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Research Article

RESEARCH ARTICLE

Spatial autocorrelation of microbial communities atop a debris-covered glacier is evidence of a supraglacial chronosequence

John L. Darcy[1,†], Andrew J. King[2], Eli M.S. Gendron[1,3] and Steven K. Schmidt[1,*]

[1]Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309, USA, [2]King Ecological Consulting, 2504 Scarlett Oak Ct, Knoxville, TN 37909, USA and [3]Molecular, Cellular, and Developmental Biology Department, University of Colorado, Boulder, CO 80309, USA

*Corresponding author: Department of Ecology and Evolutionary Biology, Campus Box 334, Boulder, CO 80309, USA. Tel: +1 303 492 6248; Fax: +1 303 492 8699; Email: steve.schmidt@colorado.edu

One sentence summary: Bacteria and eukaryotes living on top of a debris-covered glacier show strong spatial patterns, which, combined with biogeochemical data, are evidence that the glacier's surface harbors a hidden chronosequence.

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†John L. Darcy, http://orcid.org/0000-0003-4900-1891

ABSTRACT

Although microbial communities from many glacial environments have been analyzed, microbes living in the debris atop debris-covered glaciers represent an understudied frontier in the cryosphere. The few previous molecular studies of microbes in supraglacial debris have either had limited phylogenetic resolution, limited spatial resolution (e.g. only one sample site on the glacier) or both. Here, we present the microbiome of a debris-covered glacier across all three domains of life, using a spatially-explicit sampling scheme to characterize the Middle Fork Toklat Glacier's microbiome from its terminus to sites high on the glacier. Our results show that microbial communities differ across the supraglacial transect, but surprisingly these communities are strongly spatially autocorrelated, suggesting the presence of a supraglacial chronosequence. This pattern is dominated by phototrophic microbes (both bacteria and eukaryotes) which are less abundant near the terminus and more abundant higher on the glacier. We use these data to refute the hypothesis that the inhabitants of the glacier are randomly deposited atmospheric microbes, and to provide evidence that succession from a predominantly photosynthetic to a more heterotrophic community is occurring on the glacier.

Keywords: glacier; succession; biogeography; microbial eukaryotes; Cyanobacteria; microbial phototrophs

INTRODUCTION

Glaciers are an important and popular system of study for ecologists, especially considering their importance in various fields of research, such as global warming (Oerlemans 1994; Kaser *et al.* 2004), ecological theory development (Chapin *et al.* 1994; Kaštovská *et al.* 2005; Nemergut *et al.* 2016) and resource management (Bury *et al.* 2011). While much research on the ecology of glacial microbes has focused on glacial forefields (Kaštovská *et al.* 2005; Bradley, Singarayer and Anesio 2014; Castle *et al.* 2016; Nemergut *et al.* 2016), these environments are geographically and physically distinct from the environment on or in the glacier's surface. While microbiological studies of glacial

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surfaces exist, almost all to date have been glaciers with exposed ice surfaces (Stibal, Šabacká and Žárský 2012; Edwards *et al.* 2014; Boetius *et al.* 2015), rather than debris-covered glaciers. Debris-covered glaciers are common throughout the cryosphere, especially in the Alaska Range and the Himalayas. The debris atop these glaciers are added to a glacier's surface from its surroundings, and contain unique microbial communities that have only been described in a few studies (Darcy *et al.* 2011; Franzetti *et al.* 2013; Azzoni *et al.* 2015; Schmidt and Darcy 2015).

Preliminary work characterizing the indigenous microbes of debris-covered glaciers has focused on microbes that are abundant within debris samples. Bacteria from the genus *Polaromonas* have been found to be prevalent on debris-covered glaciers by multiple studies (Darcy *et al.* 2011; Franzetti *et al.* 2013); however, their role in these environments is still unknown. It is possible that they are dormant propagules landing in an otherwise low biomass environment, and it is also possible that *Polaromonas* are somehow functioning *in situ*. Other microbes such as the green alga *Pseudendocloniopsis* (Ulvophyceae) have been detected in abundance from debris-covered glacier samples (Schmidt and Darcy 2015) and also from microcosms made from glacial debris (Darcy and Schmidt 2016). From an even more taxon-specific perspective, both nematodes and rotifers have been surveyed on debris-covered glaciers (Azzoni *et al.* 2015).

However, there is very little evidence addressing whether these organisms are functioning on debris-covered glaciers, or whether they are just inactive (dormant) atmospheric transients that are easily detectable in a low biomass environment (Darcy *et al.* 2011). Thus, our null hypothesis is that microbial communities in supraglacial sediment are random assemblages of dormant propagules and therefore should show no correlation with spatial variation in environmental variables, such as nutrient availability, pH or other biogeochemical factors. Alternatively, microbial communities that are functionally active on the glacier should be shaped by, and correlate with, spatial patterns in these environmental variables, as is often the case in soil microbial communities, which can be strongly shaped by nutrient and water availability (King *et al.* 2010; Manzoni, Schimel and Porporato 2012; Darcy and Schmidt 2016).

In glacial forefield chronosequences, these soil biogeochemical variables are often structured by substrate age (Schmidt *et al.* 2016), but this may or may not be the case atop debris-covered glaciers. Some authors have speculated that debris-covered glaciers *are* chronosequences, where recently deposited debris high on the glacier give way to older substrate at the terminus (Gobbi, Isaia and De Bernardi 2011; Franzetti *et al.* 2013). If this is the case, this supraglacial chronosequence hypothesis makes two predictions about the microbial communities atop debris-covered glaciers. First, since chronosequences are space-for-time substitutions, we expect microbial communities atop the glacier to be highly spatially structured. Sites that are geographically proximate are also temporally proximate, and are therefore in similar successional stages. Thus, the microbial communities of sites close to each other should be more similar (in terms of beta-diversity) than geographically/temporally distant sites. Second, the nature of these spatially/temporally structured communities should reflect their age, especially in terms of the relative abundance of microbial phototrophs. Ecosystem succession begins with primary producers (although there may be exceptions; Bardgett *et al.* 2007), and as such we expect chronosequences to exhibit a pattern where phototrophic organisms are more proportionally abundant at young sites, and less so at older sites.

