer snow ablation as dissolved nutrients and gases become accessible (Stibal et al., 2007; Remias, 2012; Hoham & Duval, 2001; Hoham, 1980) followed by red cyst formation toward the end of the growing season. *Sanguina* is currently comprised of two known species, the red snow algae *S. nivaloides* and the orange-hued *S. aurantia*, which have diverging ranges, cell sizes, astaxanthin-to-chlorophyll-a ratios and fatty acid profiles (Procházková et al., 2020). Red snow algae have been a historical curiosity for centuries (Bauer, 1820; Gibbs, 1871; Clark, 1875; Aristotle, 1878; Darwin, 1878; Wille, 1903; Brown, 2015) and more recently, snow algae have been the focus of examinations on algal biodiversity (Procházková et al., 2019; Engstrom et al., 2020; Gray et al., 2020) and biogeochemistry (Lutz, 2015b; Havig and Hamilton, 2017; Lutz et al., 2017; Havig and Hamilton, 2019). Red snow algae blooms are also occupied by a broad array of diverse psychrophilic and psychrotolerant microbes including fungi, bacteria, and archaea (Lutz et al., 2015a; Brown et al., 2015a; Brown & Jumpponen, 2019; Hoham & Remias, 2020). While red snow algae blooms are known to be composed of diverse assemblages of microorganisms there is

68 currently a poor understanding of drivers of community assembly and dynamics within these
69 snows.

70 Red snow algae blooms are found globally in polar and alpine environments (Brown et
71 al., 2016; Segawa, 2018; Procházková et al., 2019; Hoham & Remias, 2020; Brown & Tucker,
72 2020). Over the course of the last century, *Sanguina nivaloides* has been documented in
73 numerous surveys and studies in western North America. *S. nivaloides* snow blooms are
74 characteristic of open snow fields above timberline (Engstrom et al., 2020), in contrast to other
75 snow algae such as *Chloromonas pichincha*, which are often observed in shaded snow patches
76 below timberline in and adjacent to forests (Procházková et al., 2018; Hoham, 1980). Snow algae
77 populations within blooms of *S. nivaloides* are not restricted to *Sanguina* species and can be
78 taxonomically diverse that presumably consist of clonal haplotypes of several algal species
79 (Brown et al., 2016). Other algal genera often observed in North American red snow algae
80 blooms include *Carteria sp.*, *Chloromonas sp.*, *Chodatella sp., Cryosistis sp.*, *Haematococcus*
81 *sp.*, *Raphidonema sp.* and *Trebouxia sp.* among others (Kol, 1941; Garric, 1965; Stein &
82 Amundsen, 1967; Sutton, 1972; Wharton & Vineyard, 1983; Hamilton & Havig, 2017). While
83 not visually apparent, 'white' snows with no apparent algal colonization have been demonstrated
84 to contain a multitude of diverse algae, including *Sanguina* spp. as well as other microbes
85 (Brown et al., 2015a; Brown & Jumpponen, 2019).

86 Microscopy, culture, and metabarcoding-based surveys indicate that fungi and bacteria
87 are common components of snow communities associated with snow algae blooms (Stein &
88 Amundsen, 1967; Hoham et al., 1993; Schmidt et al., 2012; Brown & Jumpponen, 2015; Brown
89 et al., 2015a; Lutz et al, 2016; Azzoni et al., 2018; Perini et al., 2019; Yakimovich et al., 2020).
90 In the Cascade and Rocky Mountains of the United States, diverse fungi belonging to disparate

lineages including Chytridiomycetes, Dothideomycetes, Eurotiomycetes, Microbotryomycetes, Monoblepharidomycetes are commonly found (all of which were observed in this study). This includes unique and divergent clades of snow chytrids that have been found in snows across the Western United States that may parasitize snow algae, but whose full taxonomy and functions are unresolved (Naff et al., 2013; Brown et al., 2015a). Additionally, Fungi such as *Chionaster nivalis*, *Chionaster bicornis* and *Selenotila nivalis*, all of which are currently lacking any genetic accessions, are common in snows across the globe and are primarily recorded in association with snow algae blooms (Kobayashi & Fukushima, 1952; Garric, 1965; Stein & Amundsen, 1967; Light & Belcher, 1968; Merchant, 1972; Hoham et al., 1983; Lukavský & Cepák, 2010). *S. nivaloides* have also been previously observed in close association with gram-negative bacteria (Weiss, 1983), but studies of direct symbioses have not been possible as *S. nivaloides* has as of yet resisted culturing efforts. However, synergistic effects between other algae and bacteria have been documented from algal cultures (Terashima et al., 2017) and from co-culturing experiments that demonstrate enhanced algal growth in the presence of snow bacteria isolates (Harrold et al., 2018; Krug et al., 2020).

Snow algae and associated microbial communities have been documented to have a broad and underestimated impact on global biogeochemical cycles (Havig & Hamilton, 2019; Williamson et al., 2019). Recent remote sensing studies have underscored the importance of snow algae as a carbon sink, although sparse respiration rate information may indicate only ephemeral sequestration (Gray et al., 2020). Further, snow algae have bioalbedo feedback effects that increase snow ablation rates (Ganey et al., 2017; Lutz et al., 2016). While many studies have examined algal, fungal and/or bacteria taxonomic composition of red snow algae blooms, it is less understood what temporal trophic patterns and environmental factors drive community

assembly of these microbes. Trophic profiles of snows, generally, are subject to punctuated and continuous shifts due to dry aeolian deposition, wet meteoric precipitation and primary productivity by endemic taxa (Hoham & Remias, 2020).

Red snow algae blooms typically occur from one to several weeks during spring and summer when air temperatures remain above 0°C (Kawecka, 1986) in semi-permanent or perineal snowfields located in temperate alpine and polar regions. Often they are seen occurring in higher density at the base of sun cups, depressions, or natural gullies in the snow surface (Thomas 1972). Estimates of nutrient release from recent snows suggest the first 30% of meltwater contains *ca.* 50-80% of the nutrients (Johannessen & Henriksen, 1978), suggesting that endogenous nutrient availability peaks early in the season as snows begin to ablate. Nutrient release may also be facilitated by saprotrophs that potentiate release through substrate breakdown of pollen, algae and other biologic material (Naff et al., 2013; Brown & Jumpponen, 2019) or dissolved organic matter, although it is not known if this release is concurrent with or proceeds bloom development. In some cases, snow algae growth can be induced *in situ* by applications of fertilizer (Ganey et al., 2017) and, for certain taxa, is thought to be inhibited under acidic conditions, such as those induced by prevailing winds from volcanic eruptions (Novis et al., 2008) which likely have strong impacts on snows with limited pH buffering capability (Spijkerman et al., 2012).

A study of alpine snow algae in the High Sierra Nevada of California (Thomas 1972) and one in the Pacific Northwest (Hamilton & Havig, 2017) concluded that dissolved inorganic carbon (DIC) is a limiting factor for snow algae primary production, where the latter study concluded that heterotrophs appear to contribute to active remineralization of organic carbon, potentially limiting snow algae growth. In the same study, bioavailable P and N did not stimulate

bloom growth, but DIC did, while elevated ratios of Fe, Mn and P compared to the local geology were observed, indicating active sequestration of local weathered rock minerals and/or utilization of diverse substrates. While the above study explored the effects of fertilizer (N and P) additions to snow algae blooms the growth results are counter to a study using NPK (Ganey et al., 2017).

A hypothetical food web of red snow algae blooms was proposed by Yakimovich et al. (2020) showing heterotrophic fungi and bacteria assemble based on organic matter inputs from allochthonous inputs and autochthonous algal inputs. Higher resolution studies of foodwebs in adjacent aquatic ecosystems have demonstrated that trophic partitioning of allochthonous pollen can occur between neustonic zooplankton that have a high contribution of pollen organic matter and their sestonic counterparts that derive organic matter from a mosaic of sources including pollen and algae (Masclaux et al., 2013). Another trophic partitioning study of the microbial detritusphere of soils showed fungi and bacteria had no discernable energy channel separation, highlighting the importance of successional inter-community dynamics and trophic interactions over substrate defined energy channels (Kramer et al., 2016). One aim of this current study is to examine the novel question of whether trophic partitioning occurs within the snow community between two primary sources of organic carbon inputs in red snow algae blooms — allochthonous pollen and autochthonous algae.

To address questions of physiochemical and microbial dynamics that are diagnostic of red snow algae blooms, we utilized a triad sampling strategy whereby we collected samples from independent red snow algae blooms consisting of deep red snows, snows at the periphery of visible blooms and snows without any apparent snow algae but adjacent to blooms (expanding on Hoham 1987). This design was used as sampling zones are easily observed in the field and are indicative of differing concentrations of snow algae. The research here is guided by the

overarching question of whether snow algae (Brown et al., 2015a) and/or pollen facilitate community-wide shifts across zones in the snow microbial community and whether sub-OTU networks show trophic partitioning between these two primary carbon sources. Taxonomic and physiochemical distinctions between adjacent zones is also assessed. We address several questions regarding snow algae bloom establishment and growth: (1) how is the overall microbial community structured regionally (Cascade Mountains vs. Rocky Mountains) and within individual bloom zones; (2) what changes in snow physiochemistry accompany bloom development; and (3) what co-associations exist between taxa and do certain taxa suggest the existence of a core snow algae bloom microbiome?

Methods and Materials

Sample Locations and Collection - Late-season red snow algae blooms were sampled in 2018 (eight independent snow algae blooms from Glacier Peak Wilderness Area, Wenatchee National Forest, Washington, USA [August 2018], and four from the Arapaho and Medicine Bow-Routt National Forest, Wyoming and Colorado, USA [July 2018]) across three bloom zones using a triad sampling scheme (Supplementary Figure 1). At each location, all accessible red snow algal blooms that can be safely sampled were sampled, most snow algae blooms are in inaccessible locations (Müller et al., 1998; Brown et al., 2015a). At each independent bloom location, snows were collected from three zones: deep red (M - Medial; center of algal bloom), pink (P - Peripheral; edge of algal bloom), and white, visually uncolonized snows immediately adjacent to the bloom (A - Adjacent; ~3m distance from visible algal colonization) for a total of 30 samples. All samples were collected from sites in full sun and open landscapes above tree line

(GPS coordinates, elevations, and slope faces were recorded, (Supplementary Table 1) and were visually free from anthropomorphic or animal disturbances. A total of 10 volumetric surface subsamples of ~85 cm^3 were taken to a depth of ~5 cm from M, P and A were collected and placed in clean 1-gallon zip-top plastic bags (following Brown et al., 2015a; Brown & Jumpponen, 2019); collection devices were surface sterilized using 70% denatured alcohol between samplings. Snows were melted at ambient temperatures and agitated for homogenization and a total of 100mL of snowmelt was passed through a 2.0 µm Nuclepore Track-Etch Membrane filter (25 mm; Whatman®, Kent, UK) encased in a 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 field-sterilized with denatured ethanol. Collected filtrate was then filtered through a 0.22-µm filter as described above. Dual filtration permits size fractionation of particles in melt water (Brown & Jumpponen, 2019) to enrich samples for larger particles (fungi and algae) in the first filter and bacteria in the second. Membrane filter discs were transferred into Qiagen PowerBead Tubes and first stored on ice until shipment then stored at -20° C until gDNA extractions.

Environmental Physiochemistry Variables and Cell Enumeration - From the melted snows, we measured the following: ammonium (mV), conductivity (µS/cm), dissolved oxygen (ppm), oxidation-reduction potential (mV), pH, resistivity (MΩ·cm), salinity (PSU), and total dissolved solids (ppm) using a portable meter (HI 9829 Multiparameter Meter; Hanna® Instruments; Woonsocket, RI, USA). Nitrate (NO_3^-) and potassium (K^+) were measured with LAQUAtwin pocket meters NO3-11 and K-11 (Horiba Scientific; Kyoto, Japan) respectively. Due to low nitrate and potassium concentrations, which are common to snowmelt (Lutz et al., 2015b; Havig

206 & Hamilton, 2019), mV values are given. While measurements of free phosphorus were
207 attempted (HI96706 phosphorus photometer; Hanna® Instruments; Woonsocket, RI, USA), the
208 free-P concentrations were below the instrument detection limit, so data are not included. Density
209 of snow algae (cells/mL) and pollen (grains/mL) was determined using disposable Fuchs-
210 Rosenthal hemocytometers (NCYTO C-Chip™; INCYTO, Cheonan, South Chungcheong, South
211 Korea). Identified pollen grains only included counts of distinctly bisaccate anemophilous pollen.
212
213 *gDNA Extraction and Sequencing* - DNA was extracted using a DNeasy PowerSoil® DNA
214 Isolation Kit (QIAGEN, Germantown, MD, USA) following standard procedures with
215 modifications. First, membrane filters were sonicated for 10 min (Bransonic 2800 Ultrasonic
216 Bath, St. Louis, MO) in extraction kits to maximize cellular resuspension (*following* Brown &
217 Jumpponen, 2019), then filters were removed using sterile technique. To maximize physical lysis
218 as red snow algae cysts are difficulty to break, 0.25 mL of sterile 1.0 mm zirconium oxide beads
219 (Next Advance, Troy, NY) were added to extraction tubes and samples were beaten twice for 30
220 sec. at maximum speed using a Fisherbrand™ Bead Mill 24 Homogenizer (Fisher Scientific,
221 Waltham, MA).
222 To generate amplicon sequencing libraries, we utilized a two-step PCR approach
223 (*following* Brown et al., 2018). Primary amplicon libraries for algae and fungi were generated by
224 targeting the Internal Transcribed Spacer 2 (ITS2) of the rDNA region with the primers fITS7
225 (Ihrmark et al., 2012) and ITS4 (White et al., 1990); these primers were previously shown to
226 amplify snow algae and fungi concurrently with minimal biases (Brown & Jumpponen, 2019).
227 For bacteria, we targeted the V4 hypervariable region of the 16S rDNA with the modified
228 primers 515 and 806R (Caporaso et al., 2011; Apprill et al., 2015; Parada et al., 2016). Primary

PCRs were conducted using the primer constructs that includes primers and Nextera sequencing primers (nexF and nexR) as well as a range of ambiguous nucleotides (N[3-6]) and were constructed as follows: nexF-N[3-6]-fITS7 and nexR-N[3-6]-ITS4 for algae and fungi; and nexF-N[3-6]-515f and nexR-N[3-6]-806r for bacteria. PCRs were conducted in duplicate on a SimpliAmp™ Thermal Cycler (Fisher Scientific, Waltham, MA) in 25 µL reactions containing: 2 µL DNA template, 12.5 µL Thermo Scientific™ Phusion Green™ Hot Start II High-Fidelity PCR Master Mix, 5.5 µL molecular grade nuclease-free H_2O, and 2.5 µL each of the forward and reverse primers for a total concentration of 1 µM for each primer. PCR parameters were: initial denaturation (98 °C; 30 s), then 25 cycles of denaturation (98 °C; 10 s), primer annealing (51 °C for algae/fungi and 52.5 °C for bacteria; 30 s), and extension (72 °C; 40 s), and then a final extension step (72 °C; 10 min). The ramp rate was set to 1.0 °C/s for annealing steps and to 4.0 °C/s for all other steps. All primary PCR products were visualized to confirm amplification using gel electrophoresis.

Secondary PCR was conducted using the respective forward and reverse primer pairs P5-i5-overlap and P7-i7-overlap, comprised of unique dual-barcode MIDs (i5 and i7), Illumina Adaptor sequence (P5 and P7) and partial overlap with the nexF and nexR sequences that acts as the annihilation site for primers. Forward and reverse secondary primers were mixed to generate unique dual-barcoded primers with a PCR reaction concentration of 5 µM per primer (10 µM total). Multiplexed amplicons were generated by PCR in a 25 µL reaction containing: 2.5 µL primary PCR product, 12.5 µL Thermo Scientific™ Phusion Green™ Hot Start II High-Fidelity PCR Master Mix, 7.5 µL molecular grade nuclease-free H_2O, and 2.5 µL of dual-barcoded primer mix (0.5 µM). PCR thermocycler parameters were set as follows: an initial denaturation (98 °C; 30 s), then 10 cycles of denaturation (98 °C; 20 s), primer annealing (50 °C; 20 s), and elongation

(72 °C; 50 s), and then a final extension step (72 °C; 10 min). In total, 35 cycles were run. All amplification was confirmed visually on electrophoresed gels. Negative controls consisting of sterile water were extracted, amplified, and sequenced and remained free of appreciable contamination.

PCR products were cleaned using Axygen® AxyPrep™ Mag PCR Clean-up solution (Corning Life Sciences, Tewksbury, MA) per protocol with the modification of a 1:1 ratio of PCR product to beads (Brown & Jumpponen, 2014). Cleaned PCR products were quantified using a Qubit 3.0 fluorometer with Qubit® dsDNA HS (Thermo Fisher Scientific, Waltham, MA) assay kits and pooled into a eukaryotic library and a bacterial library for sequencing and cleaned once again as above. Libraries were sequenced on a single reaction of Illumina MiSeq (300PE) with a 70% Eukarya (Fungi/Algae): 30% Bacteria loading ratio at the Kansas State University Integrated Genomics Facility (Manhattan, KS, USA). Raw sequence data were demultiplexed using the unique i5 and i7 sequence combinations yielding individual paired fastq files for each of the samples (for algal, bacterial, and fungal libraries see Supplementary Table 2)

Bioinformatics - Sequence processing was primarily done using the program *mothur* (*v.1.42.0;* Schloss et al., 2009). Forward and reverse sequences were contiged and then screened to remove sequences with ambiguous bases or those with a homopolymer length greater than 6 for fungi and algae, and 12 for bacteria. Primer sequences were trimmed using the program *cutadapt v.1.17* (Martin, 2011). Bacterial sequences were aligned against the referent database *SILVA v132.8* (Quast et al., 2013) and screened to exclude non-V4 regions. Fungal and algal ITS sequences cannot be reliably globally aligned. Sequences were pre-clustered using a *mothur* implemented

pseudo-single linkage clustering (Huse et al., 2010) and chimeras were identified and removed using *VSEARCH* as implemented in *mothur* (Rognes et al., 2016). Bacterial sequences were taxonomically identified against the RDP training set (v10) and eukaryotic sequences were identified against the UNITE non-redundant database (v6; Kõljalg et al., 2013) using the *mothur* implemented Naïve Bayesian Classifier (Wang et al., 2007); and any off-target sequences were culled. Since UNITE is a fungal database with minimal representation of non-fungal eukaryotes, sequences identified as non-fungal but eukaryotic were retained so that algal subsets could be queried and identified subsequently (see below). For bacteria, an uncorrected pairwise distance matrix was calculated (not punishing terminal gaps) between aligned sequences and the resultant distance matrix was used for OTU demarcation at a 3% dissimilarity threshold using the OptiClust method (Westcott & Schloss, 2017). For algae and fungi, OTUs were demarcated using *VSEARCH* (abundance based on unaligned sequences) (Rognes et al., 2016). For all lineages, OTUs with fewer than 10 sequences were considered potentially spurious and removed (Brown et al., 2015b; Oliver et al., 2015). Consensus taxonomic identifications and representative sequences for each OTU were generated and three separate OTU x sample matrices were created for algae, bacteria and fungi. Fungal OTUs without 100% bootstrap support at the phylum level were queried against GenBank (nr/nt) using BLASTn to confirm that these were indeed fungal. All algal OTUs were queried similarly to include only algal OTUs (culling of non-algae eukaryotes). All BLASTn queries were run with the exclusion of environmental samples. After sequence quality control, total sequence counts were: 2,094,357 for algae, 3,178,201 for bacteria and 2,632,246 for fungi. In total, 91 algal, 224 bacterial, 532 fungal OTUs were demarcated. We analyzed these communities separately (within Kingdom/Domain) and combined (across

296 Kingdom/Domain), whereby we combined all demarcated OTUs to examine whole community
297 dynamics; comprising 847 unique community OTUs.
298
299 *Statistical Analysis* - OTU richness (S_{obs}), Simpson's evenness (E_D), Gini-Simpson's diversity (1
300 - D), and Bray-Curtis dissimilarity values were calculated for algae, bacteria, fungi and the whole
301 community using 1000 iterations and mean values were used for all analyses. Matrices were
302 subsampled at a sequence depth set to the size of the smallest sample for algae (10,609), bacteria
303 (50,542), fungi (29,950) and whole community (129,506).
304 To visualize community membership, relative abundance stacked histograms were
305 generated in R v3.4.5 (all subsequent R code was also run in this version) with packages *RAM*
306 (Chen et al., 2018) and *ggplot2* (Wickham, 2019). Lineages corresponding to zones M, P and A
307 from the Cascades and Rockies are shown, either at the family (bacteria and fungi) or species
308 (algae) level. Lineages were derived based on OTU taxonomic assignments. Taxa with relative
309 abundances of less than 1% were combined into a single group.
310 A non-parametric two-sample Wilcoxon Rank-Sum test was used to test whether the most
311 abundant taxa (top 33.3%) differed significantly between the Cascade and Rocky Mountains and
312 whether physiochemical parameters also differed. Median difference (MD) values are also given,
313 where positive values indicate higher abundance in the Rockies and negative values indicate
314 higher abundance in the Cascades (Supplementary Table 3). Mean physiochemical values were
315 also calculated from the Cascades ($\bar{X}_C$) and Rockies ($\bar{X}_R$). Separate heat-matrices for the
316 Cascades and Rockies were generated using *metacoder* (Foster et al., 2017) to detail pairwise
317 OTU differences in abundance between zones M, P and A for abundant taxa. Differences
318 between OTU abundances are expressed as the log-2 median ratio.

A permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) on average Bray-Curtis dissimilarity values tested if communities (algae, bacteria, fungi or the entire community) differ with physiochemical (NH_4^+, DO, NO_3^-, ORP, pH, K^+, resistivity and TDS), region or biological (pollen and algae concentration) parameters. PERMANOVAs were run in R using the adonis function in the package *vegan* (Oksanen et al., 2016).

Paired Wilcoxon Sign-Rank tests were conducted between environmental characteristics and diversity estimators in snow zone pairs (M-P, M-A and P-A) and were used to generate parallel plots. Plots were generated in R using the following packages: *dplyr, GGally, ggplot2, ggsignif, ggstance, gridExtra, hrbrthemes, patchwork, tidyverse*, *scales* and *viridis* (Auguié, 2017; Garnier, 2018; Schloerke, et al. 2018; Wickham, 2018; Ahlmann-Eltze, 2019; Henry et al., 2019; Rudis, 2019; Wickham, 2019a; Wickam, et al. 2019b). Pairwise Wilcoxon Sign-Rank tests were conducted using JMP® Pro (v.14; SAS Institute Inc., 2009).

Weighted-gene correlation network analyses (WGCNA) were performed on community OTUs from the Cascades and Rockies (separately) in R using the *WGCNA* package (Langfelder & Horvath, 2007; Langfelder & Horvath, 2008) and as implemented with recommendations by Wilson (Wilson et al., 2018) with several modifications designed for optimization. WGCNA was used in order to understand how community wide modules responded to environmental parameters, where modules represent a collection of OTUs (OTU clusters) that are highly correlated and co-occur across multiple samples. Interconnected OTU modules represent networks that may be induced by a similar set of environmental parameters and they may also be viewed as microbial community constructs (or sub-communities) that have similar biological properties in an ecosystem context. The network is modularized into a set of first principal components or module eigengene vectors whereupon modules can be correlated with

342 environmental traits or OTUs of interest. The Cascade and Rocky Mountain samples were
343 analyzed separately as PERMANOVA analyses indicated significant regional differences (Table
344 1). Additional R packages used here were *cluster*, *entropy*, *GO.db*, *impute*, *infotheo*,
345 *preprocessCore*, *vegan* (Hausser & Strimmer, 2014; Meyer 2014; Bolstad, 2019; Carlson, 2019;
346 Hastie et al., 2019; Maechler et al., 2019). Raw counts were transformed to Hellinger distances,
347 which gives higher weight to the more abundant OTUs. Hellinger distances were clustered to
348 identify sample outliers (none were detected) as recommended by Langfelder and Horvath
349 (2008). Strong correlations between OTUs were emphasized and weak correlations punished
350 using soft-thresholding powers (β). To minimize noise from low abundance OTUs, β was
351 selected from the smallest set (OTU_N) of highly abundant OTUs that maintained a scale-free
352 topology model fit (signed R^2) > 0.80 and a mean connectivity score (κ) > 1.0 (OTU_N = 241, R^2 =
353 0.84, β = 7 and κ = 2.10 for the Cascades and OTU_N = 110, R^2 = 0.81, β = 9 and κ = 1.32 for the
354 Rockies). Unsigned adjacency matrices were then generated using these designated soft powers
355 and transposed to unsigned topological overlap dissimilarity matrices (TOM) to reduce noise and
356 spurious associations and to relate co-expression similarity to the broader community level
357 networks. Unsupervised hierarchical clusters, using Ward's method for agglomeration, were used
358 to generate dendrograms. Ward's method returns visually delineated clusters corresponding to
359 regions of high density in PCA ordinated space (Murtagh & Legendre, 2014). To generate
360 modules, a dynamic cut approach was used allowing for improved module detection (Langfelder
361 et al., 2008). OTU module sizes (Mod_{min}) and a max tree cut height (Mod_{maxcut}) were set for the
362 dynamic tree cut, which yielded a set of colorized OTU modules (Mod_N) for the Cascades
363 (Mod_{min} = 14, Mod_{maxcut} = 2.25 and Mod_N = 7) and Rockies (Mod_{min} = 6, Mod_{maxcut} = 1.75 and
364 Mod_N = 8). Modules were then assessed for stability by identifying the clusterwise Jaccard

similarity, resampled 100 times, in an approach similar to SABRE (Shannon et al., 2016) and using the R package *clusterboot* (Deen & de Rooij, 2020) modified for use with dynamic tree cutting. Quantifying network module stability allows for prioritization of modules if further experimental interrogation is desired. Module stability for the Cascades (turquoise = 0.37 , blue = 0.12, brown = 0.12, yellow = 0.11, green = 0.11, black = 0.10, red = 0.10) and Rockies (turquoise = 0.32, blue = 0.16, brown = 0.17, yellow = 0.17, green = 0.17, black = 0.17, red = 0.15, pink = 0.15) was fairly low, likely due to relatively few samples and possibly due to the stochastic nature of the system, but no evidence was presented to doubt the veracity of these module bins. Modules were then reduced in dimensionality into eigengenes (i.e. the first principal components) and eigengene clusters were merged with their respective OTU modules. Eigengenes were calculated for each module color using the original Hellinger transformed relative abundance scores and multi-dimensional scaling plots of module eigengenes, using first and second principal components were generated. Nonparametric signed Kendall-Tau correlations were then generated between eigengenes and environmental traits or abundant OTUs of interest for the Cascades and Rockies to generate heatmaps. Module eigengenes with high correlation to environmental traits or abundant OTUs are typically dominated by a single or few OTUs. Module vs. highly abundant OTU correlations were explored under the assumption that nutrient competition, resource sharing or saprotrophic utilization by groups of individual snow microbes underlie these associations. Correlations give an idea for how OTU consortia with similar sample abundance patterns associate with environmental traits or other OTUs. In module eigengene to OTU comparisons, the abundant OTU was purged from its respective module so as not to improperly skew correlations.

Kendall-Tau co-association first-order neighborhood network graphs were generated for several highly abundant OTUs from the Cascades and Rockies. Only vertices with $K \geq |0.40|$ and p-value ≤ 0.1 were incorporated into the subnetworks. Graphs and summary statistics were generated in R using a modified plot_network function from the package *phyloseq* (McMurdie & Holmes 2013). Graphic generation also utilized the packages: *GGally, ggraph, SpiecEasi, igraph, intergraph, Matrix, network, SpiecEasi, sna,* and *stringr*. Co-association networks included the full community of OTUs and were constrained by OTU absolute abundance (AA > 100) and by region (Cascades or Rockies).

Results

Regional Differences between Snow Algae Blooms - Snow algae bloom OTU abundances (RA%) were dominated by *Sanguina nivaloides* (86%), and to a lesser extent, the *Sanguina aurantia* (5%) (Figure 1a). Additionally, *Raphidonema nivale* (4.5%) was more abundant in the Cascades (MD = -0.006; p = 0.00025) and can be highly abundant in individual samples (Figure 1a). Algae showed no appreciable differences between the Rockies and Cascades for evenness (S = 2; p = 0.91) richness (S = -13; p = 0.34) or diversity (S = -17; p = 0.20) (Supplementary Table 4).

Bacterial communities were dominated by four families (Figure 1b): Sphingobacteriaceae (23% RA; MD = -0.14; $p = 1.10 \times 10^{-04}$), which were more abundant in the Cascades, Burkholderiaceae (20% RA), Hymenobacteraceae (20% RA), and Chitinophagaceae (19% RA; MD = 0.15; $p = 2.60 \times 10^{-04}$) were more abundant in the Rocky Mountains. Subdominant taxa (between 1% and 10% RA) included: Microbacteriaceae (4.4%; MD = 0.05; $p = 3.00 \times 10^{-06}$, Acetobacteraceae (3.5%; MD = -0.05; $p = 6.50 \times 10^{-06}$, uncultured (2.1%), Rhodanobacteraceae

(1.9%; MD = -0.01; p = 1.00 x 10^{-04}), Bacillaceae (1.23%), and Micrococcaceae (1%). Bacteria diversity estimates were similar across both regions: evenness (S = 7; p = 0.62), richness (S = 20; p = 0.13) and diversity (S = 17; p = 0.20) (Supplementary Table 4).

Nearly one-third of all fungal sequences could not be resolved at the genus level with currently databased accessions (Supplementary Table 5) which is common for snow fungal analyses (Brown & Jumpponen 2019) and is indicative of the poorly understood nival mycosphere. Fungi had higher richness in the Rockies than the Cascades (S = 25; p = 0.026), which is due to a multitude of rare OTUs (<1% RA) in the Rockies (Figure 1c). Unclassified families within the phylum Chytridiomycota, which included 3 OTUs, were dominant in the Cascades (38% RA) but nearly absent in the Rockies (0.04% RA). Further, the Microbotryomycetes were abundant within both regions, but significantly more so in the Rockies for a single unclassified Microbotryomycete OTU (MD = 0.18; p = 0.02) and unclassified families within Microbotryomycetes (MD = 0.16; p = 3.47 x 10^{-06}). These two unclassified family level lineages within the Microbotryomycetes are the most abundant taxa across all samples (40% RA). Other dominant families including the Aureobasidiaceae (MD = 0.02; p = 3.30 x 10^{-07}), Dothioraceae (MD = 0.02; p = 9.20 x 10^{-06}), and Mycosphaerellaceae (MD = 0.10; p = 3.30 x 10^{-07}), which were significantly more abundant in the Rockies, while the Leucosporidiaceae (MD = -0.1; p = 1.30 x 10^{-04}), Leucosporidiales sp. (MD = 0.05; p = 5.90 x 10^{-04}), Monoblepharidales *incertae sedis* (MD = -0.02; p = 1.40 x 10^{-03}), and Trigonopsidaceae (MD = -0.01; p = 1.10 x 10^{-05}) were significantly more abundant in the Cascades (Supplementary Table 3).

Environmental parameters between the Rockies and Cascades (Table 2) also show several significant differences for ORP (S = -33; p = 0.007), DO (S = 39; p = 0.0005), Conductivity (S =

28.5; p = 0.04), NO_3^- (S = -38; p = 0.001) and pH (S = 23; p = 0.04). Where ORP, NO_3^- have higher values in the Cascade Mountains and DO, Conductivity, and pH have higher values in the Rocky Mountains.

Zonal Differences of Snow Algae Blooms - By evaluating differences between pairwise zones within the same algae bloom, several interesting patterns emerge (Figure 2). Unsurprisingly, the concentration of algal cells differed between zones M-P (S = 22.5; p = 0.0039), M-A (S = 22.5; p = 0.0039) and P-A (S = 22.5; p = 0.0039), increasing from an average concentration of 2.10 x 10^3 cells/mL in A, to 1.40 x 10^4 cells/mL in P to 1.68 x 10^5 cells/mL in M, an approximate order of magnitude difference between zones. Pollen concentrations also showed a similar pattern, differing significantly between zones M-A (S = 23; p = 0.0078) and M-P (S = 23.5; p = 0.0078). Zones A and P had average concentrations of 320 and 420 grains/mL respectively, while zone M had an average concentration of 1.44 x 10^3 grains/mL.

Oxidation-reduction potential (ORP) and other electrochemical measures illuminate which zones might have higher potential for the decomposition of organic matter. As is common in other aquatic systems ORP is a proxy for microbial activity, indicating whether the system's potential is toward anaerobic (negative) or aerobic (positive) output (Scholz 2019). Rank-sum testing found significant pairwise zonal differences for electrochemical measures (Figure 2). ORP was found to be lower in M than in P (S = 21.5; p = 0.0137), while resistivity was lower in M than P (S = -25; p = 0.0078) and A (S = -26.5; p = 0.0039). Conductivity, the reciprocal of resistivity, was significantly higher in M than A (S = 23; p = 0.0156). Dissolved oxygen (DO) levels were also measured to gauge if a trend toward anaerobic activity in zone M compared to zone P and A was plausible. While not statistically significant (S = -11.25; p = 0.1377), average

DO levels did show lower levels in M (46.85%) compared with P (50.8%) and A (49.35%). A significant increase in potassium levels was also observed in zone M compared to P ($S = 23.5$; $p = 0.0137$) and A ($S = 17.5$; $p = 0.042$). Clear differences in electrochemical measures between snow algae bloom zones underscores the presence of an active microbial community.

Diversity estimates were also shown to differ between zones (Figure 2). Between zones A and P, we see a decrease in algal richness ($S = -22.5$; $p = 0.0098$) and Simpson's Diversity (1-D; $S = -21.5$; $p = 0.0137$) which coincided with an increase in bacterial richness ($S = 19.5$; $p = 0.0244$) and algal richness was also shown to decrease from zone A to M ($S = -19.5$; $p = 0.0244$).

As broad taxonomic differences were observed across snow algae bloom zones, we sought to identify specific community members that differed across zones. To do so, we used Wilcoxon Rank-Sum tests across all zone pairs (M-P, M-A and P-A) for each group and a small subset of the most significant taxa are shown in (Table 3) ($p < 0.10$) (full dataset in Supplementary Table 6). Relatively few OTUs showed significant differences between zones. We see that *Sanguina nivaloides* are found in higher numbers in P than A ($MD = 0.096$; $p = 0.05$), but this is not the case between M and P ($MD = -0.013$; $p = 0.58$) or M and A ($MD = 0.083$; $p = 0.14$). Interestingly, a bacterial OTU within *Polaromonas* showed higher levels in M than A ($MD = 0.02$; $p = 0.003$) and in P than A ($MD = 0.016$; $p = 0.011$). This indicates an increased OTU abundance for *Sanguina* and *Polaromonas* in peripheral snows as compared to 'white' adjacent snows. For fungi, an unclassified Microbotryomycete showed lower levels in P than A ($MD = -0.03$; $p = 0.11$). Metacoder heat trees (Foster et al., 2017) were also generated to visually show differences in OTU abundances between zones M, P, and A for the Cascade (Supplementary Figure 2a) and Rocky Mountains (Supplementary Figure 2b).

PERMANOVA - The PERMANOVA analyses for algae, bacteria, fungi, and the entire community (Table 1) show differential responses to environmental parameters. Algae showed no significant community variation for any environmental traits, whereas bacterial communities shift with oxidation-reduction potential (ORP) ($R^2 = 0.105$; $p = 0.05$) and dissolved oxygen (DO) ($R^2 = 0.071$; $p = 0.044$). Fungi also showed significant variation with ORP ($R^2 = 0.122$; $p = 0.005$), with some variation also potentially explained by DO ($R^2 = 0.06$; $p = 0.073$). Regional differences were observed for bacteria ($R^2 = 0.114$; $p = 0.011$) and fungi ($R^2 = 0.154$; $p = 0.002$) between the Cascade and Rocky Mountains. Fungal communities also responded to pH ($R^2 = 0.169$; $p = 0.001$), conductivity ($R^2 = 0.059$; $p = 0.003$), TDS ($R^2 = 0.056$; $p = 0.013$), nitrate ($R^2 = 0.078$; $p = 0.016$), salinity ($R^2 = 0.05$; $p = 0.032$), and potassium ($R^2 = 0.073$; $p = 0.043$) whereas bacteria showed a marginal response to potassium ($R^2 = 0.062$; $p = 0.060$). The entire community PERMANOVA showed significant between-group variation for ORP, region ($R^2 = 0.116$; $p = 0.007$), pH ($R^2 = 0.109$; $p = 0.008$) and potassium ($R^2 = 0.088$; $p = 0.025$) where nitrate ($R^2 = 0.058$; $p = 0.055$) also likely plays a role but may only influence a sub-community of microbes (see below).

Taxonomic/Environmental WGCNA Analysis - Here we use WGCNA to assess red snow algae bloom sub-network structuring as related to environmental parameters, nutrient concentrations, and to select highly abundant taxa. In understanding taxonomic shifts within snow algae blooms and which microorganisms predominate we can begin to assess whether alpine red snow algae blooms possess core taxa and whether sub-network modules of OTUs might suggest patterns of niche or trophic partitioning.

Sample sets were clustered according to Hellinger distances to assess whether samples clustered by snow zones (M, P and A) or by bloom site for the Cascade and Rocky Mountains (Figure 3a-b). Generally, the microbial community in zones M, P and A showed greater tendency to cluster with adjacent zones within a local sample site, rather than across sampling sites with zones of the same category. This suggests that the local sampling sites may be more important than the algae bloom zone in determining community structure. In incomplete clusters, M tended to be displaced more often than A and P.

To assess whether underlying subsets of OTUs co-associate across sampling sites, hierarchical trees of OTUs were clustered and cut to check for stable forming modules. TOM dissimilarities, a measure of interconnectedness based on shared network neighbors (Li & Horvath, 2009), were used as it considers OTU pairs in the context of the entire network rather than in isolation as with a standard adjacency matrix (Yip & Horvath, 2007). Seven modules were identified for the Cascade Mountains and eight for the Rocky Mountains (Supplementary Figure 3) Each module set for the Cascade and Rocky Mountains had a dominant turquoise module with a high bootstrap stability measure of 37% and 32% respectively where remaining modules comprised of fewer OTUs showed lower stability. Multi-dimensional scaling (MDS) of the modules show groupings that appear in a core-tendril configuration for both the Cascade Mountains and Rocky Mountains (Supplementary Figure 4). In both regions a core cluster (turquoise) was identified with two or three tendrils, where the tendrils indicate a high degree of similarity in one dimension and little similarity in the other.

Modules were correlated using Kendall-Tau (K) with environmental parameters and presented in a heat matrix for the Cascade (Figure 4a) and Rocky Mountains (Figure 4b). It should be noted that modules of the same color in the Cascade and Rocky Mountains do not

necessarily share the same OTUs. In the Cascade Mountains the strongest module associations are with ammonium and nitrate (Figure 4a). Two modules (green and yellow) are negatively correlated (K = -0.51 and K = -0.35) with nitrate. The green module from the Cascades comprised of several highly abundant OTUs best identified to the following genera: *Caldalkalibacillus* (BOTU0014 and BOTU0028), *Nesterenkonia* (BOTU0015 and BOTU0032), *Nakamurella* (BOTU0022), *Halomonas* (BOTU0026), Bacillaceae unclassified (BOTU0038) and *Trebouxia* (AOTU0028). Interestingly, a near identical set of OTUs were also observed to cluster together in the Rocky Mountains black module (all except *Trebouxia* - AOTU0028) indicating similar OTU networks between regions. Significant module correlations were limited in the Cascade Mountains compared with the Rocky Mountains.

Modules in the Rocky Mountains (Figure 4b) showed weak to moderate associations with ammonium (red, green blue, yellow) and nitrate (yellow, green, blue), and stronger associations with DO, ORP and elevation. The red module was negatively correlated with DO (K = -0.39; p = 0.2) and ORP (K = -0.5; p = 0.09). Yellow and blue modules showed positive correlation with DO (K = 0.36; p = 0.2) (K = 0.27; p = 0.4) and ORP (K = 0.41; p = 0.2) (K = 0.63; p = 0.03) respectively; the yellow module was composed entirely of fungi and contained an abundant Microbotryomycete (FOTU0006) and unclassified fungi (FOTU0008); and the blue module was composed of abundant Solitelia (BOTU0001), Rhizocarpon (FOTU0023), and Aureobasidium (FOTU0032). Green and pink modules also showed negative correlation with elevation (K = -0.7; p = 0.01) (K = -0.5; p = 0.1).

In addition to explorations of module-environment associations, highly abundant OTUs were parametrized to explore OTU-module relationships in the Cascade (Figure 5a) and Rocky (Figure 5b) Mountains. This was done to ascertain whether module-OTU association might

547 suggest trophic partitioning. In the Cascade and Rocky Mountains, we observed more significant
548 associations of modules with fungal OTUs, compared with bacterial or algal taxa suggesting
549 fungi in these snows may be highly important for trophic functionality. For all modules in both
550 regions, *Sanguina sp.* showed weak non-significant correlations with all OTUs. In the Cascades,
551 the red module (N = 14) consisted entirely of fungi and included the highly abundant but
552 unclassified chytrid (FOTU0001) and Leucosporidiales (FOTU0005) as well as less abundant
553 Leucosporidiales and Microbotryomycetes. This red module showed moderate positive
554 associations with the unclassified chytrid (FOTU0001) and two Leucosporidiales (FOTU0003
555 and FOTU0005) while showing moderate negative association with a Microbotryomycete
556 (FOTU0006).
557
558 *OTU Co-association Network Subsampling* - Given the granular resolution of the WGCNA, first-
559 order neighborhood OTU co-association networks were generated to assess regional relationships
560 between individual highly abundant taxa and the red snow algae bloom community (Figure 6a-g).
561 *S. nivaloides* had limited connectivity in the Cascades and Rocky Mountains (Figure 6a-b). This
562 is in strong contrast with the high connectivity and significant correlations between *Solitalea* sp.
563 (BOTU0001) and unclassified Chytridiomycota (FOTU0001) (Figure 6c-e). Of these positively
564 correlated OTUs, several members of the order Leucosporidiales (FOTU0003, FOTU0005 and
565 FOTU0008) were shown to positively and significantly co-associate with this Chytrid. These
566 OTUs were also dominant and together comprise about 18% of the Cascades total relative
567 abundance. While Leucosporidiales and Chytridiomycota are known to frequently co-occur in
568 snow, ice and marine systems (Rämä et al., 2014; Brown et al., 2015a; Perini et al., 2019a; Perini
569 et al., 2019b), such a strong and significant positive co-association is first demonstrated here.

570 *Polaromonas* sp. (BOTU0004) had few associations in the Cascade Mountains (Figure 6f), but
571 showed a robust and interconnected network in the Rocky Mountains (Figure 6f), which
572 associated with several highly abundant bacterial and fungal OTUs.
573
574 **Discussion**
575
576 Here we present the first study of red snow algae bloom dynamics across spatial zones
577 coupled with investigations into associated microbial communities and physiochemical attributes.
578 In doing so we demonstrate there is little evidence of biogeographic structuring across semi-
579 continental distances for algae and bacteria, whereas fungi are regionally structured. Similar
580 biogeographic patters were previously observed in Northern Hemispheric 'white' snows (Brown
581 & Jumpponen, 2019) and may be due to environmental filtering of fungi (Rime et al., 2016). As
582 seen here and with other studies (as noted in the introduction), we see abundant bacteria taxa that
583 belong to the α-proteobacteria, Actinobacteria, Bacilli, Bacteroidia and ɣ-proteobacteria and
584 abundant fungal classes including the Microbotryomycetes, Chytridiomycetes, Dothideomycetes
585 and Monoblepharidomycetes. While observed biodiversity of snow algae blooms is high, the
586 number of true cryobionts that grow and/or reproduce in this niche space may be a small fraction
587 of the OTUs observed, but this needs to be further interrogated.
588 This study shows increasing potassium levels from A to P to M, suggesting potassium
589 may be required for snow algae bloom growth. A previous study of dissolved snowpack in the
590 Cascade-Sierra Nevada Range showed significant correlation between dissolved organic carbon
591 and potassium (Laird et al. 1986), which may mean that pollen is a significant source of
592 potassium. Additionally, sub-community modules in the Cascade and Rocky Mountains show

moderate and significant correlation with ammonium and nitrate measures indicating sub-community networks may have differential nutrient limitations. Evidence that inorganic carbon is a limiting factor continues to emerge (Thomas 1972; Hamilton & Havig, 2017) along with evidence of minimal effects of NO_3^- and phosphorous or NH_4^+ and phosphorous supplements on *S. nivaloides* growth (Hamilton & Havig, 2017). Surface gas-exchange experiments in the Snowy Range of the Rocky Mountains proposed that *S. nivaloides* may derive CO_2 not from atmospheric gas exchange, but from respiration occurring within or beneath the snow (Williams et al., 2003) derived from heterotrophic soil and/or snow fungi or bacteria. Higher photosynthetic rates were found to occur in unmelted snows (Thomas 1972), suggesting multiphasic nutrient absorption may be an important strategy for *S. nivaloides* survival in snows. Our study demonstrates that snow algae are associated, albeit minimally with NO_3^- and NH_4^+ across zones M, P and A and that K^+ appears to increase from zones A-M and P-M, having the highest concentrations in M (middle of the bloom). Given that K^+ concentrations were highest in M and that two previous studies exploring fertilizer effects showed that NP fertilizer did not induce growth (Hamilton & Havig, 2017) while NPK fertilizer did (Ganey et al., 2017), further exploration of the role of K^+ is warranted as it is known to be rate limiting for some aquatic systems (Talling, 2010). Moreover, low levels of potassium have been found to correspond with *Chytridium sp.* infections of green algae (Abeliovich and Dikbuck 1977), however, this study did not find evidence of significant negative spatial correlation across zones between K^+ and putative chytrid OTUs in the Cascade or Rocky Mountains.

S. nivaloides was observed to be marginally negatively associated with ORP in the Cascades (K = -0.39; p-value = 0.107; $\bar{X}_C$ = 16.22) and positively associated in the Rockies (K = 0.65; p-value = 0.021; $\bar{X}_R$ = -6.40), which may reflect broadly aerobic or anaerobic communities,

616 respectively. The differences between these sites are intriguing and requires more
617 experimentation to disentangle but may simply be due to sampling lag. For logistical purposes,
618 the Cascades were sampled one month later than the Rockies, anaerobic activity from fungi such
619 as the unclassified Chytridiomycota seen to predominate in the Cascades may have lowered DO
620 (%) levels. However, DO (%) does not appear to differ significantly across bloom zones. This
621 poses a broader question of whether environmental parameters, altered by biological activity, can
622 maintain differential levels across snow zones, and if so, how long do measurable differences last
623 during snow ablation.

624 Snow algae blooms typically occur in low conductivity environments (Hoham & Remias,
625 2020) where cell lysis through osmotic stress and increased freeze/thaw cycles during summer is
626 thought to increase conductivity (Harding et al., 2011; Larose et al., 2013). While this study
627 revealed elevated conductivity measures in M compared to A, it is not clear if this difference is
628 due to differential deposition of allochthonous material or differential release of organic and
629 inorganic material. Additional temporal studies, tracking red snow algae blooms over days or
630 weeks, that include *in situ* manipulations with fertilizers and organic matter, such as pollen, are
631 needed to bring greater resolution to these electrochemical fluctuations.

632 Bacteria within the genus *Polaromonas* were previously shown to be abundant in snow
633 algae blooms (Hamilton & Havig, 2017) and commonly in association with algae in a diversity of
634 habitats. Across the genus *Polaromonas*, diversification of genetic and metabolic traits may be
635 driven by adaptation to local conditions and algal hosts (Gawor et al., 2016). In the present study,
636 *Polaromonas* OTUs increased concomitantly in abundance with *S. nivaloides* across bloom
637 zones. This supports the idea that snow algae blooms may facilitate niche expansion of select
638 taxa within the community. Conversely, snow algae acclimation to snow and ice might also be

639 facilitated by algae-associated bacteria that confer improved ice nucleating ability (Kvíderová et
640 al. 2013). While *Polaromonas sp.* appear to be facilitated by red snow algae blooms, or vice
641 versa, this may not be the case for all taxa in the community. For example, two WGCNA
642 modules in the Cascades (green) and Rockies (black) form intramodal hubs of OTUs that are
643 nearly identical across regions at the genus level. These genera include *Caldalkalibacillus*
644 (BOTU0014 and BOTU0028), *Nesterenkonia* (BOTU0015 and BOTU0032), *Nakamurella*
645 (BOTU0022), *Halomonas* (BOTU0026), Bacillaceae unclassified (BOTU0038) and *Trebouxia*
646 (AOTU0028; Rockies only), many of which have extremophilic relatives adapted to alkali-, halo-
647 , psychro-, or thermophilic conditions (Mata et al. 2002; Kalamorz et al. 2011; Chander et al.
648 2017; Da et al. 2019). None of these genera co-occurred with *S. nivaloides* (AOTU0001) nor
649 were they found to vary significantly from M to P to A. Given their abundance in the Cascade
650 and Rocky Mountains, and the independent formation of near identical modules based on highly
651 similar co-occurrence patterns in both localities, these taxa may occupy a discrete niche
652 unoccupied by the snow algae bloom. Further evidence beyond co-occurrence and modulization
653 would be needed to investigate this claim.

654 Examining network connections of *S. nivaloides* (AOTU0001) and other dominant
655 bacteria and fungi (top 90%), we see that most bacteria and fungi are disjoint from these OTUs
656 in red snow algae blooms (suggesting that these community members may simply co-occur in
657 these systems but not actively interact in the environment. This is in direct opposition to our
658 hypothesis that algae may facilitate community formation and/or functionality. Demonstrated
659 here (Figure 6e), the most abundant fungal OTU (FOTU0001) was an unclassified
660 Chytridiomycota and has a network highly connected with other abundant fungal and bacterial
661 OTUs (Supplementary Table 7), but not with snow algae. This supports the hypothesis of disjunct

662 and independent networks between heterotroph dominated networks (sub-communities) and
663 photoautotroph networks dominated by *Sanguina* spp. in support of trophic partitioning. If these
664 networks are truly discrete this would indicate that another carbon source, such as pollen, may
665 underpin a large segment of the community. Functionally, this would mean that pollen facilitates
666 a segment of the sampled community. It remains to be explored whether pollen might actually
667 play a role in facilitation of snow algae. Future investigations into snow algae bloom food webs
668 will help determine whether these predominantly heterotrophic co-association networks are
669 inducible by a common carbon source such as pollen or algae.

670 Chytridiomycota are seen to be a major component of these red snow algae bloom
671 communities and have previously been shown to co-occur with snow algae (Naff et al., 2013;
672 Brown et al., 2015a; Brown & Jumpponen, 2019). Naff et al. (2013) identified a novel clade of
673 snow chytrids (SC1; later placed into the Mesochytriales (Karpov et al., 2014)) and posed
674 hypotheses of chytrid parasitism on snow algae. Other research has highlighted the evolutionary
675 significance of Chytridiomycota in fostering early diverging Streptophyte colonization of
676 terrestrial habitats (Knack et al. 2015). Additional work (Brown et al., 2015a; Brown &
677 Jumpponen, 2019) has suggested that these snow chytrids are found in abundance in the absence
678 of snow algae colonization, casting doubt on the connection between these chytrids and algae.
679 Here, we provide evidence these snow chytrids may underpin independent but concurrent
680 heterotrophic networks from algae, suggesting that trophic partitioning dynamics drive
681 community connections and assembly, likely driven by carbon inputs from degradation of pollen.
682 Many chytrids are known saprotrophs of Pinaceae pollen (Amon, 1984; Freeman et al., 2009),
683 which was found in increasing concentrations from zones A to P to M. Anemophilous bisaccate
684 pollen, identified to the genus level as *Abies*, *Pinus* or *Picea*, were prevalent in our samples.

685 However, increases in pollen concentration were not associated with increases in Chytrid OTU
686 sequence abundances in this study, but the potential for pollen induction of chytrid populations
687 should not be precluded based on this evidence. For this study Chytridiomycota OTUs
688 constituted the largest relative abundance of all OTU sequences (28%), where a higher relative
689 abundance was observed in the Cascades (91%) than the Rockies (9%). Diversity of
690 Chytridiomycota across both regions included OTUs best identified as: Chytridiomycota
691 unclassified (~97%), *Hyaloraphidium curvatum* (3%), and *Rhizophydium* sp. (<0.001%). The
692 reason for the high abundance of chytrids in the Cascade Mountains and the relatively sparse
693 representation in the Rocky Mountains is unclear but may be due to an abundance of nutritional
694 resources, possibly due to the lower elevation of the Cascades or the presence of polyploidy or
695 aneuploidy resulting in higher ITS2 copy numbers, which is a known feature of many
696 Chytridiomycota (Berman, 2016; Farrer et al., 2017). Given that these abundant chytrids are
697 taxonomically unresolved at levels below phylum and have thus far been unable to be cultured, to
698 fully disentangle chytrid dominated trophic partitioning, additional investigations are needed.
699 We also see numerous and abundant representation of fungi within the Septobasidiales
700 (Pucciniomycetes) and Microbotryales (Microbotryomycetes). These taxa are often cryptic and
701 diminutive, lacking unifying structural features (Frieders et al. 2008). Microbotryomycetes are
702 common in numerous ‘extreme’ environments including: Icelandic snows (Lutz et al., 2015a);
703 clear ice, dark ice and supraglacial water in Greenland (Perini et al., 2019); nival (3,200-3,400m)
704 and alpine (2,600-2,900) soils of Mount Schrankogel in the Central Alps of Tyrol Austria, but not
705 in alpine-nival zones (3,000-3,100m) (Praeg et al., 2019); alpine glacier cryoconites from the
706 Stubaier glacier in Tyrol, Austria (Margesin et al., 2007); and, hypersaline permafrost tarn flats in
707 Northern Victoria Land (Borruso et al., 2018). Microbotryomycetes, which includes the genus

Rhodotorula, a basidiomycetous yeast that is frequently found in snow and ice systems (Brown et al., 2015, Brown & Jumpponen, 2019) have shown to be preferential to recently deglaciated winter soils over summer soils and they appear more similar to communities of snow, ice or cryoconite than to soil communities even further from the glacier (Dresch et al., 2019).

Recent sampling of aeolian dust microbiomes in the Sierra Nevada Mountains in California showed that Microbotryomycetes, specifically the genera *Rhodotorula* and *Rhodosporidiobolus*, relative abundance was positively correlated with increasing elevation. These samples, verified using radioisotope signatures, originated in Asia and traveled thousands of miles to reach the Sierra Nevada Mountains (Maltz et al., 2021). Given the findings in this work it might be hypothesized that the range of diversity within the Microbotryomycetes seen in snows of the Cascades and Rockies may be tied to atmospheric air currents and the subsequent deposition of Microbotryomycetes sourced from far ranging trans-oceanic locales but experiments to test this hypothesis are needed. Conceivably then, snow algae bloom communities in their entirety may be assembled of OTU sub-networks that originate seasonally from discrete locations such as the atmosphere, the underlying soil and from the mature firn of the previous season. If borne out, a question to pose is what degree of interaction exists between snow community sub-networks that have disparate origins.

Certain taxa present in snows also appear to be in a continuum with the underlying soil (Dresch et al., 2019) and atmospheric (Els et al., 2020) microbial communities. These events are interspersed with continuous dry deposition that also infuse snows with nutrients. Interestingly, some pollen, fungal spores and bacteria may have evolved to have low supersaturation levels and/or wind transport potential (Fröhlich-Nowoisky et al., 2016), which may increase the potential for bioorganic deposition. Genera found in an extensive atmospheric survey that overlap

731 with our snows, albeit with scant representation include: *Alternaria*, *Aureobasidium*, *Didymella*,
732 *Knufia Mycosphaerella*, *Rhodotorula* and *Sistotrema* (Woo et al., 2018). This may support the
733 idea that atmospheric seeding of some transient taxa in snows occurs. However, it remains
734 uncertain if this potential atmospheric transfer consists of species that can occupy active niche
735 space and are viable in snows, but if demonstrated, this could extend our understanding of snow-
736 microbe sources.

737 Bisaccate pollen grains were predominant in our snow samples and are shown to
738 positively correlate with *S. nivaloides* cell counts. It is not entirely clear if pollen facilitates algal
739 bloom development via saprotrophic nutrient release, but evidence suggests that trophic
740 partitioning is likely occurring among heterotrophic networks reliant on either autochthonous
741 snow algae *S. nivaloides* or allochthonous pollen sources as primary production inputs. The
742 occurrence of trophic partitioning in these snows suggests the existence of discrete feeding
743 strategies and nutrient requirements among community members (or sub-communities) that
744 results in flows of primary production carbon through separate heterotrophic sub-networks. This
745 is supported by our findings that the *S. nivaloides* (AOTU0001) taxonomic co-association
746 network is largely separate and disconnected from dominant fungi (Figure 6a-b) such as
747 Chytridiomycota unclassified (FOTU0001), unclassified Microbotryomycetes (FOTU0002),
748 Leucosporidiaceae (FOTU0003), and bacteria, such as *Solitalea* sp. (BOTU0001) and
749 *Hymenobacter* sp. (BOTU0002). A similar trend of limited co-association was shown previously
750 by Yakimovich et al. (2020). In the present study, only three of the twenty most abundant OTUs
751 were found to be associated with *S. nivaloides* in the Cascades and none were found to correlate
752 in the Rockies. Similar trophic partitioning patterns have been demonstrated in freshwater lake
753 neustons where chytrids and some actinobacteria initiate a pollen-based microbial food webs by

754 increasing the digestibility of pollen for other microbes (Masclaux et al., 2013); this also may be
755 what is occurring in snows. Here, we have two co-occurring but independent food webs whereby
756 algae and pollen drive trophic networks. Interestingly, natural abundance biomass $\delta^{13}C$ for snow
757 algae (Hamilton & Havig, 2017) and *Pinus* pollen (Descolas-Gros & Schölzel, 2007) do not
758 overlap in range (−24.1 to −26.9‰ and -27.39 to -30.5‰ respectively) which necessitates future
759 spatiotemporal studies of snow algae bloom pulse/press dynamics, using varied nutrient regimes,
760 to discern whether trophic partition networks derive from algal and/or pollen sources. It also
761 indicates the need for development of trophic indexes specific to snows.