Here, we test the above hypotheses, and also present the most thorough picture so far of life atop a debris-covered glacier, the Middle Fork Toklat Glacier in Denali National Park and Preserve. Although previous sequencing studies of debris-covered glaciers were limited to bacteria or select taxa therein (Darcy *et al.* 2011; Franzetti *et al.* 2013) or limited by spatial resolution (Schmidt and Darcy 2015; Darcy and Schmidt 2016), here we present an in-depth analysis of life in the Middle Fork Toklat Glacier's supraglacial debris, using both 16S and 18S high-throughput rRNA gene sequencing to look at all three domains of life.

MATERIALS AND METHODS

Sample collection

Samples were collected from the surface of the Middle Fork Toklat Glacier in the summer of 2009, along a transect that started on top of the glacier near its terminus and ended ~600 m up-glacier (Fig. 1). Sample sites near the terminus were high enough on the glacier and far enough away from recently exposed subglacial sediment to be sure of their supraglacial origin. Samples consisted of roughly 50 g from the top 4 cm of sediment. During sampling, GPS coordinates and elevations for each sample point were recorded using a Garmin 60CSx GPS unit. Samples were frozen on ice in the field and were shipped to the University of Colorado, where they were kept at –80°C until DNA was extracted in 2010. Figure 1 shows the spatial origins of the 21 samples collected in this manner. A thorough description of the Middle Fork Toklat Glacier and pictures of the site can be found elsewhere (Schmidt and Darcy 2015; Darcy and Schmidt 2016; Schmidt *et al.* 2016).

DNA extraction and sequencing

DNA was extracted from each of the 21 samples using the MoBio PowerSoil DNA isolation kit (Carlsbad, CA, USA), and was kept frozen at –20°C until 2014, when each extraction was amplified in triplicate using 515F/806R (Caporaso *et al.* 2012) for bacteria and archaea, and 1391F/EukBR (Amaral-Zettler *et al.* 2009) for eukaryotes. Samples were amplified with barcoded versions of these primers, which also contained Illumina flowcell adapters. Both primer sets can be accessed from the Earth Microbiome Project website (http://press.igsb.anl.gov/earthmicrobiome/emp-standard-protocols). Triplicate reactions were pooled, and amplified DNA concentrations were assayed using Pico Green fluorimetry using a BioTek Synergy 2 microplate reader (BioTek, Winooski, VT, USA). Reactions were diluted to equimolar concentration and pooled, then sequenced using an Illumina MiSeq (Illumina, Inc.) with 2 × 150 bp chemistry. A 30% phiX spike was added to the run, in order to increase read variability (Caporaso *et al.* 2012).

Sequence data processing and bioinformatics

Raw sequence reads were demultiplexed using QIIME (Caporaso *et al.* 2010). 16S rRNA gene paired-end reads were joined, but this process did not work for 18S rRNA gene reads due to insufficient overlap, so only one end was used. Sequences were clustered at the 97% similarity level using UCLUST (Edgar 2010), and taxonomy was assigned with QIIME using the SILVA Ref NR 99 database's taxonomy for 18S rRNA gene sequences and the GreenGenes database's taxonomy (DeSantis *et al.* 2006) for 16S rRNA gene sequences. We used different databases because