762 We initially hypothesized that algae may be facilitating microbial community dynamics,
763 but our pollen and co-association data suggest that pollen may be the main driver of diversity and
764 algae may be relegated to a more minor role independent of primary producing pollen. If pollen
765 does facilitate algal bloom development than it is reasonable to expect they would overlap
766 spatially. However, there are two non-mutually exclusive reasons for why algae and pollen would
767 be coupled: (1) airborne particulates, including non-pollen nutrient sources necessary to promote
768 snow algae growth, are heterogeneously deposited to alpine snows during snow deposition and
769 snow drifting events due to local topological variations; and (2), snow melt dynamics causes an
770 uneven distribution of biomaterial (including algae, pollen and other taxa) that lead to snow algae
771 blooms of varying intensity and minimal taxonomic differences across snow zones as shown in
772 this study. Both scenarios may be possible since snow deposition, distribution, drifting and melt
773 increase in variability over landscapes as spatial complexity increases (Mott et al., 2018).
774 Regional atmospheric concentrations of Pinaceae pollen (2003 – 2017) peaks in late-May in the
775 Cascades (> 100 grains/m^3) and in early-June in the Rockies (10-100 grains/m^3) and has tails
776 extending from February through October (Lo et al., 2019). While limited data exists

777 documenting bloom onset, pollen peaks appear to coincide with the onset of summer, generally
778 when visually apparent snow algae blooms begin to develop. Whether this timing is also reflected
779 in pollen deposition and whether a relationship deeper than an associative and coincident pattern
780 remains to be investigated. It is also important to consider trophic partitioning between snow
781 algae and pollen (or other spore producing plants) in an evolutionary context. Assuming that
782 snow algae divergence predates pollen deposition, then it would make sense that trophic
783 partitioned microbial networks could exist for snow algae and pollen. To help clarify any
784 relationship between pollen and snow algae blooms the authors recommend that future studies of
785 alpine snow algae blooms, consider including a quantitative pollen measure.
786 This study gives the first in depth look at the spatial dynamics of red snow algae bloom
787 microbial communities across bloom zones and reveals parameters and taxa that underly bloom
788 development. Our research suggests that snow algae blooms may have an active peripheral zone
789 and a less active medial and adjacent zone that possibly shift between autotrophic and
790 heterotrophic maxima. These zones may also consist of reliable sub-OTU networks that overlap
791 in niche space, but ultimately co-associate as larger discrete networks due to trophic partitioning
792 mechanisms based on food webs anchored by either algae or pollen. The idea that a sub-
793 community trophic network is anchored by pollen is evidenced by the increased in pollen
794 concentrations concomitantly with snow algae concentrations, thus demonstrating that pollen,
795 while observed and speculated to be of importance in previous studies (Thomas 1972; Hoham
796 1978; Hoham 1987; Nedbalová et al. 2008; Naff et al. 2013) but not typically examined, may be
797 a crucial parameter of future ecological studies of snow algae blooms. Given the overall
798 complexity of this study, these findings require detailed manipulative experiments to either
799 support, refute and/or resolve our claims and to disentangle connections between algae and pollen

800 trophic food-webs and to elucidate a mechanistic understanding of the snow algae associated
801 communities.

802 **Table 1.** PERMANOVA values for environmental traits of algal, bacterial, fungal, and
803 community taxonomic groups. Bolded characteristics were found to be significant ($p < 0.05$).
804

Algae	DF	Pseudo-F	R^2	Prob>F
Potassium	1	3.13	0.10	0.06
pH	1	1.18	0.04	0.27
TDS	1	0.84	0.03	0.36
Conductivity	1	0.78	0.03	0.37
ORP	1	0.87	0.03	0.40
Pollen	1	0.86	0.03	0.42
Nitrate	1	0.8	0.03	0.46
Region	1	0.74	0.03	0.48
Salinity	1	0.59	0.02	0.53
Resistivity	1	0.65	0.02	0.56
Snow Algae	1	0.52	0.02	0.56
Ammonium	1	0.61	0.02	0.56
DO	1	0.16	0.01	0.94

Bacteria	DF	Pseudo-F	R^2	Prob>F
ORP	**1**	**3.56**	**0.11**	**0.01**
Region	**1**	**3.88**	**0.11**	**0.01**
DO	**1**	**2.41**	**0.07**	**0.04**
Potassium	1	2.08	0.06	0.06
pH	1	2.08	0.06	0.07
Nitrate	1	1.73	0.05	0.12
Snow Algae	1	1.23	0.04	0.26
Resistivity	1	1.2	0.04	0.29
Salinity	1	1.05	0.03	0.34
Conductivity	1	1.01	0.03	0.37
TDS	1	1.05	0.03	0.38
Pollen	1	0.99	0.03	0.40
Ammonium	1	0.48	0.01	0.84

Fungi	DF	Pseudo-F	R^2	Prob>F
pH	**1**	**6.67**	**0.17**	**0.00**
Region	**1**	**6.08**	**0.15**	**0.00**
Conductivity	**1**	**2.32**	**0.06**	**0.00**
ORP	**1**	**4.84**	**0.12**	**0.01**
TDS	**1**	**2.22**	**0.06**	**0.01**
Nitrate	**1**	**3.09**	**0.08**	**0.02**
Salinity	**1**	**1.98**	**0.05**	**0.03**
Potassium	**1**	**2.88**	**0.07**	**0.04**
Pollen	1	2.52	0.06	0.06
DO	1	2.36	0.06	0.07
Resistivity	1	1.98	0.05	0.10
Snow Algae	1	1.06	0.03	0.44
Ammonium	1	0.71	0.02	0.55

Community	DF	Pseudo-F	R^2	Prob>F
ORP	**1**	**4.24**	**0.12**	**0.01**
Region	**1**	**4.08**	**0.12**	**0.01**
pH	**1**	**3.84**	**0.11**	**0.01**
Potassium	**1**	**3.11**	**0.09**	**0.03**
Nitrate	1	2.05	0.06	0.06
DO	1	1.75	0.05	0.09
Conductivity	1	1.39	0.04	0.19
TDS	1	1.37	0.04	0.20
Pollen	1	1.41	0.04	0.21
Salinity	1	1.23	0.04	0.29
Resistivity	1	1.11	0.03	0.35
Snow Algae	1	0.83	0.02	0.58
Ammonium	1	0.63	0.02	0.72

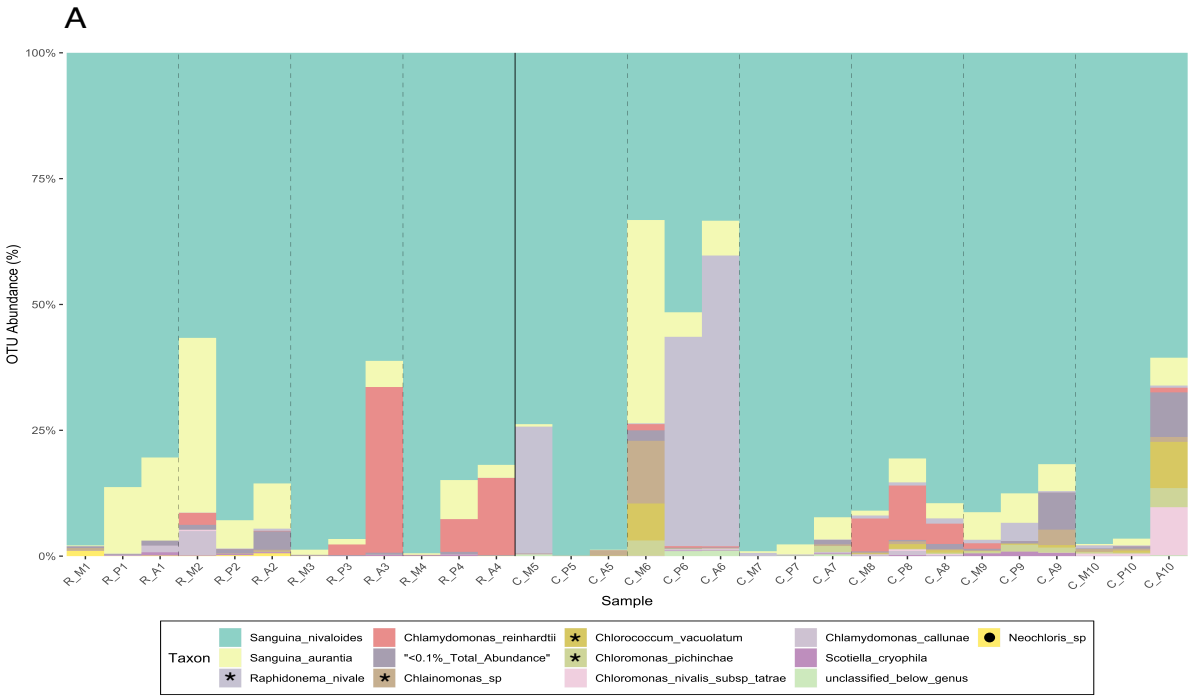
805

806 **Table 2.** Wilcoxon Rank-Sum tests of environmental parameters and mean values between the
807 Rockies and Cascades.
808

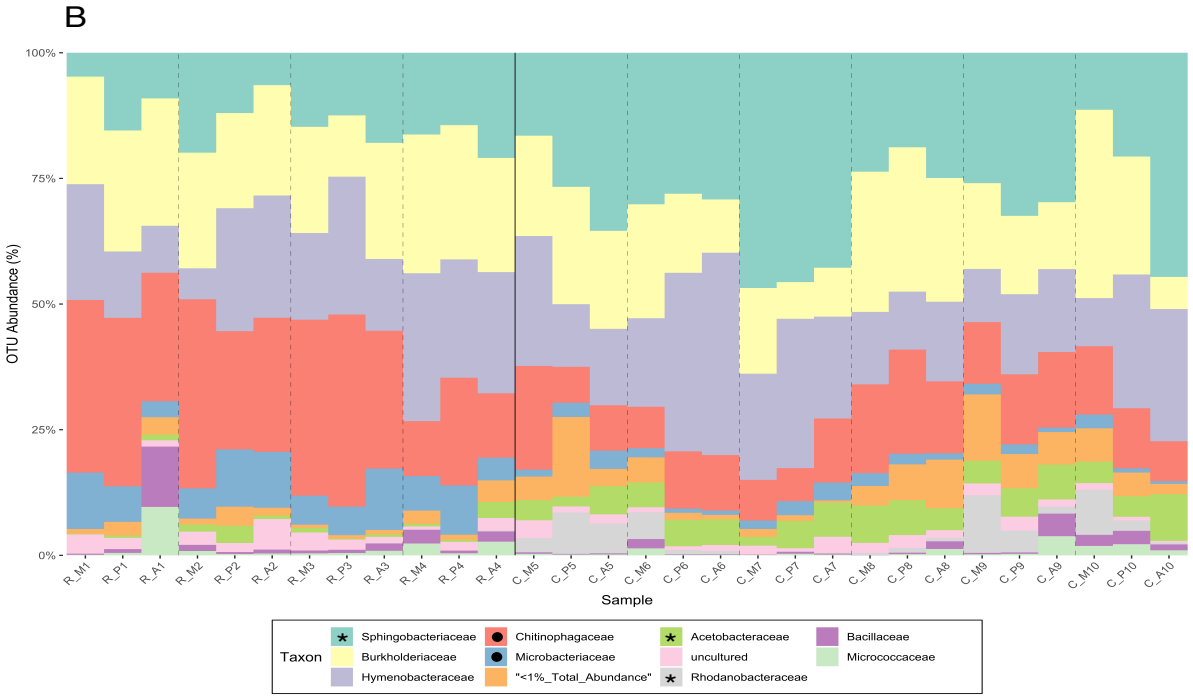
	Rockies-Cascades			
Environmental Parameter	**Test Statistic (S)**	**Prob > S**	$\bar{X}_C$	$\bar{X}_R$
DO (%)	**39**	**0**	43	57
Conductivity (µS/cm)	**28.5**	**0.02**	3.00	11.67
Resistivity (MΩ・cm)	-28.5	0.98	0.42	0.21
TDS (ppm)	**27.5**	**0.02**	1.50	5.92
Salinity (PSU)	11.5	0.25	0	0
NO_3^- (mv)	-38	1	118	83
NH_4^+ (mv)	-9	0.74	-214	-220
K^+ (mv)	17.5	0.09	-204	-197
Algae (cells/mL)	-18	0.92	79167	34667
Pollen (grains/mL)	-3.5	0.62	678	833
pH	**23**	**0.04**	6.74	7.64
ORP (mv)	-33	1	16.22	-6.40

809 **Table 3.** Significant OTU comparisons by snow zones medial (M), peripheral (P) and adjacent
810 (A) at varying taxonomic levels (Wilcoxon Rank-Sum; $p < 0.10$).
811

Taxon	Zone 1	Zone 2	Median Difference	Wilcoxon p-value
		Algae		
Sanguina_nivaloides	P	A	0.11	0.05
Viridiplantae	P	A	0.05	0.06
Chlorophyta	P	A	0.05	0.06
Sanguina	P	A	0.1	0.06
Chlorophyceae	P	A	0.07	0.11
Chlamydomonadales	P	A	0.07	0.11
Chlamydomonadaceae	P	A	0.07	0.11
		Bacteria		
Polaromonas	M	A	0.01	0
Polaromonas	P	A	0.02	0.01
Gammaproteobacteria	M	A	0.02	0.02
Proteobacteria	M	A	0.02	0.04
Betaproteobacteriales	M	A	0.02	0.04
		Fungi		
Burkholderiaceae	M	A	0.02	0.05
Microbotryomycetes	P	A	-0.07	0.06
Fungi	P	A	-0.06	0.08
Basidiomycota	P	A	-0.05	0.08
Agaricomycetes	M	A	0.01	0.09
Microbotryomycetes	P	A	-0.03	0.11


A
100%
75%
50%
25%
0%
OTU Abundance (%)
R_M1 R_P1 R_A1 R_M2 R_P2 R_A2 R_M3 R_P3 R_A3 R_M4 R_P4 R_A4 C_M5 C_P5 C_A5 C_M6 C_P6 C_A6 C_M7 C_P7 C_A7 C_M8 C_P8 C_A8 C_M9 C_P9 C_A9 C_M10 C_P10 C_A10
Sample
Taxon
Sanguina_nivaloides
Sanguina_aurantia
Raphidonema_nivale
Chlamydomonas_reinhardtii
"<0.1%_Total_Abundance"
Chlainomonas_sp
Chlorococcum_vacuolatum
Chloromonas_pichinchae
Chloromonas_nivalis_subsp_tatrae
Chlamydomonas_callunae
Scotiella_cryophila
unclassified_below_genus
Neochloris_sp

812


B
100%
75%
50%
25%
0%
OTU Abundance (%)
R_M1 R_P1 R_A1 R_M2 R_P2 R_A2 R_M3 R_P3 R_A3 R_M4 R_P4 R_A4 C_M5 C_P5 C_A5 C_M6 C_P6 C_A6 C_M7 C_P7 C_A7 C_M8 C_P8 C_A8 C_M9 C_P9 C_A9 C_M10 C_P10 C_A10
Sample
Taxon
Sphingobacteriaceae
Burkholderiaceae
Hymenobacteraceae
Chitinophagaceae
Microbacteriaceae
"<1%_Total_Abundance"
Acetobacteraceae
uncultured
Rhodanobacteraceae
Bacillaceae
Micrococcaceae

813

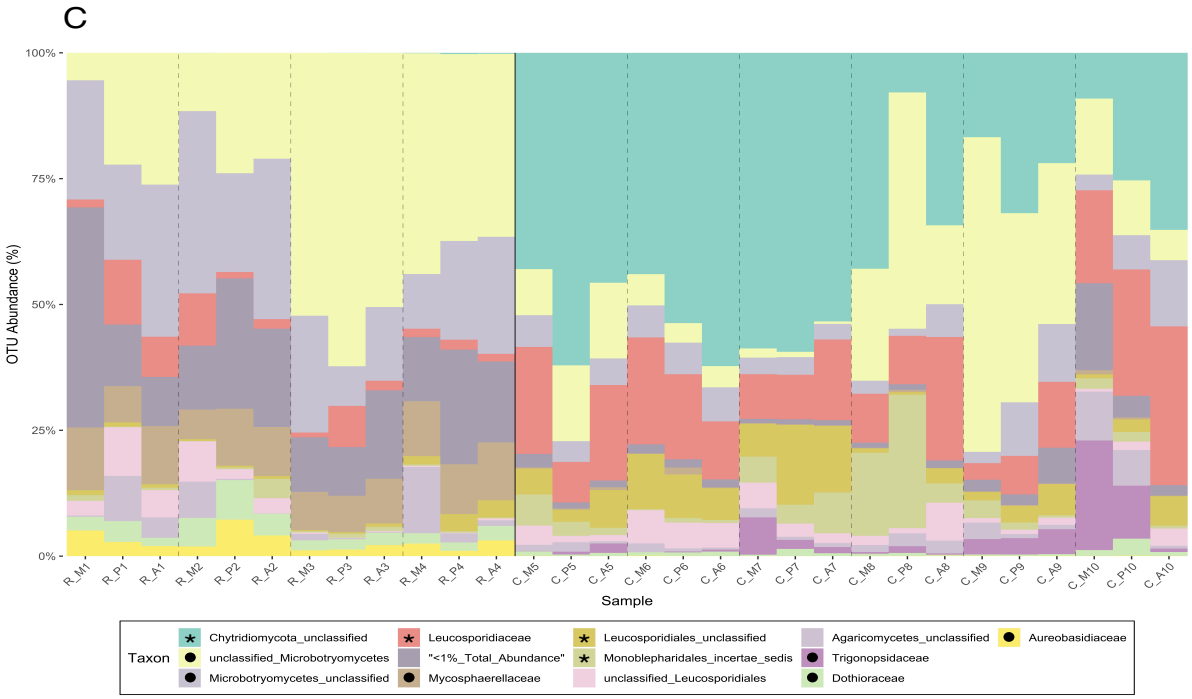


Figure 1 (A-C). Algal (A), bacterial (B), and fungal (C) relative abundance chart resolved at the family, family, and species levels respectively. Samples from the Cascades (C_), Rockies (R_) and snow bloom zones (M, P, or A) are noted on the x-axis. Taxa below 0.1% of the total OTU abundance were grouped together. Legend asterisks (*) and circles (•) denote taxa with OTU counts found to be significantly higher between the Cascades or Rockies samples respectively (Wilcoxon Rank Sum; $p < 0.05$). Differences for "<0.1% Total Abundance" were not tested for significance.