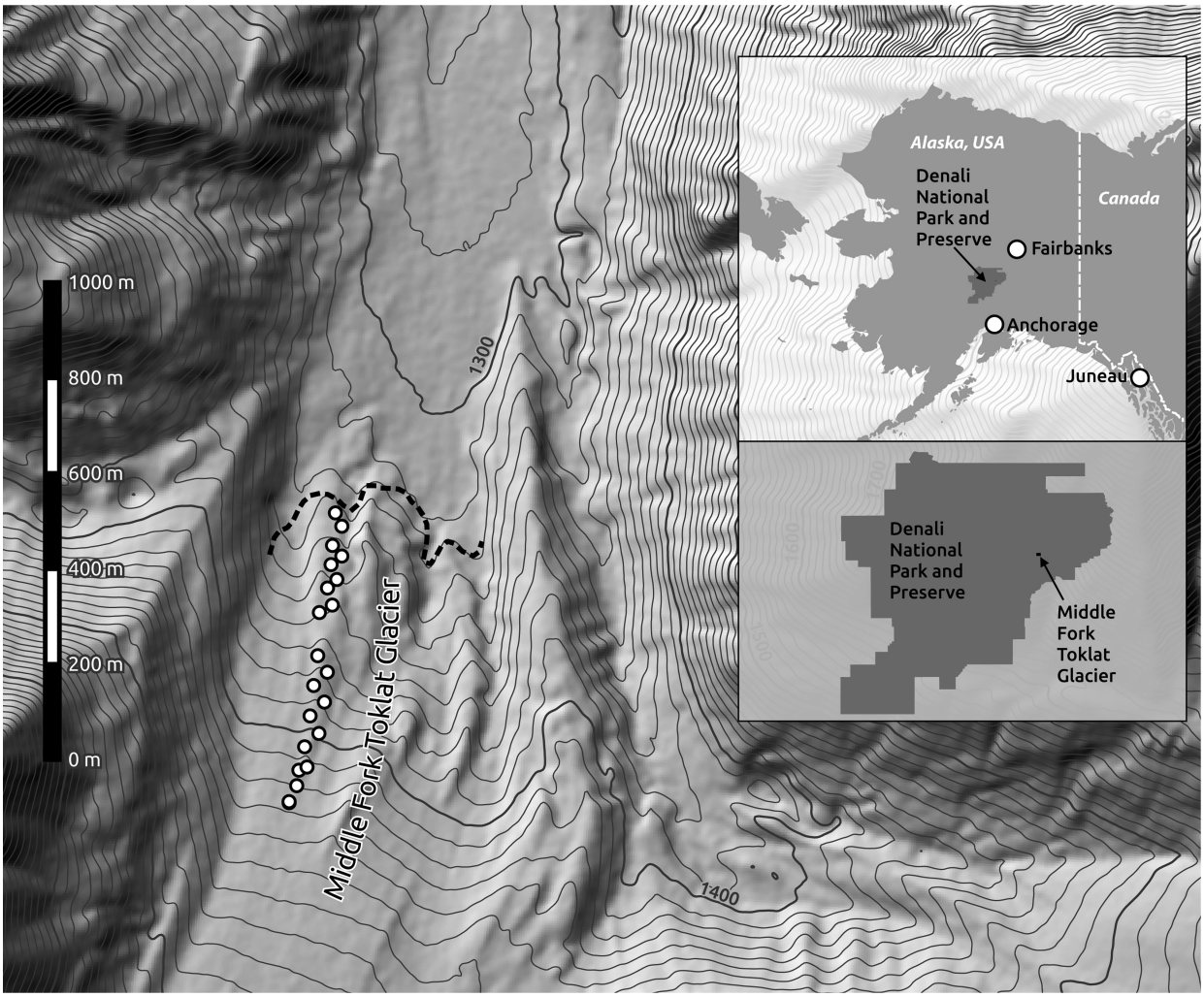

Figure 1. Topographic map of sampling sites across the Middle Fork Toklat Glacier. Sample sites (white dots) were located along a staggered transect, beginning at the glacier's terminus (dashed black line) and ending slightly more than 600 m to the south, corresponding to an elevation increase of 100 m (contours represent 10-m elevation differential). Inset, top: Map of Alaska, showing the location of Denali National Park and Preserve. Inset, bottom: Map of Denali National Park and Preserve, showing location of the Middle Fork Toklat Glacier within the park.

the GreenGenes taxonomy is the most commonly used taxonomy for 16S rRNA gene sequences and we wanted our results to be comparable to other publications. However, the GreenGenes database does not contain taxonomy for 18S rRNA gene sequences, so the SILVA database was used instead. All mitochondrial and chloroplast OTUs were removed from the 16S rRNA gene library, and archaeal sequences were separated. Bacterial and archaeal OTUs were removed from the 18S rRNA gene data set where present (possibly due to PCR primer promiscuity). The filtered data sets were each rarefied to a depth of 6000 sequences per sample, except for the archaeal data set which had 0 sequence per sample for most samples and only 10 sequences for the most populous sample. Weighted UniFrac (Lozupone and Knight 2005) was used to calculate beta-diversity matrices for the 16S and 18S rRNA gene data sets. BLAST (Altschul *et al.* 1990) was used to compare sequence data to the NCBI nucleotide database and to find phylotypes similar to common OTUs from our data sets.

Sediment biogeochemical measurements

Soil water content was measured gravimetrically by determining weight loss of roughly 1 g of soil after 48 h at 60°C. Dissolved organic carbon (DOC), dissolved organic nitrogen (DON), NH_4^+ and NO_x were extracted from ~5 g soil samples by adding 25 ml of 0.5 M K_2SO_4 and shaking at 150 rpm for 1 h. Samples were centrifuged for 1 h at 4000 rpm. A blank sample that contained no soil was also processed. NO_x concentration was analyzed for each extraction using a Lachat QuickChem 8500 (Hach Ltd, Loveland, CO). NH_4^+ concentration was analyzed for each sample colorimetrically using a BioTek Synergy 2 microplate reader (BioTek, Winooski VT). DOC and DON were analyzed using a Shimadzu total organic carbon analyzer (TOC 5000) equipped with a total dissolved nitrogen (TDN) module (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). DON was calculated as TDN-DIN. All concentrations were corrected using measurements from blanks. Concentrations of C and N were calculated stoichiometrically using the original soil masses, and were corrected for background levels of C and N using the blank sample data.

Statistical analyses

Pairwise geographic distances were calculated for sample locations (Fig. 1) using the R package 'Fields' (Nychka *et al.* 2016).