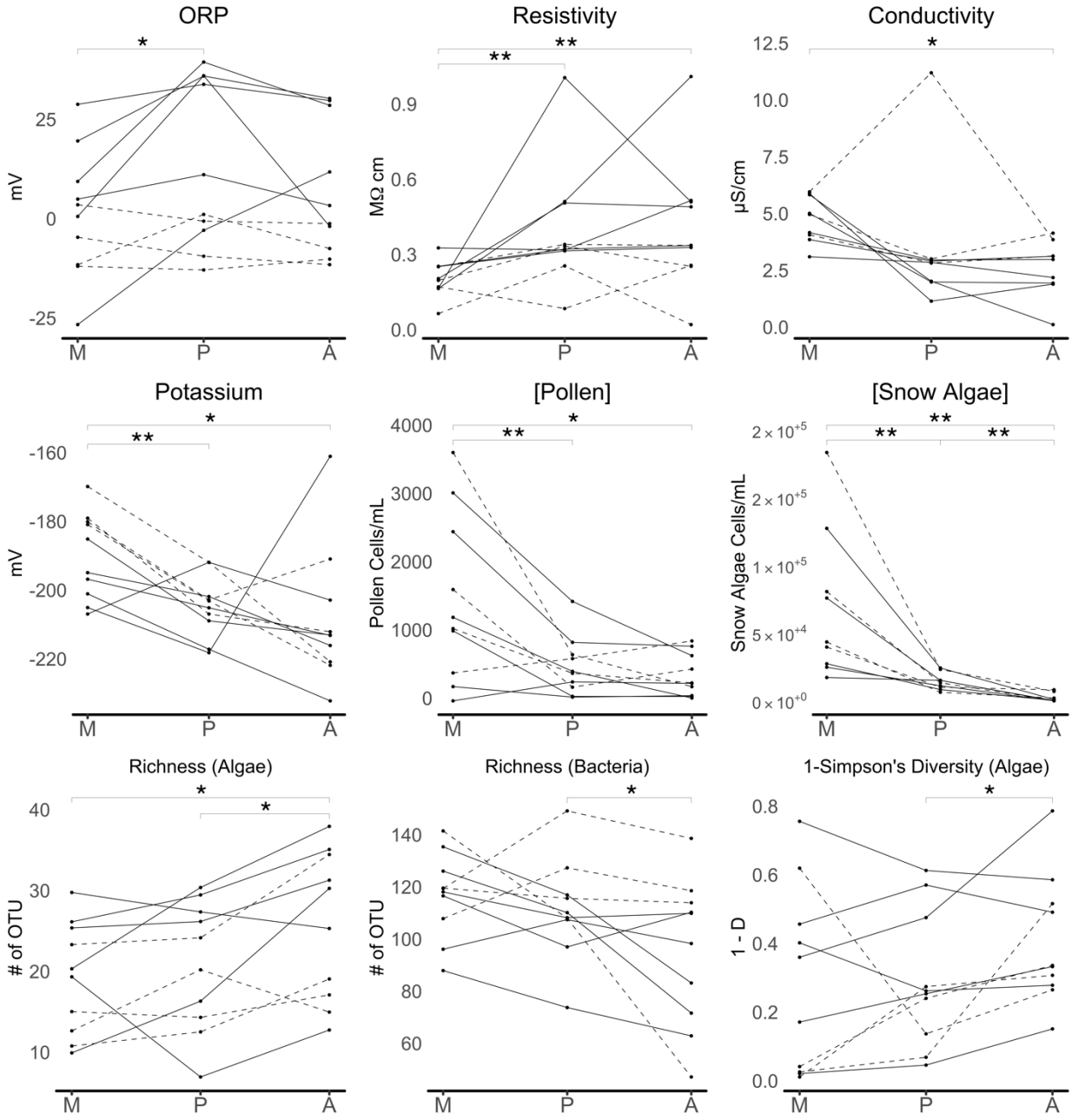
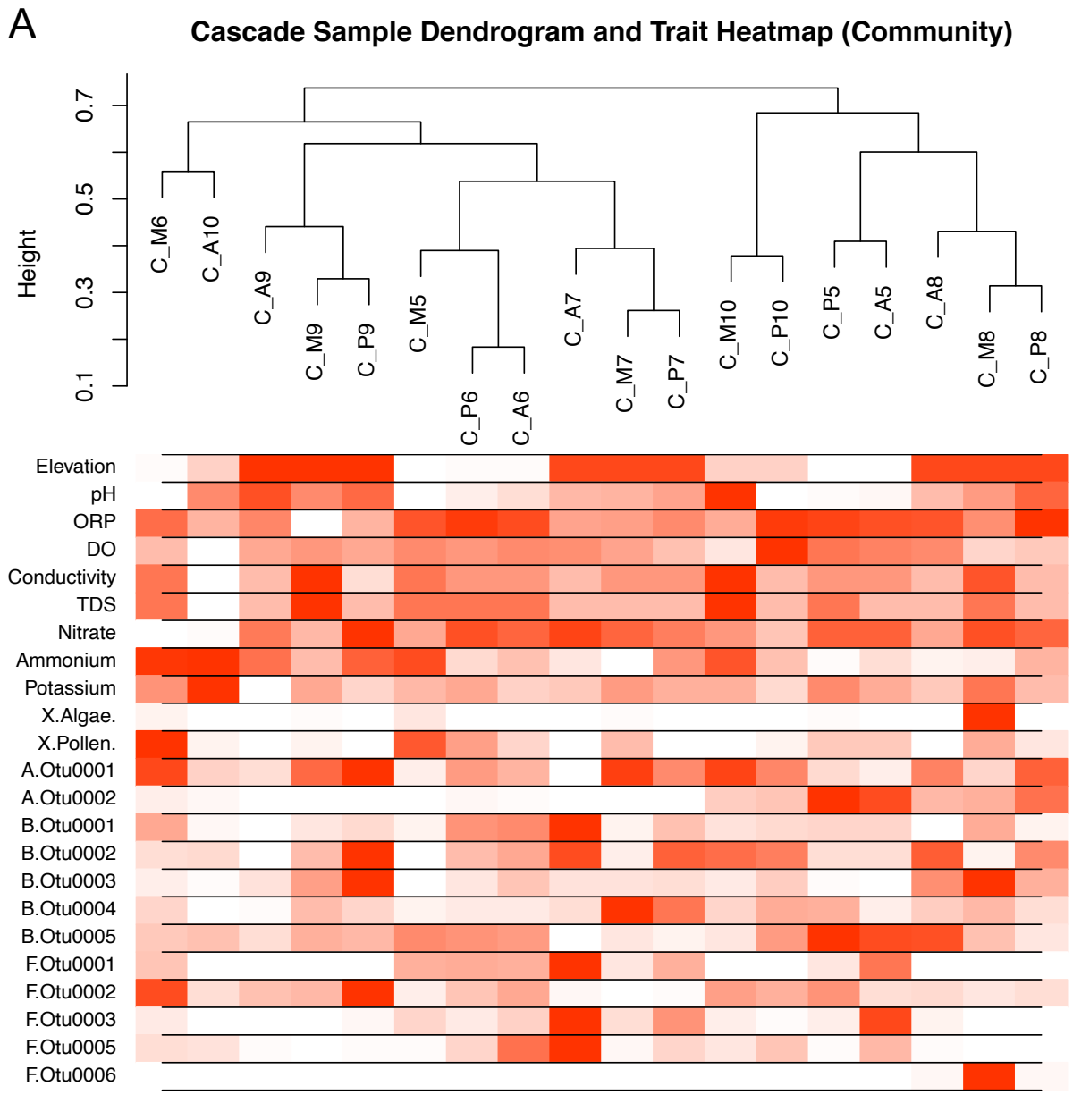


Figure 2. Parallel plots showing significantly different Wilcoxon Rank-Sum tests of environmental traits and diversity estimators between paired snow algae bloom zones M, P and A (* for $p<0.05$ and ** for $p<0.01$) and the Cascades and Rockies (solid and dashed lines respectively). Parameters not included were not found to have pairwise significance. Plots were jittered to better represent individual samples.

A


Cascade Sample Dendrogram and Trait Heatmap (Community)
Height
0.1
0.3
0.5
0.7
C_M6
C_A10
C_A9
C_M9
C_P9
C_M5
C_P6
C_A6
C_A7
C_M7
C_P7
C_M10
C_P10
C_P5
C_A5
C_A8
C_M8
C_P8
Elevation
pH
ORP
DO
Conductivity
TDS
Nitrate
Ammonium
Potassium
X.Algae.
X.Pollen.
A.Otu0001
A.Otu0002
B.Otu0001
B.Otu0002
B.Otu0003
B.Otu0004
B.Otu0005
F.Otu0001
F.Otu0002
F.Otu0003
F.Otu0005
F.Otu0006

830

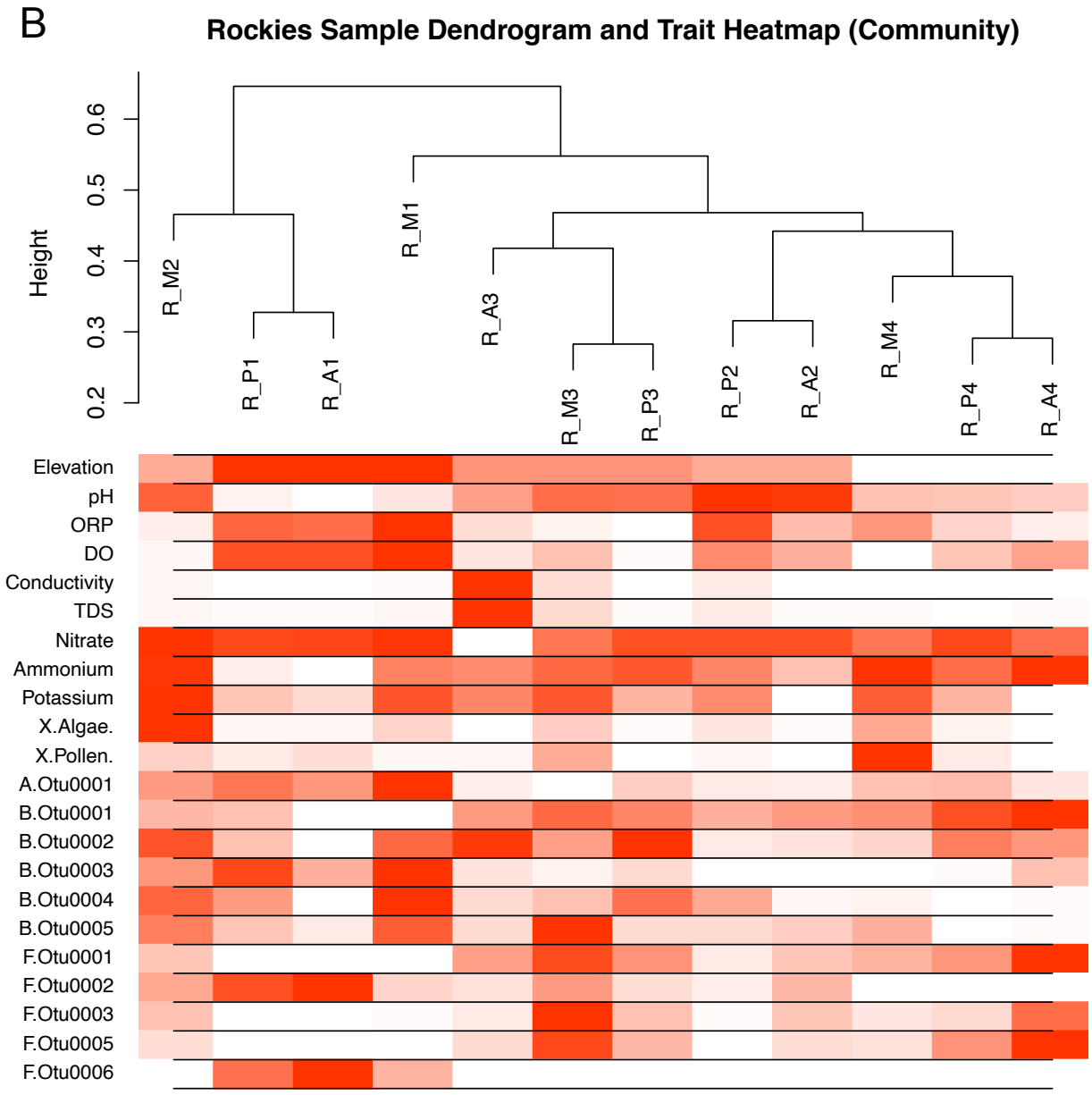
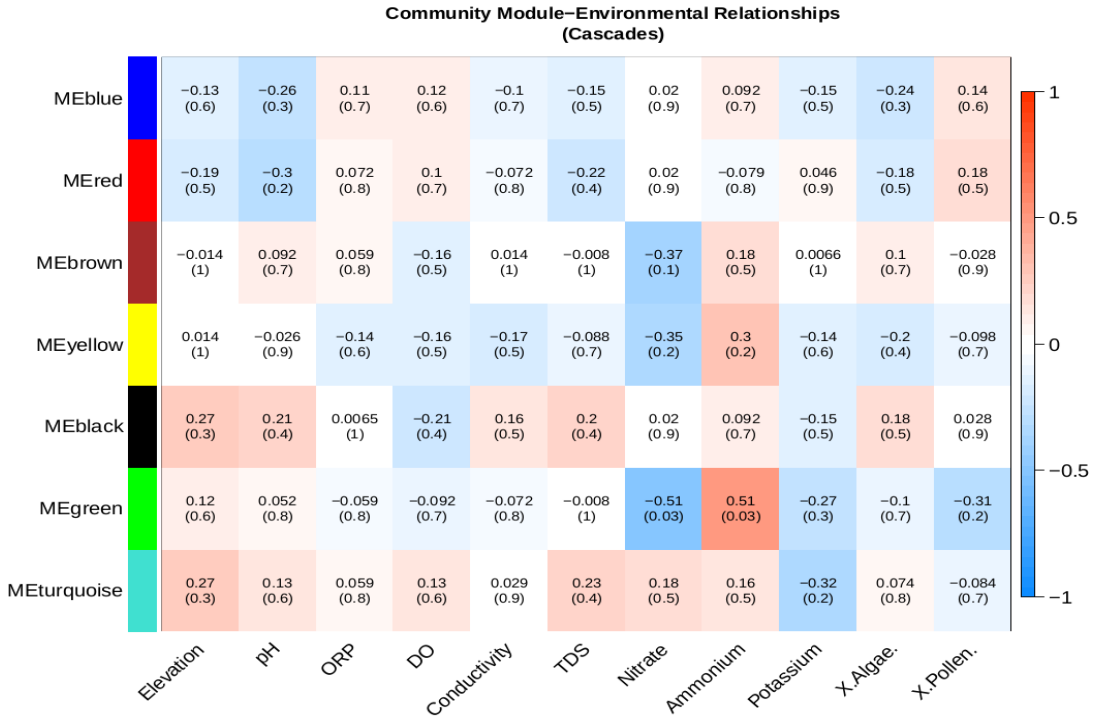


Figure 3 (A-B). Hierarchical clustering of Cascade (A) and Rocky Mountain (B) snow community samples plotted against environmental traits and OTU relative abundances. Grey values depict the ordering for hierarchical tree assembly. White squares indicate a low numeric value for an environmental trait or OTU relative abundance and an increasing intensity of red indicates a higher numeric value.



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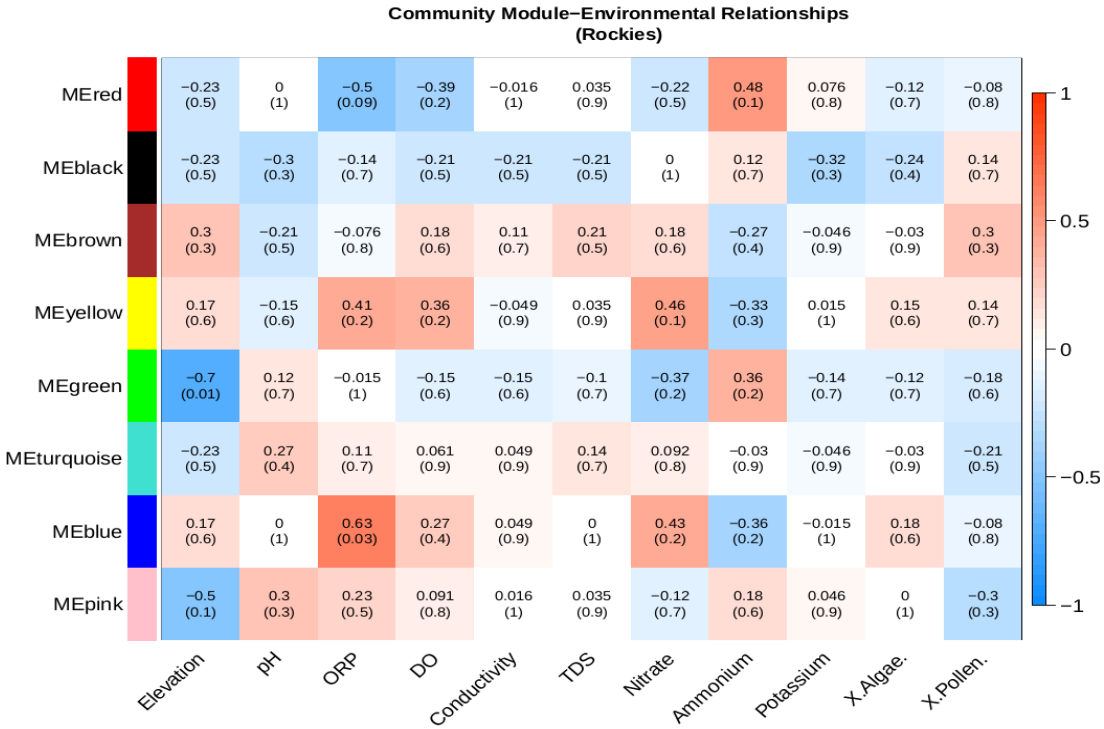
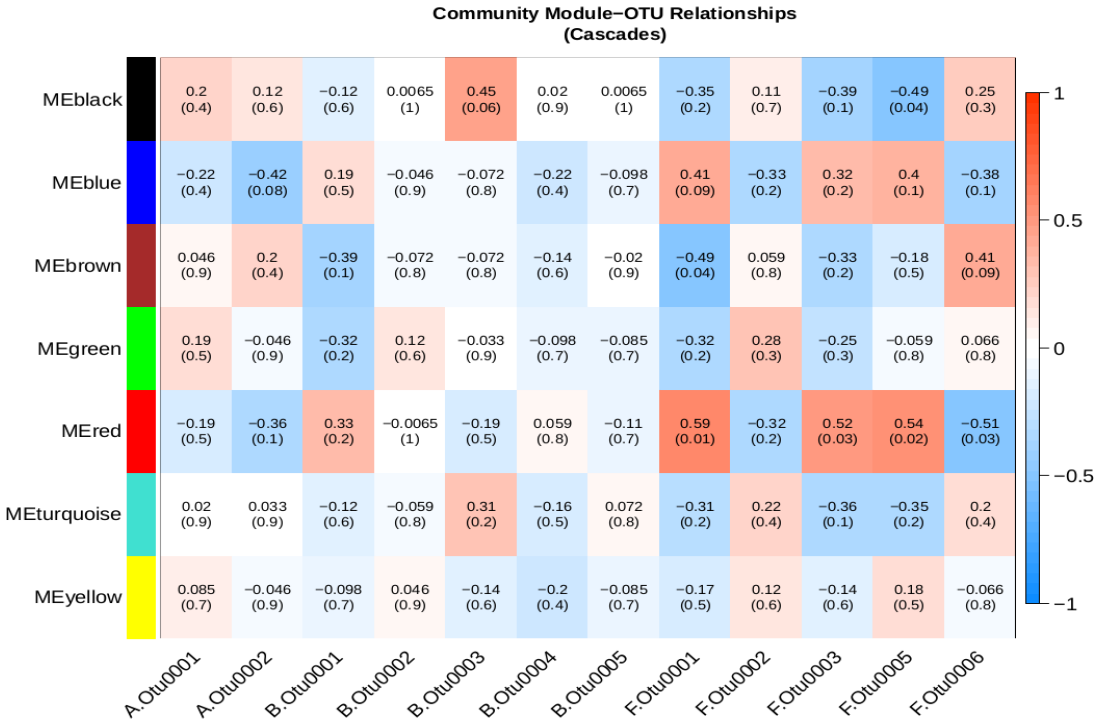


838
839

840 **Figure 4 (A-B).** Heat matrix showing snow community modules in the Cascades (A) and
841 Rockies (B) correlated (Kendall-Tau) with environmental parameters. The upper value in each
842 individual square is the correlation and the lower value is the p-values. Modules of the same color
843 in different regions are not comprised of the same OTUs.