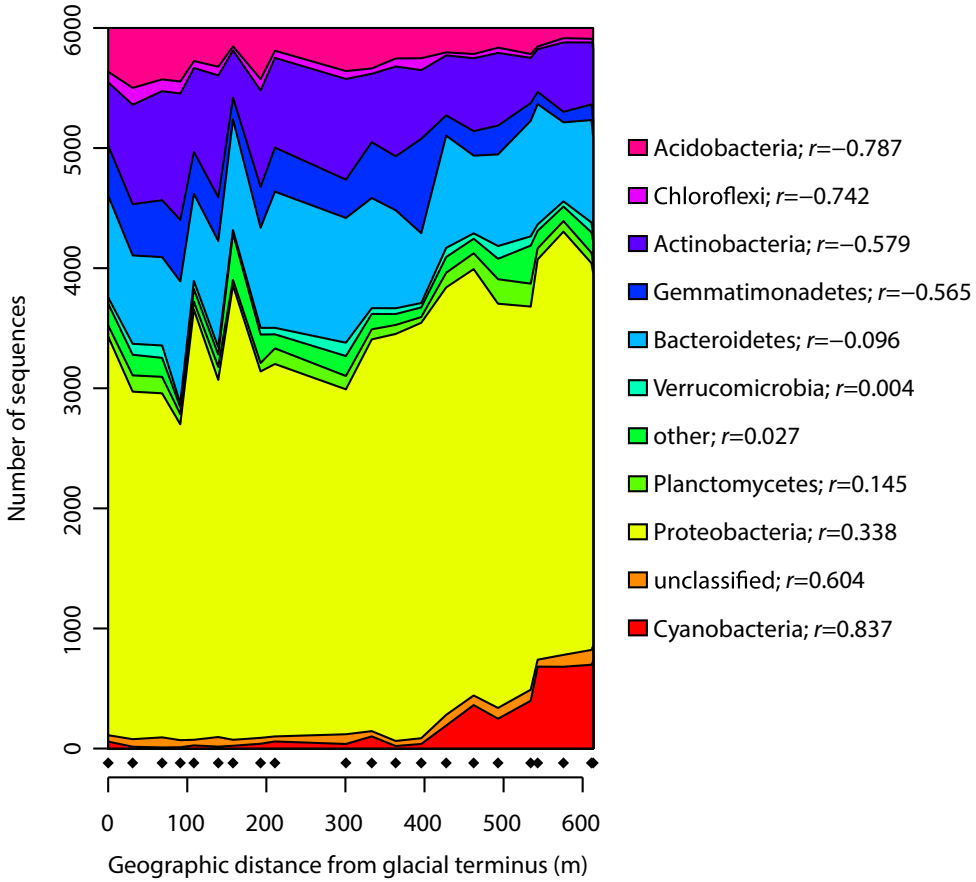

Figure 2. Relative abundance of bacterial phyla across the supraglacial transect. In this plot, each polygon represents a different bacterial phylum. The vertical width of the polygon for any sample location (x-axis) represents the relative abundance of that phylum within the rarefied sample. Polygons are ordered vertically by their correlation coefficient with geographic distance. Cyanobacteria (bottom, red) were the most positively correlated with geographic distance, meaning that they were not abundant near the glacier's terminus but were more abundant higher on the glacier. Acidobacteria showed the opposite trend, and were more abundant near the terminus and less abundant higher on the glacier.

Semivariograms (Bachmaier and Backes 2008) were then made in R by combining 16S and 18S rRNA gene beta-diversity matrices with their geographic distances. We used a sliding-window approach to geographic distance classes within the semivariograms in order to better visualize the spatial structuring of bacterial and eukaryote beta-diversity, with a window overlap of 2/3 and even sample size between bins; this means that all distance classes contain the same number of pairwise points, and each class shares 2/3 of its points with the class before it. Versions of these figures without the sliding-window approach are available as supplementary material (Fig. S1, Supporting Information). To test whether individual taxa exhibited spatial patterns, correlations between taxon relative abundance and geographic distance were calculated in R, tested against a *t*-distribution and corrected for multiple hypothesis testing using the false discovery rate (FDR) algorithm. Non-metric multidimensional scaling (NMDS) was calculated from 16S and 18S rRNA gene beta-diversity matrices using the R package 'vegan' (Oksanen *et al.* 2016) to test how the biogeochemical parameters we measured were related to bacterial and eukaryote beta-diversity, and correlations between geographic distance and biogeochemical parameters were calculated in R. Principal coordinates of neighbor matrices (PCNM; Brocard and Legendre 2002) was used to account for non-linear spatial patterns in beta-diversity. Resulting PCNM vectors were tested permutationally against UniFrac distance matrices and visualized using NMDS (Oksanen *et al.* 2016); *P*-values were corrected using FDR.

Data accessibility

Sequence data and metadata from this study are available at FigShare: https://figshare.com/s/07d6a70e199f99f612e9

RESULTS

Bacterial biogeography

The most striking biogeographic pattern was that of cyanobacteria, which comprised over 13% of the bacterial communities at sites high on the glacier, but were almost absent near the terminus (Fig. 2). The most common cyanobacteria were in the class Synechococcophycideae, and clustered into three main OTUs which were most similar to sequences in GenBank that originated from polar and alpine environments. Cyanobacteria in the Chamaesiphonaceae and Phormidiaceae were also present and showed similar spatial patterns, although they were less abundant than the Synechococcophycideae (Fig. S2, Supporting Information).

Other bacteria showed patterns over the glacier as well, including the Acidobacteria (Fig. 2) which were slightly more abundant near the terminus. Within the Acidobacteria, OTUs in the orders DS-18 and iii1–15 were responsible for this trend. Betaproteobacteria were generally less abundant near the terminus and more abundant higher on the glacier, with the genera *Polaromonas*, *Methylibium* and *Methylotenera* contributing greatly to this pattern, but the *Thiobacillus* exhibited the