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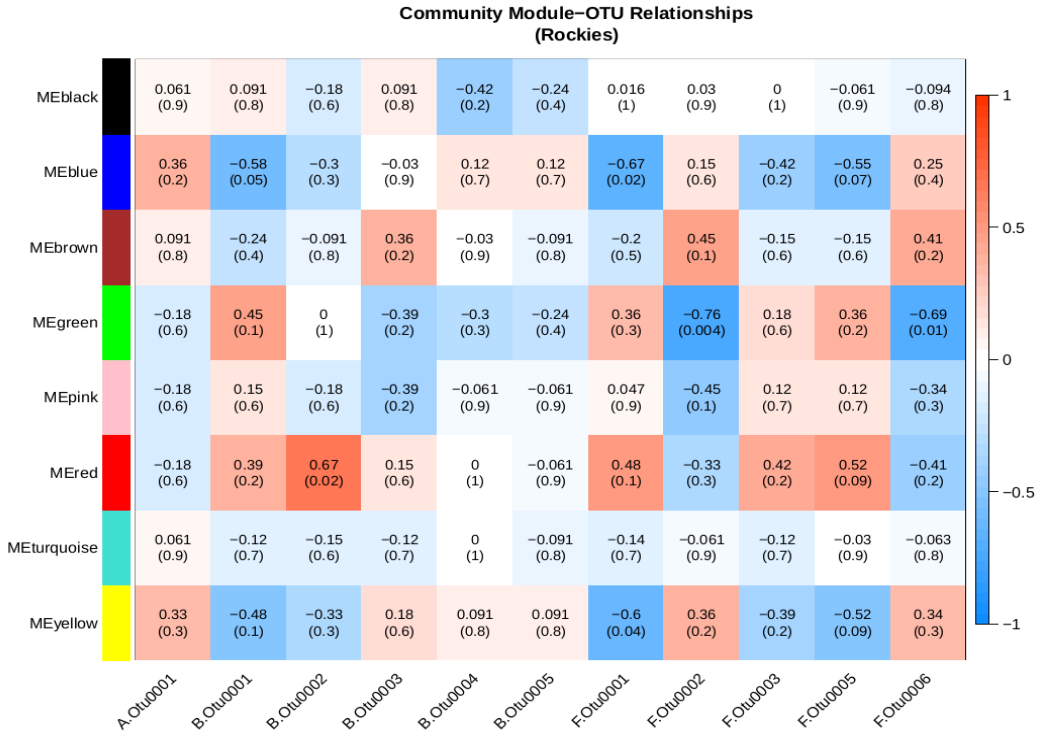

845
846

847 **Figure 5 (A-B).** Heat matrix showing snow community modules in the Cascades (A) and
848 Rockies (B) correlated (Kendall-Tau) with user selected highly abundant OTUs of interest. The
849 upper value in each individual square is the correlation and the lower value is the p-values.
850 Modules of the same color in different regions are not comprised of the same OTUs.

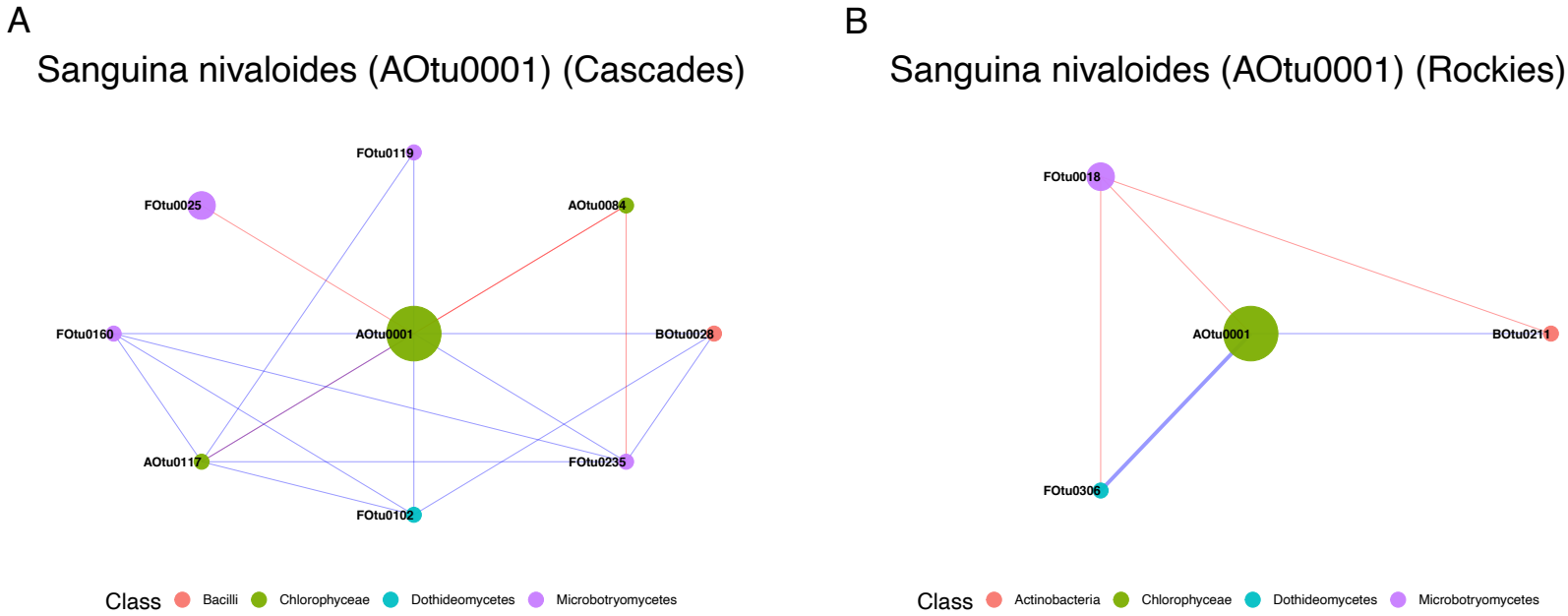

Figure 6. (A-B) *Sanguina nivaloides* (AOtu0001) co-association network from the Cascade (Edges = 18; Vertices = 9; Transitivity = 0.81; Average-Path Length = 1.5) and (Edges = 5; Vertices = 4; Transitivity = 0.83; Average-Path Length = 1.17) Rocky Mountains. Red lines indicate a negative correlation while blue lines indicate a positive correlation. Thin lines have a $|K| < 0.6$; moderate lines have a $|K| \geq 0.6$ and < 0.8; thick lines have a $|K| \geq 0.8$. Vertex colors are randomly generated and not necessarily the same across graphics.

C

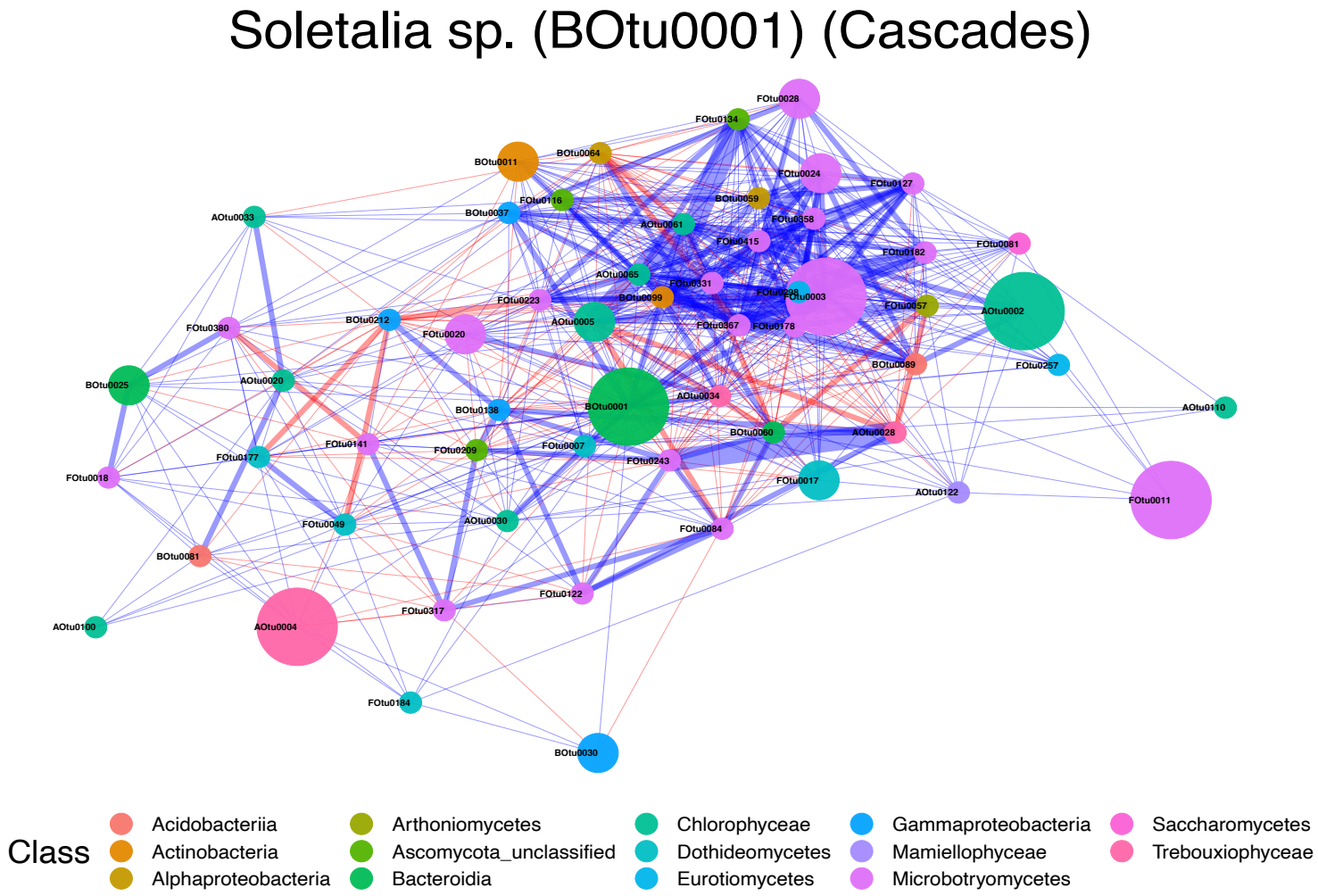

Figure 6. (C) *Solitalea* sp. (BOtu0001) co-association network from the Cascade Mountains (Edges = 655; Vertices = 58; Transitivity = 0.68; Average-Path Length = 1.6).

D

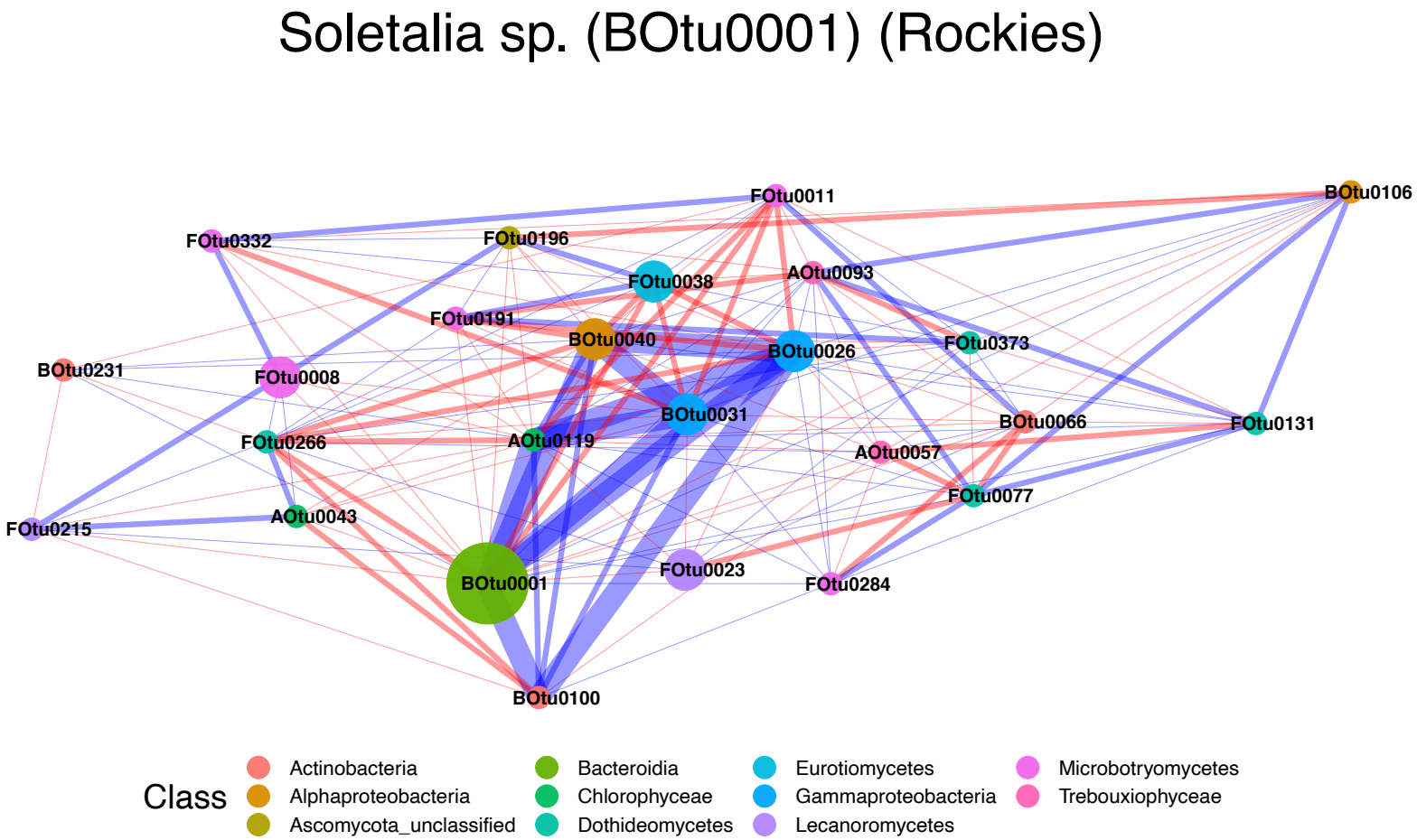

Figure 6. (D) *Solitalea* sp. (BOtu0001) co-association network from the Rocky Mountains (Edges = 161; Vertices = 25; Transitivity = 0.72; Average-Path Length = 1.4).

E

unclassified Chytridiomycota (FOtu0001) (Cascades)

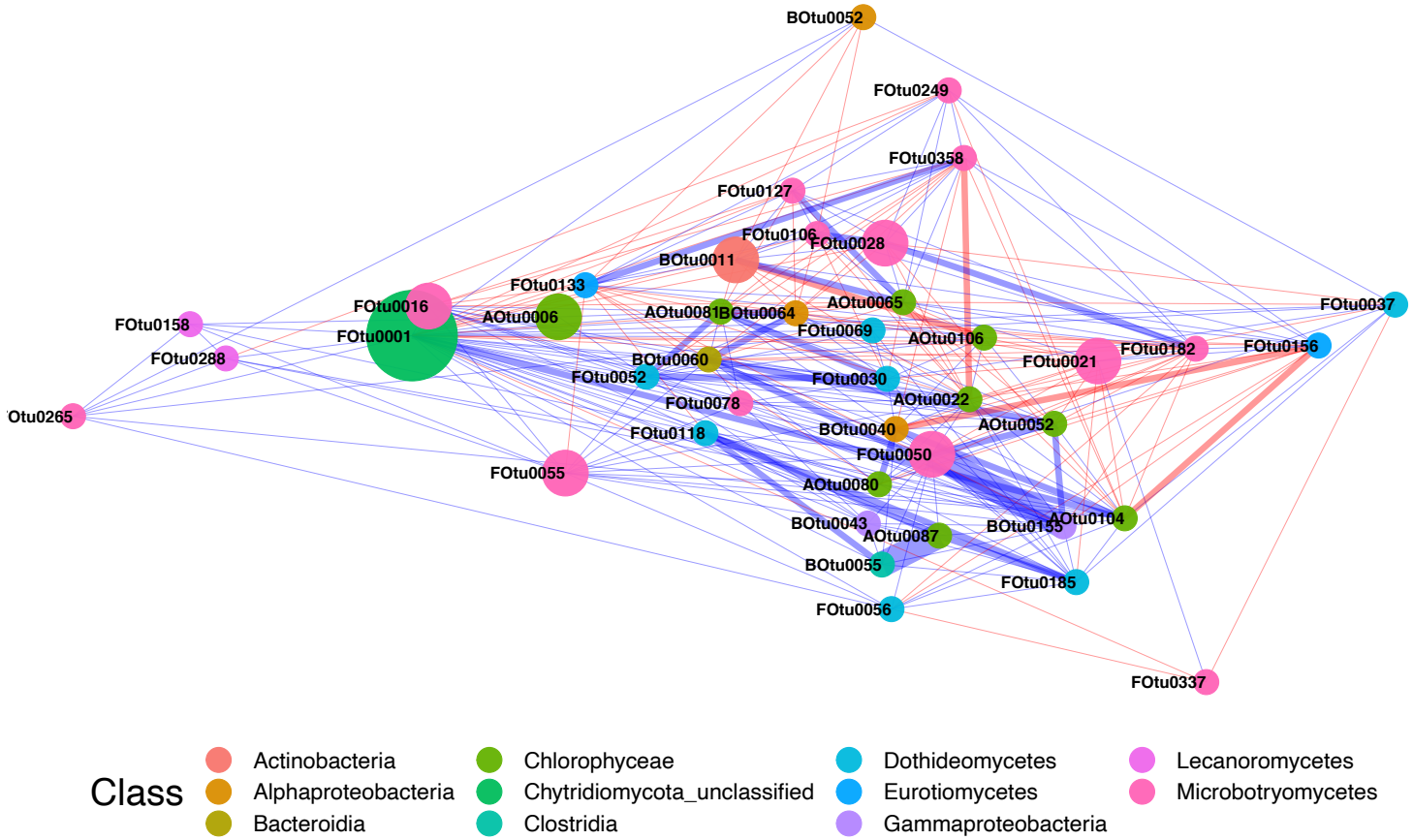

Figure 6. (E) unclassified Chytridiomycota (FOtu0001) co-association network from the Cascade Mountains (Edges = 655; Vertices = 58; Transitivity = 0.68; Average-Path Length = 1.6).

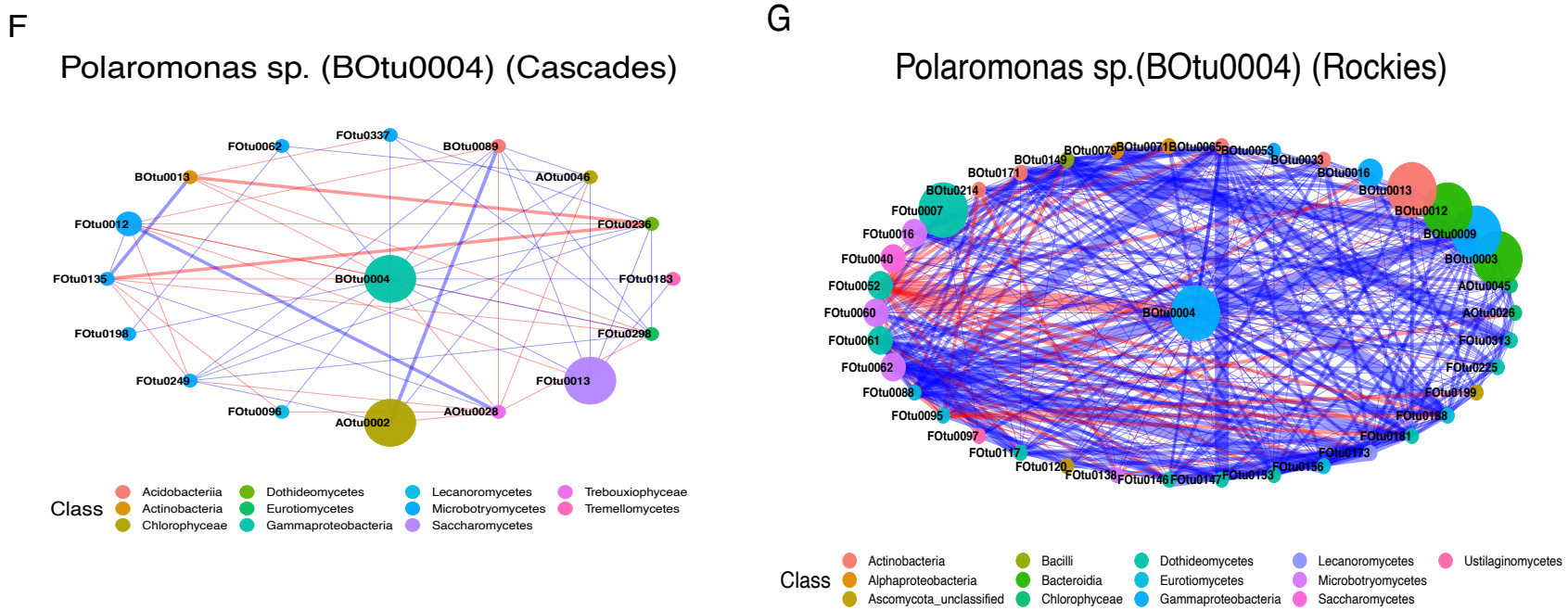

Figure 6. (F-G) *Polaromonas* sp. co-association networks from the Cascade (Edges = 55; Vertices = 517; Transitivity = 0.75; Average-Path Length = 1.6) and Rocky Mountains (Edges = 376; Vertices = 39; Transitivity = 0.72; Average-Path Length = 1.49).