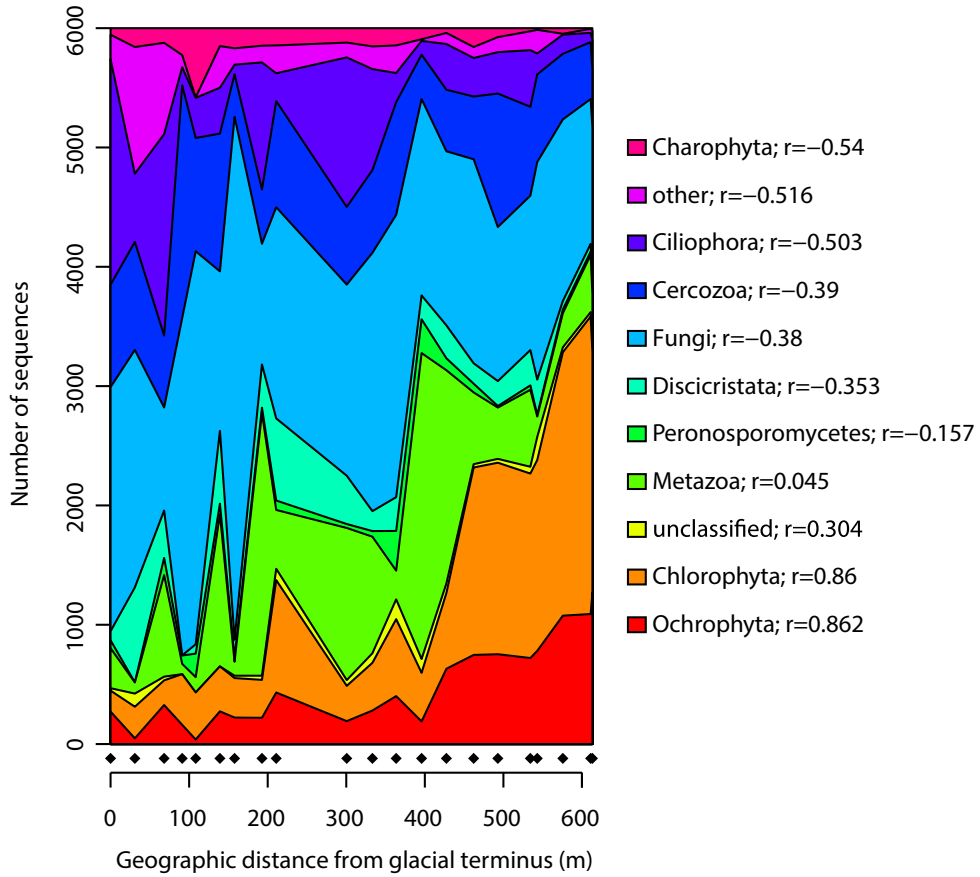

Figure 3. Relative abundance of eukaryote phyla across the supraglacial transect. Similar to the pattern observed for phototrophic cyanobacteria (Fig. 2), phototrophic eukaryotes (Ochrophyta, Chlorophyta) were much less abundant near the glacier's terminus than they were high on the glacier. Please see the caption of Fig. 2 regarding the interpretation of this figure.

opposite pattern with higher abundances near the terminus (Fig. S3, Supporting Information).

Eukaryote biogeography

Similar to the bacteria, phototrophic eukaryotes had much higher relative abundance at sites high on the glacier compared to sites near the terminus (Fig. 3). This was the case for both Chlorophyta (green algae) and Ochrophyta (mostly Chrysophyceae), which together made up over 50% of 18S rRNA gene sequences high on the glacier, but less than 8% at the terminus. Within the Chlorophyta, the Ulvophyceae decreased along the transect (from top of glacier toward terminus) by the largest magnitude, but Trebouxiophyceae and Chlorophyceae decreased as well (Fig. S4, Supporting Information). The Ulvophyceae were dominated (>99%) by just one OTU, which was in the genus *Pseudendocloniopsis*.

Heterotrophic eukaryotes did not show obvious spatial patterns, but in aggregate heterotrophs exhibited the opposite pattern of the phototrophs (above) in terms of relative abundance; however, this pattern may be driven solely by the lack of phototrophs near the terminus. Chytridiomycetes were the most abundant fungal clade, and while they were generally more prevalent near the terminus, this pattern had high variance. Within the Chytridiomycetes, the Chytridiales were absent at the terminus but present higher on the glacier, although they never made up more than 5% of any site's community. Pezizomycotina were also abundant, and within them the Leotiomycetes were more abundant near the terminus, but the Peziziomycetes were more abundant higher on the glacier. Metazoa sequences were also abundant, but like the Fungi they did not appear to be spatially structured. The most common metazoa were either rotifers (Philodina) or hexapods (Collembola).

Archaeal community

Although we detected very few archaeal sequences, they were only observed in samples in the upper half of the transect (not near the terminus). Because we detected so few archaeal 16S rRNA gene sequences, this is not enough information to indicate a biogeographic signal in archaea; we only detected 22 archaeal 16S rRNA gene sequences which clustered into eight OTUs, out of a total sampling depth of 449 630 sequences. Nevertheless, these few Archaeal phylotypes were mostly unclassified, but the remainder were in the Nitrososphaeraceae, Methanoregulaceae and Methanobacteriaceae families. The scarcity of archaeal phylotypes was not solely due to primer bias, because many similar archaea (also in the Nitrososphaeraceae) were successfully amplified and sequenced from other soil samples that were included on the same MiSeq run (data not shown).

Spatial structuring of microbial communities

Beta-diversity analysis revealed that both bacterial and eukaryote communities were strongly spatially structured (Fig. 4). Our

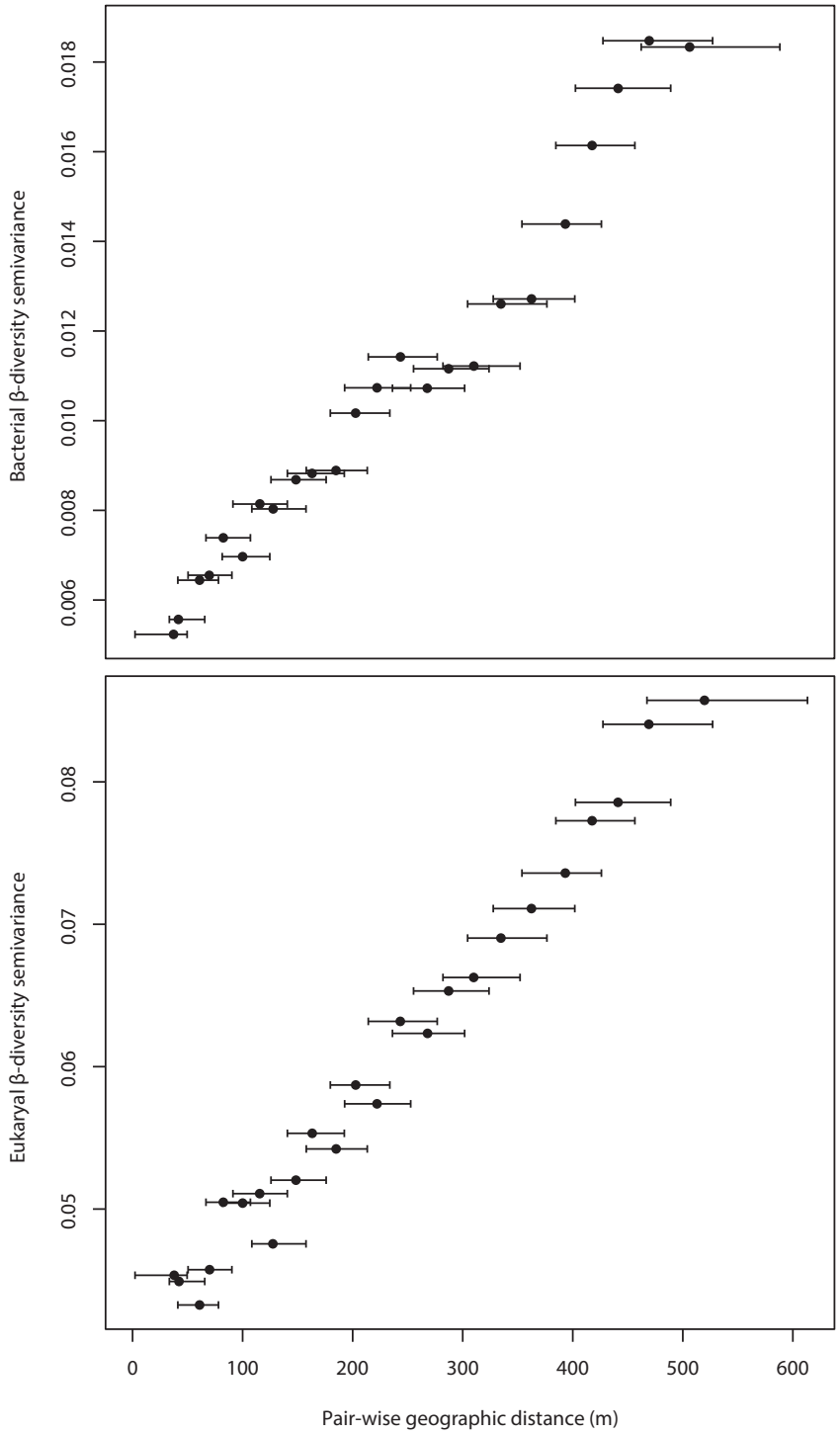

Figure 4. Semivariograms of bacterial and eukaryote communities. These semivariograms show that for both bacterial communities and eukaryote communities, community composition is highly spatially autocorrelated across the entire transect. If these semivariograms were to plateau, it would indicate that at some distance the difference between communities becomes random. Instead, both semivariograms show a strong spatial signal across the entire transect, indicating that the phylogenetic difference between communities is related to the spatial distance between them, even at our largest scale (~600 m, Fig. 1). Horizontal bars indicate the widths of geographic distance classes (bins), and points are placed at the mean geographic distance within each class.

beta-diversity semivariograms did not plateau, which would have indicated a distance at which spatial structuring breaks down (King *et al.* 2010; Robeson *et al.* 2011). Said another way, a plateau would indicate that at some distance, the difference between communities becomes random instead of being structured by space. Since we did not observe this pattern in our semivariograms, the microbial communities we sampled across the Middle Fork Toklat Glacier are spatially structured across the entire transect. PCNM analysis of community spatial structuring only produced one significant ($P_{corrected} < 0.05$) spatial

vector for bacterial communities and one significant spatial vector for eukaryote communities. In both cases, this PCNM vector was nearly identical to regular geographic distance in terms of its relationship to beta-diversity (Fig. S5, Supporting Information), strongly supporting linear geographic structure of microbial communities instead of non-linear structures. For both bacterial and eukaryote communities, significant PCNM vectors explained less beta-diversity variance than regular geographic distance did.

Biogeochemical structuring of microbial communities

NMDS analysis showed that soil water content, DOC, DON and spatial distance all affected the bacterial community composition similarly, but inorganic nitrogen (NO_x and NH_4^+) was found to have an opposite relationship with bacterial beta-diversity than other measured variables (Fig. 5A). The same was true for eukaryote communities, but to an even greater extent. Both NO_x and NH_4^+ concentrations were negatively correlated with geographic distance from the glacial terminus (Table 1, Fig. S6, Supporting Information), meaning that NO_x concentration is generally high near the glacier's terminus (dashed line, Fig. 1), and is low higher up on the glacier. The other biogeochemical parameters we measured were positively correlated with distance from the glacial terminus (Table 1, Fig. S6), meaning that they were low at the terminus and higher on top of the glacier.

DISCUSSION

Although many studies have examined microbial communities on glacier ice surfaces, relatively few studies have focused on microbes on debris-covered glaciers. Here we present the most comprehensive view of the debris-covered glacier microbiome to date, across all three domains of life, and at a large enough spatial scale to capture community variation across the Middle Fork Toklat Glacier. We found that both bacterial (Fig. 2) and eukaryote (Fig. 3) communities were strongly structured by geographic distance (Fig. 4), along the entire length of the exposed debris on top of the glacier (Fig. 1). This finding is evidence against the hypothesis (Darcy *et al.* 2011) that organisms (e.g. *Polaromonas*) found on this glacier are uniformly distributed dormant propagules from the atmosphere that remain inactive atop the glacier, and are only detected because they landed in an otherwise low biomass environment. If a uniformly distributed rain of dormant atmospheric propagules were the driving force structuring microbial community composition, there should be no spatial autocorrelation in beta-diversity (Fig. 4).