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877 **References**
878
879 Abeliovich, A., & Dikbuck, S. (1977). Factors Affecting Infection of *Scenedesmus obliquus* by a
880 *Chytridium* sp. in Sewage Oxidation Ponds. *Applied and Environmental Microbiology*,
881 *34*(6), 832–836. https://doi: 10.1128/AEM.34.6.832-836.1977
882 Ahlmann-Eltze, C. (2019). *ggsignif: Significance Brackets for "ggplot2."* R package version
883 0.5.0.
884 Amon, J.P. (1984). *Rhizophydium littoreum*: A Chytrid from Siphonaceous Marine Algae: An
885 Ultrastructural Examination. *Mycologia*, *76*(1), 132–139. doi:
886 10.1080/00275514.1984.12023817
887 Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance.
888 *Austral ecology*, *26*(1), 32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x
889 Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU
890 rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic*
891 *Microbial Ecology*, *75*(2), 129–137. doi: 10.3354/ame01753
892 Aristotle (1878). Book the Fifth: Chapter XVII. In R. Cresswell (tran.), *Aristotle's History of*
893 *Animals in Ten Books* (p. 126). London, UK: George Bell & Sons.
894 Auguié, B. (2017). *gridExtra: Miscellaneous Functions for "Grid" Graphics.* R package version
895 2.3.
896 Azzoni, R. S., Tagliaferri, I., Franzetti, A., Mayer, C., Lambrecht, A., Compostella, C., ...
897 Ambrosini, R. (2018). Bacterial diversity in snow from mid-latitude mountain areas:
898 Alps, Eastern Anatolia, Karakoram and Himalaya. *Annals of Glaciology*, *59*(77), 10–20.
899 doi: 10.1017/aog.2018.18
900 Bauer, F. (1820). Red snow of Baffin's Bay. *American Journal of Science and Arts*, *2*, p. 356.
901 Berman, J. (2016). Ploidy plasticity: A rapid and reversible strategy for adaptation to stress.
902 *FEMS Yeast Research*, *16*(3), 1–5. doi: 10.1093/femsyr/fow020.
903 Bolstad, B. (2019). *preprocessCore: A collection of pre-processing functions*. R package version
904 1.46.0.
905 Borruso, L., Sannino, C., Selbmann, L., Battistel, D., Zucconi, L., Azzaro, M., Turchetti, B., ...
906 Guglielmin, M. (2018). A thin ice layer segregates two distinct fungal communities in
907 Antarctic brines from Tarn Flat (Northern Victoria Land). *Scientific Reports*, *8*, 6582. doi:
908 10.1038/s41598-018-25079-3
909 Bourgeois, J. C., Gajewski, K., & Koerner, R. M. (2001). Spatial patterns of pollen deposition in
910 arctic snow. *Journal of Geophysical Research*, *106*(D6), 5255–5265. doi:
911 10.1029/2000JD900708
912 Brown, R. (2015). List of Plants collected by the Officers, & c., in Captain Ross's voyage, on the
913 coasts of Baffin's Bay. In J.J. Bennett (Ed.), *The miscellaneous botanical works of Robert*
914 *Brown* (pp. 175–178). Cambridge, UK: Cambridge University Press.
915 Brown, S. P., & Jumpponen, A. (2014). Contrasting primary successional trajectories of fungi
916 and bacteria in retreating glacier soils. *Molecular Ecology*, *23*(2), 481–497. doi:
917 10.1111/mec.12487
918 Brown, S. P., & Jumpponen, A. (2015). Phylogenetic diversity analyses reveal disparity between
919 fungal and bacterial communities during microbial primary succession. *Soil Biology and*
920 *Biochemistry*, *89*, 52–60. doi: 10.1016/j.soilbio.2015.06.025
921 Brown, S. P., Olson, B. J. S. C., & Jumpponen, A. (2015a). Fungi and Algae Co-Occur in Snow:

922 An Issue of Shared Habitat or Algal Facilitation of Heterotrophs? *Arctic, Antarctic, and*
923 *Alpine Research*, *47*(4), 729–749. doi: 10.1657/AAAR0014-071
924 Brown, S. P., Veach, A. M., Rigdon-Huss, A. R., Grond, K., Lickteig, S. K., Lothamer, K.,
925 Oliver, A. K., & Jumpponen, A. (2015b). Scraping the bottom of the barrel: are rare high
926 throughput sequences artifacts? *Fungal Ecology*, *13*, 221–225. doi:
927 10.1016/j.funeco.2014.08.006
928 Brown, S. P., & Jumpponen, A. (2019). Microbial Ecology of Snow Reveals Taxa-Specific
929 Biogeographical Structure. *Microbial Ecology*, *77*(4), 946–958. doi: 10.1007/s00248-019-
930 01357-z
931 Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P.
932 ... Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of
933 sequences per sample. *Proceedings of the National Academy of Sciences of the United*
934 *States of America*, *108 Suppl 1*(Suppl 1), 4516–4522. doi: 10.1073/pnas.1000080107
935 Carlson, M. (2019). *GO.db: A set of annotation maps describing the entire Gene Ontology*. R
936 package version 3.8.2.
937 Chander, A. M., Nair, R. G., Kaur, G., Kochhar, R., Dhawan, D. K., Bhadada, S. K., & Mayilraj,
938 S. (2017). Genome Insight and Comparative Pathogenomic Analysis of *Nesterenkonia*
939 *Jeotgali* Strain CD08_7 Isolated from Duodenal Mucosa of Celiac Disease Patient.
940 *Frontiers in Microbiology*, *8*, 129. doi: 10.3389/fmicb.2017.00129
941 Chen, W., Simpson, J., & Levesque, C.A. (2018). *RAM: R for Amplicon-Sequencing-Based*
942 *Microbial-Ecology*. R package version 1.2.1.7.
943 Clark, F.C. (1875). Red Snow. *American Naturalist*, *9*(3), 129–135. doi: 10.1086/271455
944 Cohen, P. A., & Macdonald, F. A. (2015). The Proterozoic Record of Eukaryotes. *Paleobiology*,
945 *41*(4), 610–632. doi: 10.1017/pab.2015.25
946 Da, X., Zhao, Y., Zheng, R., Wang, L., Chang, X., Zhang, Y., Yang, J., & Peng, F. (2019).
947 Nakamurella Antarctica Sp. Nov., Isolated from Antarctica South Shetland Islands Soil.
948 *International Journal of Systematic and Evolutionary Microbiology*, *69*(12), 3710–3715.
949 doi: 10.1099/ijsem.0.003507
950 Darwin, C. (1878). Chapter XV: Red Snow. *Journal of researches into the natural history and*
951 *geology of the countries visited during the voyage of H.M.S. Beagle round the world,*
952 *under the command of Capt. Fitz Roy / by Charles Darwin* (pp. 322-323). New York,
953 New York: D. Appleton and Company.
954 Davey, M. P., Norman, L., Sterk, P., Huete-Ortega, M., Bunbury, F., Loh, ... Smith, A. G. (2019).
955 Snow algae communities in Antarctica: Metabolic and taxonomic composition. *The New*
956 *Phytologist*, *222*(3), 1242–1255. doi: 10.1111/nph.15701
957 Deen, M., & de Rooij, M. (2020). *ClusterBootstrap: Analyze Clustered Data with Generalized*
958 *Linear Models using the Cluster Bootstrap*. R package version 1.1.0.
959 Descolas-Gros, C., & Schölzel, C. (2007). Stable isotope ratios of carbon and nitrogen in pollen
960 grains in order to characterize plant functional groups and photosynthetic pathway types.
961 *The New Phytologist*, *176*(2), 390–401. doi: 10.1111/j.1469-8137.2007.02176.x
962 Dresch, P., Falbesoner, J., Ennemoser, C., Hittorf, M., Kuhnert, R., & Peintner, U. (2019).
963 Emerging from the ice-fungal communities are diverse and dynamic in earliest soil
964 developmental stages of a receding glacier. *Environmental Microbiology*, *21*(5), 1864–
965 1880. doi: 10.1111/1462-2920.14598
966 Els, N., Greilinger, M., Reisecker, M., Tignat-Perrier, R., Baumann-Stanzer, K., Kasper-Giebl,

A., ... Larose, C. (2020). Comparison of bacterial and fungal composition and their chemical interaction in free tropospheric air and snow over an entire winter season at Mount Sonnblick, Austria. *Frontiers in Microbiology*, *11*, 980. doi: 10.3389/fmicb.2020.00980

Engstrom, C. B., Yakimovich, K. M., & Quarmby, L. M. (2020). Variation in snow algae blooms in the coast range of British Columbia. *Frontiers in Microbiology*, *11*, 569. doi: 10.3389/fmicb.2020.00569

Farrer, R. A., Martel, A., Verbrugghe, E., Abouelleil, A., Ducatelle, R., Longcore, J. E., ... Cuomo, C. A. (2017). Genomic innovations linked to infection strategies across emerging pathogenic chytrid fungi. *Nature Communications*, *8*, 14742. doi: 10.1038/ncomms14742

Foster, Z. S. L., Sharpton, T. J., & Grünwald, N. J. (2017). Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. *PLoS Computational Biology*, *13*(2), e1005404. doi: 10.1371/journal.pcbi.1005404

Freeman, K. R., Martin, A. P., Karki, D., Lynch, R. C., Mitter, M. S., Meyer, A. F., ... Schmidt, S. K. (2009). Evidence that chytrids dominate fungal communities in high-elevation soils. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(43), 18315–18320. doi: 10.1073/pnas.0907303106

Fröhlich-Nowoisky, J., Kampf, C. J., Weber, B., Huffman, J. A., Pöhlker, C., Andreae, M. O., ... Pöschl, U. (2016). Bioaerosols in the Earth System: Climate, health, and ecosystem interactions. *Atmospheric Research*, *182*, 346–376. doi: 10.1016/j.atmosres.2016.07.018

Ganey, G. Q., Loso, M. G., Burgess, A. B., & Dial, R. J. (2017). The role of microbes in snowmelt and radiative forcing on an Alaskan icefield. *Nature Geoscience*, *10*(10), 754–759. doi: 10.1038/ngeo3027

Garnier, S. (2018). *viridis: Default Color Maps from 'matplotlib'*. R package version 0.5.1.

Garric, R. K. (1965). The cryoflora of the pacific northwest. *American Journal of Botany*, *52*(1), 1–8. doi: 10.1002/j.1537-2197.1965.tb06750.x

Gawor, J., Grzesiak, J., Sasin-Kurowska, J., Borsuk, P., Gromadka, R., Górniak, D., ... Zdanowski M. K. (2016). Evidence of adaptation, niche separation and microevolution within the genus *Polaromonas* on Arctic and Antarctic glacial surfaces. *Extremophiles*, *20*(4), 403–413. doi: 10.1007/s00792-016-0831-0

Gibbs, G. (1871). Red Snow in Washington Territory. *The American Naturalist*, *5*(2), 116–117. Retrieved from https://www.jstor.org/stable/2447422

Gorton, H. L., & Vogelmann, T. C. (2003). Ultraviolet radiation and the snow alga *Chlamydomonas nivalis* (Bauer) Wille. *Photochemistry and Photobiology*, *77*(6), 608–615. doi: 10.1562/0031-8655(2003)077<0608:uratsa>2.0.co;2

Gray, A., Krolikowski, M., Fretwell, P., Convey, P., Peck, L. S., Mendelova, M., ... Davey, M. P. (2020). Remote sensing reveals Antarctic green snow algae as important terrestrial carbon sink. *Nature Communications*, *11*(1), 2527. doi:10.1038/s41467-020-16018-w,

Hamilton, T. L., & Havig, J. (2017). Primary productivity of snow algae communities on stratovolcanoes of the Pacific Northwest. Geobiology, *15*(2), 280–295. doi: 10.1111/gbi.12219

Harding, T., Jungblut, A.D., Lovejoy, C., & Vincent, W. F. (2011). Microbes in high arctic snow and implications for the cold biosphere. *Applied and Environmental Microbiology*, *77*(10), 3234–3243. doi: 10.1128/AEM.02611-10

Harrold, Z. R., Hausrath, E. M., Garcia, A. H., Murray, A. E., Tschauner, O., Raymond, J. A., &

Huang, S. (2018). Bioavailability of Mineral-Bound Iron to a Snow Algal-Bacterial Coculture and Implications for Albedo-Altering Snow Algal Blooms. *Applied and Environmental Microbiology*, *84*(7), e02322-17. doi: 10.1128/AEM.02322-17

Hassett, B. T., & Gradinger, R. (2016). Chytrids dominate arctic marine fungal communities. *Environmental Microbiology*, *18*(6), 2001–2009. doi: 10.1111/1462-2920.13216

Hastie, T., Tibshirani, R., Narasimhan, B., & Chu, G. (2019). *impute: Imputation for microarray data*. R package version 1.58.0.

Hausser, J., & Strimmer, K. (2014). *entropy: Estimation of Entropy, Mutual Information and Related Quantities*. R package version 1.2.1.

Havig, J. R., & Hamilton, T. L. (2019). Snow algae drive productivity and weathering at volcanic rock-hosted glaciers. *Geochimica et Cosmochimica Acta*, *247*, 220–242. doi: 10.1016/j.gca.2018.12.024

Hennig, C. (2007). Cluster-wise assessment of cluster stability. *Computational Statistics & Data Analysis*, *52*(1), 258–271. doi: 10.1016/j.csda.2006.11.025

Henry, L., Wickam, H., & Chang, W. (2019). *ggstance: Horizontal "ggplot2" Components*. R package version 0.3.2.

Hoham, R. W. (1976) The Effect of Coniferous Litter and Different Snow Meltwaters upon the Growth of Two Species of Snow Algae in Axenic Culture. *Arctic and Alpine Research*, *8*(4), 377–386. doi: 10.1080/00040851.1976.12003886

Hoham, R. W., Mullet, J. E., & Roemer, S. C. (1983). The life history and ecology of the snow alga *Chloromonas polyptera* comb. nov. (Chlorophyta, Volvocales). *Canadian Journal of Botany*, *61*(9), 2416–2429. doi: 10.1139/b83-266

Hoham, R. W. (1987). Snow Algae from High-Elevation, Temperate Latitudes and Semi-Permanent Snow: Their Interaction with the Environment. In *Proceedings of the Eastern Snow Conference Fourty-fourth Annual Meeting*. New Brunswick: Canada. Retrieved from https://www.easternsnow.org/esc-1987

Hoham, R. W., Laursen, A. E., Clive, S. O., & Duval, B. (1993). Snow algae and other microbes in several alpine areas in New England. In *Proceedings of the Fiftieth Annual Eastern Snow Conference.* Quebec City: Quebec. Retrieved from https://www.easternsnow.org/esc-1993

Hoham, R. W., & Duval, B. (2001). Microbial ecology of snow and freshwater ice with emphasis on snow algae. In Jones, H.G., Pomeroy, J.W., Walker, D.A. & Hoham, R.W. (Eds.), *Snow Ecology: An Interdisciplinary Examination of Snow-Covered Ecosystems* (pp. 168–228). Cambridge, UK: Cambridge University Press,

Hoham, R. W., & Remias, D. (2020). Snow and glacial algae: A Review. *Journal of Phycology*, *56*(2), 264–282. doi: 10.1111/jpy.12952

Huse, S. M., Welch, D. M., Morrison, H. G., & Sogin M. L. (2010). Ironing out the wrinkles in the rare biosphere through improved OTU clustering. *Environmental Microbiology*, *12*(7), 1889–1898. doi: 10.1111/j.1462-2920.2010.02193.x

Ihrmark, K., Bödeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., ... Lindahl, B. D. (2012). New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology*, *82*(3), 666–677. doi: 10.1111/j.1574-6941.2012.01437.x

Light, J. J., & Belcher, J. H. (1968). A snow microflora in the Cairngorm Mountains, Scotland. *British Phycological Bulletin*, *3*(3), 471–473. doi: 10.1080/00071616800650061

Johannessen, M., & Henriksen, A. (1978). Chemistry of snow meltwater: Changes in concentration during melting. *Water Resources Research*, *14*(4), 615–619. doi: 10.1029/WR014i004p00615

Kalamorz, F., Keis, S., McMillan, D. G. G., Olsson, K., Stanton, J.-A., Stockwell, P., ... Cook, G. M. (2011). Draft Genome Sequence of the Thermoalkaliphilic *Caldalkalibacillus Thermarum* Strain TA2.A1. *Journal of Bacteriology*, *193*(16), 4290–4291. doi: 10.1128/JB.05035-11

Karpov, S. A., Mamkaeva, M. A., Aleoshin, V. V., Nassonova, E., Lilje, O., & Gleason, F. H. (2014). Morphology, phylogeny, and ecology of the aphelids (Aphelidea, Opisthokonta) and proposal for the new superphylum Opisthosporidia. *Frontiers in Microbiology*, *5*, 112. doi: 10.3389/fmicb.2014.00112

Kawecka, B. (1986). Ecology of snow algae. *Polish Polar Research*, *7*(4), 407–415.

Kelley, J. L., Peyton, J. T., Fiston-Lavier, A.-S., Teets, N. M., Yee, M.-C., Johnston, J. S., ... Denlinger, D. L. (2014). Compact genome of the Antarctic midge is likely an adaptation to an extreme environment. *Nature Communications*, *5*, 4611. doi: 10.1038/ncomms5611

Knack, J. J., Wilcox, L. W., Delaux, P. M., Ané, J. M., Piotrowski, M. J., Cook, M. E., ... Graham, L. E. (2015). Microbiomes of streptophyte algae and bryophytes suggest that a functional suite of microbiota fostered plant colonization of land. *International Journal of Plant Sciences*, *176*(5), 405–420. doi: 10.1086/681161

Knox, J. S., & Paterson, R. A. (1973). The occurrence and distribution of some aquatic phycomycetes on ross island and the dry valleys of victoria land, antarctica. *Mycologia*, *65*(2), 373–387. doi: 10.2307/3758109

Kobayashi, H., & Fukushima, H. (1952). On the red and green snow newly found in Japan I. *Report of Botanical Magazine-Tokyo*, *65*, 765–766.

Kol, E. (1941). The Green Snow of Yellowstone National Park. *American Journal of Botany*, *28*(3), 185–191. doi: 10.1002/j.1537-2197.1941.tb07959.x

Kol, E. (1942). Snow and Ice Algae of Alaska. *Smithsonian Miscellaneous Collections*, *101*(16), 1–36.

Kol, E. (1968). Kryobiologie. Biologie und Limnologie des Schnees und Eises I. Kryovegetation. Die Binnengewasser, v. 24. E. Schweizerbart'sche (Nagele & Obermiller), Stuttgart. viii + 216 p., 16 Plates. DM 66.

Kramer, S., Dibbern, D., Moll, J., Huenninghaus, M., Koller, R., Krueger, D., ... Kandeler, E. (2016). Resource Partitioning between Bacteria, Fungi, and Protists in the Detritusphere of an Agricultural Soil. *Frontiers in Microbiology*, *7*. doi: 10.3389/fmicb.2016.01524

Krug, L., Erlacher, A., Markut, K., Berg, G., & Cernava, T. (2020). The microbiome of alpine snow algae shows a specific inter-kingdom connectivity and algae-bacteria interactions with supportive capacities. *The ISME Journal*, *14*, 2197–2210. doi: 10.1038/s41396-020-0677-4

Kvíderová, Jana, Josef Hájek, and Roger M. Worland. (2013). The Ice Nucleation Activity of Extremophilic Algae. *Cryo Letters*, *34*(2), 137–148.