Relative abundance of microbial phototrophs contributed greatly to the spatial structuring of microbial communities across the glacier. Cyanobacteria comprised a significant part of bacterial communities at sites high on the glacier, but were almost absent near the terminus (Fig. 2). A similar pattern was observed for eukaryote phototrophs, but for eukaryotes, phototrophic OTUs made up a much larger proportion of 18S rRNA gene sequences high on the glacier (over half) than bacterial phototrophs did (Fig. 3). These biogeographic patterns of microbial phototrophs are surprising, in part because the only other spatial study of debris-covered glacier bacteria did not detect significant phototroph (cyanobacteiral) abundance (Franzetti *et al.* 2013). However, the authors of that study only sequenced the bacterial 16S rRNA gene, and it may have been that algal phototrophs were more abundant.

The strong spatial patterning of both eukaryote and bacterial communities across the Middle Fork Toklat Glacier not only increases our understanding of the biogeographic structure of an extreme environment, but also provides insight into the interactions between biogeography and biogeochemical functioning of this system. Especially relevant are the patterns of inorganic nitrogen availability observed in this study, which show large increases in availability near the glacier terminus (Table 1, Fig. S6) coinciding with the observed decline in both bacterial and eukaryote phototrophs (Figs 2 and 3). A likely cause for both the decline of phototrophs and the spike in inorganic N availability is that the glacier is undergoing severe disturbance in the form of break-up at the terminus, whereas the upper reaches of the glacier have a more uniform surface (AJK personal observation, 2009). Thus, it is likely that inorganic N is not structuring the microbial community as much as the microbial community is structuring the supply of inorganic N, that is, disturbance disrupts the functioning of microbial phototrophs (e.g. by burying them), ending inputs of newly photosynthesized C which may result in the accumulation of inorganic N. Similar patterns of N availability linked to disturbance and disruption of microbial N demand have been observed in other ecosystems (Vitousek and Matson 1985; Vitousek 2004).

A further sign of disturbance at the toe of the glacier is the increase in relative abundance of *Thiobacillus* phylotypes. Thiobacilli thrive in environments where reduced sulfur compounds (e.g. pyrite) are exposed to the oxidative world, often due to mining or other disturbances (Male, Leduc and Ferroni 1997; Skidmore *et al.* 2005). Previous work on the valley floor near this glacier terminus also indicated a spike in the relative abundance thiobacilli near the glacier toe that corresponded to higher pyrite levels, compared to down-valley sites further from the glacier (Schmidt *et al.* 2016). Likewise, studies along the down-valley chronosequence going away from the Toklat Glacier also showed higher availability of N near the glacier terminus and a sharp decrease in inorganic N availability further from the glacier where phototrophs were again establishing on the glacial debris left behind by the retreat of the glacier. Thus, the patterns seen on top of the Middle Fork Toklat Glacier are a mirror image of the down-valley chronosequence previously described at this site (Schmidt *et al.* 2016).

This mirroring of biotic and abiotic features between the glacial forefield chronosequence (Adema, Karpilo and Molnia 2007; Schmidt *et al.* 2016) and the supraglacial transect we describe here suggests that the surface of the Middle Fork Toklat Glacier is actually a chronosequence. Indeed, the debris atop many other glaciers have been shown to have a strong spatiotemporal distribution. Models of debris-covered glacier surface flow show debris are transported downhill by underlying ice (Konrad and Humphrey 2000), with debris accumulating high on the glacier and moving downward (Potter 1972). Supraglacial debris have even been aged using techniques such as ^{14}C dating and lichenometry; on the Mendel Glacier (Sierra Nevada Range), debris were young at the glacier's cirque and older at downhill sites (Konrad and Clark 1998). Likewise, debris at higher sites on Søre Illåbreen and Hellstugubreen glaciers (Scandinavian Range) had no lichens, but lichen density increased down glacier (Griffey 1978) indicating a substrate age gradient.

Across our supraglacial transect, we observed a strong biogeographic pattern for microbes on top of the glacier (Figs 4 and 5), and similar biogeographic patterns for plants and animals have been observed on the debris-covered Miage glacier (Caccianiga *et al.* 2011; Gobbi, Isaia and De Bernardi 2011). This has led some ecologists to hypothesize that the spatiotemporal structuring of supraglacial debris leads to spatiotemporal structuring of ecological communities as well (Gobbi *et al.* 2011 Franzetti *et al.* 2013), just like in glacier forefield chronosequences. This hypothesis is supported by our observation