Laird, L. B., Taylor, H. E., & Kennedy, V. C. (1986). Snow chemistry of the Cascade-Sierra Nevada Mountains. *Environmental Science & Technology*, *20*(3), 275–290. doi: 10.1021/es00145a009

Langfelder, P., & Horvath, S. (2007). Eigengene networks for studying the relationships between co-expression modules. *BMC Systems Biology*, *1*, 54. doi: 10.1186/1752-0509-1-54

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*, 559. doi: 10.1186/1471-2105-9-559

Langfelder, P., Zhang, B., & Horvath, S. (2008). Defining clusters from a hierarchical cluster tree: The Dynamic Tree Cut package for R. *Bioinformatics*, *24*(5), 719–720. doi: 10.1093/bioinformatics/btm563

Larose, C., Dommergue, A., & Vogel, T. M. (2013). The dynamic arctic snow pack: An unexplored environment for microbial diversity and activity. *Biology*, *2*(1), 317–330. doi: 10.3390/biology2010317

Li, A., & Horvath, S. (2009). Network module detection: Affinity search technique with the multi-node topological overlap measure. *BMC Research Notes*, *2*, 142. doi: 10.1186/1756-0500-2-142

Lladó, F. S., Větrovský, T., & Baldrian, P. (2019). The concept of operational taxonomic units revisited: Genomes of bacteria that are regarded as closely related are often highly dissimilar. *Folia Microbiologica*, *64*(1), 19–23. doi: 10.1007/s12223-018-0627-y

Lo, F., Bitz, C. M., Battisti, D. S., & Hess, J. J. (2019). Pollen calendars and maps of allergenic pollen in North America. *Aerobiologia*, *35*(4), 613–633. doi: 10.1007/s10453-019-09601-2

Lukavský, J., & Cepák, V. (2010). Cryoseston in Stara Planina (Balkan) Mountains. *Acta botanica Croatica*, *69*(2), 163–171.

Lutz, S., Anesio, A. M., Edwards, A., & Benning, L. G. (2015a). Microbial diversity on Icelandic glaciers and ice caps. *Frontiers in Microbiology*, *6*, 307. doi: 10.3389/fmicb.2015.00307

Lutz, S., Anesio, A. M., Field, K., & Benning, L. G. (2015b). Integrated "Omics", Targeted Metabolite and Single-cell Analyses of Arctic Snow Algae Functionality and Adaptability. *Frontiers in Microbiology*, *6*, 1323. doi: doi.org/10.3389/fmicb.2015.01323

Lutz, S., Anesio, A. M., Raiswell, R., Edwards, A., Newton, R. J., Gill, F., & Benning, L. G. (2016). The biogeography of red snow microbiomes and their role in melting arctic glaciers. *Nature Communications*, *7*, 11968. doi: 10.1038/ncomms11968

Lutz, S., Anesio, A. M., Edwards, A., & Benning, L. G. (2017). Linking microbial diversity and functionality of arctic glacial surface habitats. *Environmental Microbiology*, *19*(2), 551–565. doi: 10.1111/1462-2920.13494

Frieders, E. M., McLaughlin, D. J., & Szabo, L. J. (2008, February 21). Pucciniomycotina (formerly Urediniomycetes): A diverse group of fungi, including rusts, yeasts, smut-like and jelly-like fungi [Tree of Life web project]. Retrieved from http://tolweb.org/Pucciniomycotina/20528/2008.02.21

Maechler, M., Rousseeuw, P., Strufy, A., Hubert, M., & Hornik, K. (2019). *cluster: Cluster Analysis Basics and Extensions*. R package version 2.1.0.

Maltz, M., Carey, C.J., Pombubpa, N., Freund, H., Stajich, J., Hart, S.C., ... Aronson, E.L. (2021). *Landscape topography and regional drought events alter dust microbiomes in California's Sierra Nevada* [Manuscript submitted for publication]. Environmental Microbiology, University of California, Riverside.

Marchant, H. J. (1982). Snow algae from the Australian Snowy Mountains. *Phycologia*, *21*(2), 178–184. doi: 10.2216/i0031-8884-21-2-178.1

Margesin, R., Fonteyne, P.-A., Schinner, F., & Sampaio, J. P. (2007). *Rhodotorula psychrophila* sp. nov., *Rhodotorula psychrophenolica* sp. nov. and *Rhodotorula glacialis* sp. nov., novel psychrophilic basidiomycetous yeast species isolated from alpine environments.

International Journal of Systematic and Evolutionary Microbiology, *57*(Pt 9), 2179–2184. doi: 10.1099/ijs.0.65111-0

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*, 17, 10.

Masclaux, H., Perga, M.-E., Kagami, M., Desvilettes, C., Bourdier, G., & Bec, A. (2013). How pollen organic matter enters freshwater food webs. *Limnology and Oceanography Letters*, *58*(4), 1185–1195. doi: 10.4319/lo.2013.58.4.1185

Mata, J. A., Martínez-Cánovas, J., Quesada, E., & Béjar, V. (2002). A detailed phenotypic characterisation of the type strains of *Halomonas* species. *Systematic and Applied Microbiology*, *25*(3), 360–375. doi:10.1078/0723-2020-00122

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *Plos One*, *8*(4), e61217. doi: 10.1371/journal.pone.0061217

Meyer, P. E. (2014). *infotheo: Information-Theoretic Measures*. R package version 1.2.0.

Müller, T., Bleiß, W., Martin, C. D., Rogaschewski, S., & Fuhr, G. (1998). Snow algae from northwest Svalbard: their identification, distribution, pigment and nutrient content. *Polar Biology*, *20*(1), 14–32. doi: 10.1007/s003000050272

Murtagh, F., & Legendre, P. (2014). Ward's Hierarchical Agglomerative Clustering Method: Which Algorithms Implement Ward's Criterion? *Journal of Classification*, *31*(3), 274–295. doi: 10.1007/s00357-014-9161-z

Naff, C. S., Darcy, J. L., & Schmidt, S. K. (2013). Phylogeny and biogeography of an uncultured clade of snow chytrids. *Environmental Microbiology*, *15*(10), 2672–2680. doi: 10.1111/1462-2920.12116

Nedbalová, L., Kociánová, M., & Lukavský, J. (2008). Ecology of snow algae in the Giant Mts. *Opera Corcontica*, *45*, 59–68.

Newton, A. P. W. (1982). Red-coloured snow algae in Svalbard – Some environmental factors determining the distribution of *Chlamydomonas nivalis* (Chlorophyta volvocales). *Polar Biology*, *1*, 167–172. doi: 10.1007/BF00287003

Novis, P. M., Hoham, R. W., Beer, T., & Dawson, M. (2008). Two snow species of the quadriflagellate green alga *Chlainomonas* (Chlorophyta, Volvocales): Ultrastructure and phylogenetic position within the Chloromonas clade(1). *Journal of Phycology*, *44*(4), 1001–1012. doi: 10.1111/j.1529-8817.2008.00545.x

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P. McGlinn, D., ... Wagner, H. (2016). vegan: Community Ecology Package. R package version 2.4-1.

Oliver, A. K., Brown, S. P., Callaham, M. A., & Jumpponen, A. (2015). Polymerase matters: Non-proofreading enzymes inflate fungal community richness estimates by up to 15 %. *Fungal Ecology*, *15*, 86–89. doi: 10.1016/j.funeco.2015.03.003

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*(5), 1403–1414. doi: 0.1111/1462-2920.13023

Pearson, D. L. (1994). Selecting indicator taxa for the quantitative assessment of biodiversity. *Philosophical Transactions of the Royal Society of London Series B - Biological Sciences*, *345*(1311), 75–79. doi: 10.1098/rstb.1994.0088

Perini, L., Gostinčar, C., Anesio, A. M., Williamson, C., Tranter, M., & Gunde-Cimerman, N.

(2019a). Darkening of the Greenland Ice Sheet: Fungal Abundance and Diversity Are Associated With Algal Bloom. *Frontiers in Microbiology*, *10*, 557. doi: 10.3389/fmicb.2019.00557

Perini, L., Gostinčar, C., & Gunde-Cimerman, N. (2019b). Fungal and bacterial diversity of Svalbard subglacial ice. *Scientific Reports*, *9*(1), 20230. doi: 10.1038/s41598-019-56290-5

Picard, K. T., Letcher, P. M., & Powell, M. J. (2013). Evidence for a facultative mutualist nutritional relationship between the green coccoid alga *Bracteacoccus* sp. (Chlorophyceae) and the zoosporic fungus *Rhizidium phycophilum* (Chytridiomycota). *Fungal Biology*, *117*(5), 319–328. doi: 10.1016/j.funbio.2013.03.003

Praeg, N., Pauli, H., & Illmer, P. (2019). Microbial Diversity in Bulk and Rhizosphere Soil of *Ranunculus glacialis* Along a High-Alpine Altitudinal Gradient. *Frontiers in Microbiology*, *10*, 1429. doi: 10.3389/fmicb.2019.01429

Procházková, L., Remias, D., Řezanka, T., & Nedbalová, L. (2018). *Chloromonas nivalis* subsp. *tatrae*, subsp. nov. (Chlamydomonadales, Chlorophyta): Re-examination of a snow alga from the High Tatra Mountains (Slovakia). *Fottea (Praha)*, *18*(1), 1–18. doi: 10.5507/fot.2017.010

Procházková, L., Leya, T., Křížková, H., & Nedbalová, L. (2019). *Sanguina nivaloides* and *Sanguina aurantia* gen. et spp. nov. (Chlorophyta): The taxonomy, phylogeny, biogeography and ecology of two newly recognized algae causing red and orange snow. *FEMS Microbiology Ecology*, *95*(6), fiz064. doi: 10.1093/femsec/fiz064

Procházková, L., Remias, D., Holzinger, A., Řezanka, T., & Nedbalová, L. (2020). Ecophysiological and ultrastructural characterisation of the circumpolar orange snow alga *Sanguina aurantia* compared to the cosmopolitan red snow alga *Sanguina nivaloides* (Chlorophyta). *Polar Biology*. doi: 10.1007/s00300-020-02778-0

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., ... Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(Database issue), D590-6. doi: 10.1093/nar/gks1219

Rämä, T., Nordén, J., Davey, M. L., Mathiassen, G. H., Spatafora, J. W., & Kauserud, H. (2014). Fungi ahoy! Diversity on marine wooden substrata in the high North. *Fungal Ecology*, *8*, 46–58. doi: 10.1016/j.funeco.2013.12.002

Remias, D. (2012). Cell Structure and Physiology of Alpine Snow and Ice Algae. In C. Lütz (Ed.), *Plants in Alpine Regions* (pp. 175–185). Vienna, Austria: Springer. doi: 10.1007/978-3-7091-0136-0_13

Rezanka, T., Nedbalová, L., Sigler, K., & Cepák, V. (2008). Identification of astaxanthin diglucoside diesters from snow alga *Chlamydomonas nivalis* by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *Phytochemistry*, *69*(2), 479–490. doi: 10.1016/j.phytochem.2007.06.025

Rime, T., Hartmann, M., & Frey, B. (2016). Potential sources of microbial colonizers in an initial soil ecosystem after retreat of an alpine glacier. *The ISME Journal*, *10*(7), 1625–1641. doi: 10.1038/ismej.2015.238

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *4*, e2584. doi: 10.7717/peerj.2584

Rudis, B. (2019). *hrbrthemes: Additional Themes, Theme Components and Utilities for*

"ggplot2". R package version 0.60.
SAS Institute Inc. 2009. JMP® 8 User Guide, Second Edition. Cary, NC: SAS Institute Inc.
Scherholz, M. L., & Curtis, W. R. (2013). Achieving pH control in microalgal cultures through fed-batch addition of stoichiometrically-balanced growth media. *BMC Biotechnology*, *13*, 39. doi: 10.1186/1472-6750-13-39
Schloerke, B., Crowley, J., Cook, D., et al. (2018). *GGally: Extension to "ggplot2"*. R package version 1.4.0.
Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., ... Weber, C. F. (2009). Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology*, *75*(23), 7537–7541. doi: 10.1128/AEM.01541-09
Schmidt, S. K., Naff, C. S., & Lynch, R. C. (2012). Fungal communities at the edge: Ecological lessons from high alpine fungi. *Fungal Ecology*, *5*(4), 443–452. doi: 10.1016/j.funeco.2011.10.005
Scholz, M. (2019). Dye Wastewater Treatment by Vertical-Flow Constructed Wetlands. In M. Scholz (Ed.), *Sustainable Water Treatment*: *Engineering Solutions for a Variable Climate* (pp. 191–213). Amsterdam, Netherlands: Elsevier. doi: 10.1016/B978-0-12-816246-0.00014-8
Seastedt, T. R., Bowman, W. D., Caine, T. N., McKnight, D., Townsend, A., & Williams, M. W. (2004). The Landscape Continuum: A Model for High-Elevation Ecosystems. *Bioscience*, *54*(2), 111. doi: 10.1641/0006-3568(2004)054[0111:TLCAMF]2.0.CO;2
Segawa, T., Matsuzaki, R., Takeuchi, N., Akiyoshi, A., Navarro, F., Sugiyama, S., ... Mori, H. (2018). Bipolar dispersal of red-snow algae. *Nature Communications*, *9*(1), 3094. doi: 10.1038/s41467-018-05521-w
Shannon, C. P., Chen, V., Takhar, M., Hollander, Z., Balshaw, R., McManus, B. M., ... Ng, R. T. (2016). SABRE: A method for assessing the stability of gene modules in complex tissues and subject populations. *BMC Bioinformatics*, *17*(1), 460. doi: 10.1186/s12859-016-1319-8
Shin, W., Boo, S. M., & Longcore, J. E. (2001). *Entophlyctis apiculata*, a chytrid parasite of *Chlamydomonas* sp. (Chlorophyceae). *Canadian Journal of Botany*, *79*(9), 1083–1089. doi: 10.1139/b01-086
Spijkerman, E., Wacker, A., Weithoff, G., & Leya, T. (2012). Elemental and fatty acid composition of snow algae in Arctic habitats. *Frontiers in Microbiology*, *3*, 380. doi: 10.3389/fmicb.2012.00380
Stein, J. R., & Amundsen, C. C. (1967). Studies on Snow Algae and Fungi From the Front Range of Colorado. *Canadian Journal of Botany*, *45*(11), 2033–2045. doi: 10.1139/b67-221
Stibal, M., Elster, J., Sabacká, M., & Kastovská, K. (2007). Seasonal and diel changes in photosynthetic activity of the snow alga *Chlamydomonas nivalis* (Chlorophyceae) from Svalbard determined by pulse amplitude modulation fluorometry. *FEMS Microbiology Ecology*, *59*(2), 265–273. doi: 10.1111/j.1574-6941.2006.00264.x
Sutton, E. A. (1972). *The Physiology and Life Histories of Selected Cryophytes of the Pacific Northwest* (PhD thesis). Oregon State University.
Talling, J. F. (2010) Potassium — A Non-Limiting Nutrient in Fresh Waters? *Freshwater Reviews*, *3*(2), 97–104. doi: 10.1608/FRJ-3.2.1
Terashima, M., Umezawa, K., Mori, S., Kojima, H., & Fukui, M. (2017). Microbial Community

Analysis of Colored Snow from an Alpine Snowfield in Northern Japan Reveals the Prevalence of *Betaproteobacteria* with Snow Algae. *Frontiers in Microbiology*, *8*, 1481. doi: 10.3389/fmicb.2017.01481

Thomas, W. H. (1972). Observations on Snow Algae in California. *Journal of Phycology*, *8*(1), 1–9. doi: 10.1111/j.1529-8817.1972.tb03994.x

Jacobus, L. M. (2014). View of First Report of the Snow Crane Fly *Chionea scita* Walker, 1848 (Diptera: Tipuloidea: Limoniidae) from Indiana. *Proceedings of the Indiana Academy of Science*, *123*(1), 65–66.

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267. doi: 10.1128/AEM.00062-07

Weiss, R. L. (1983). Fine Structure of the Snow Alga (*Chlamydomonas nivalis*) and Associated Bacteria1. *Journal of Phycology*, *19*(2), 200–204. doi: 10.1111/j.0022-3646.1983.00200.x

Westcott, S. L., & Schloss, P. D. (2017). OptiClust, an Improved Method for Assigning Amplicon-Based Sequence Data to Operational Taxonomic Units. *mSphere*, *2*(2), e00073-17. doi: 10.1128/mSphereDirect.00073-17

Wharton, R. A., & Vineyard, W. C. (1983). Distribution of Snow and Ice Algae in Western North America. *Madroño*, *30*(4), 201–209.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky (Eds.), *PCR Protocols, a Guide to Methods and Applications* (pp. 315–322). San Diego, California: Academic Press. doi: 10.1016/B978-0-12-372180-8.50001-9

Wickham, H. (2018). *scales: Scale Functions for Visualization*. R package version 1.1.0.

Wickham, H. (2019). *ggplot2: Elegant Graphics for Data Analysis*. R package version 3.1.1.

Wickham, H., François, R., Henry, L., & Müller, K. (2019a). *dplyr: A Grammar of Data Manipulation*. R package version 0.8.0.1.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., … Yutani, H. (2019b). Welcome to the tidyverse. *The Journal of Open Source Software*, *4*(43), 1686. doi: 10.21105/joss.01686

Williams, W. E., Gorton, H. L., & Vogelmann, T. C. (2003). Surface gas-exchange processes of snow algae. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(2), 562–566. doi: 10.1073/pnas.0235560100

Williamson, C. J., Cameron, K. A., Cook, J. M., Zarsky, J. D., Stibal, M., & Edwards, A. (2019). Glacier algae: A dark past and a darker future. *Frontiers in Microbiology*, *10*, 524. https://doi.org/10.3389/fmicb.2019.00524

Wilson, J. M., Litvin, S. Y., & Beman, J. M. (2018). Microbial community networks associated with variations in community respiration rates during upwelling in nearshore Monterey Bay, California. *Environmental Microbiology Reports*, *10*(3), 272–282. doi: 10.1111/1758-2229.12635

Woo, C., An, C., Xu, S., Yi, S-M., & Yamamoto, N. (2018). Taxonomic diversity of fungi deposited from the atmosphere. *The ISME Journal*, *12*(8), 2051–2060. doi: 10.1038/s41396-018-0160-7

Yakimovich, K. M., Engstrom, C. B., & Quarmby, L. M. (2020). Alpine Snow Algae Microbiome Diversity in the Coast Range of British Columbia. *Frontiers in Microbiology*, *11*, 1721. doi: 10.3389/fmicb.2020.01721

Yang, R.-H., Su, J.-H., Shang, J.-J., Wu, Y.-Y., Li, Y., Bao, D.-P., & Yao, Y.-J. (2018). Evaluation of the ribosomal DNA internal transcribed spacer (ITS), specifically ITS1 and ITS2, for the analysis of fungal diversity by deep sequencing. *PLoS One*, *13*(10), e0206428. doi: 10.1371/journal.pone.0206428

Yip, A. M., & Horvath, S. (2007). Gene network interconnectedness and the generalized topological overlap measure. *BMC Bioinformatics*, *8*, 22. doi: 10.1186/1471-2105-8-22

Recognition

We recognize the looming threat that the global climate crisis poses to snow microbiology research and the Yakima, Chelan, Cheyenne, Arapaho, and Eastern Shoshone First Nations peoples, on whose traditional lands this work was conducted (source: Native-Land.ca).

Conflicts of Interests

The authors declare no conflict of interest.

Data Accessibility

Sequence Accessions - sequence data is archived at the Sequence Read Archive (SRA) at NCBI under the accessions: BioProject (PRJNAXXXXXX) and BioSamples (SAMNXXXXXXXX-SAMNXXXXXXXX).

Code - All code used to generate figures and analyze data has been stored in the following repository: **https://github.com/tuck82er/snow.algae.bloom.2018.git**