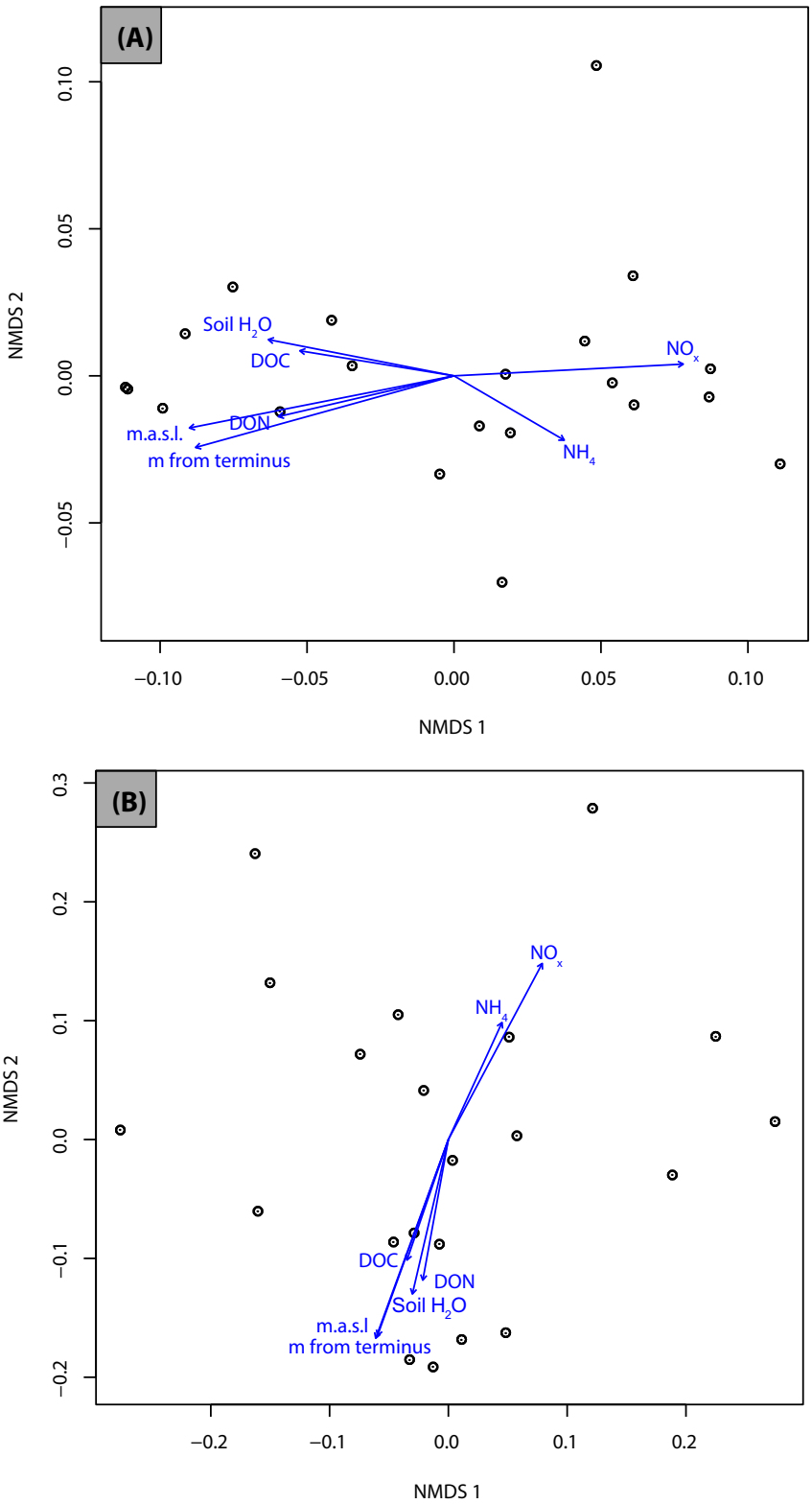

Figure 5. NMDS analysis of bacterial (**A**) and eukaryote (**B**) communities and measured biogeochemical parameters. NMDS showed that inorganic nitrogen (NO_x and NH_4) were orthogonally related to bacterial and eukaryote beta-diversity, as compared to dissolved organic carbon and nitrogen (DOC and DON). The vector arrows in these plots show how these variables relate to the beta-diversity of samples (black dots) in the NMDS ordination.

Table 1. Spatial correlations of abiotic variables.

	r-value	*P*-value
NO_x	–0.87969	4.47×10^{-7}
NH_4	–0.43833	0.047
Soil H_2O	0.607676	0.005
DOC	0.511891	0.021
DON	0.653155	0.003
m.a.s.l.	0.987865	9.88×10^{-15}

Each variable is correlated with geographic distance from the glacier's terminus. P-values were corrected for multiple hypothesis testing using the FDR algorithm. Plots can be seen in Fig. S5 (Supporting Information).

of strong spatial autocorrelation of microbial communities (Fig. 4), again, mirroring results from glacier forefield systems (Castle *et al.* 2016; Nemergut *et al.* 2016). Specifically, the strong spatial autocorrelation we observe (Fig. 4) indicates that microbial community structure changes with geographic distance from the glacier's terminus. This observation, coupled with the strong spatial pattern in microbial phototrophs, matches the predictions made by the supraglacial chronosequence hypothesis. However, it is not possible to disentangle the effects of substrate age from the effects of surface disturbance, since older sample sites near the terminus were more disturbed by glacial melting than newer sites higher on the glacier. Indeed, one of the major processes of debris-covered glacier formation is the collapse of lateral moraines (Nawako *et al.* 1986; Konrad and Humphrey 2000), which spreads debris over the glacier's surface. In this case, the glacier's surface would be a chronosequence of disturbance (*sensu* Boerner, Scherzer and Brinkman 1998) instead of a simple substrate age chronosequence.

It is unlikely that the Middle Fork Toklat glacier is unique, as other debris-covered glacier surfaces have been shown to be chronosequence-like as well (Caccianiga *et al.* 2011; Gobbi, Isaia and De Bernardi 2011), just not yet for microbes (Franzetti *et al.* 2013). Furthermore, the indigenous microbiota of the Middle Fork Toklat Glacier resembles those of other cold and dry environments, especially the cyanobacteria and algae. The most abundant cyanobacterial OTU in our sequence library was phylogenetically similar to cyanobacteria found on other glacial surfaces (Simon *et al.* 2009; Xiang *et al.* 2009; Segawa and Takeuchi 2010; Ni *et al.* 2014) and near glaciers (Nemergut *et al.* 2007; Michaud, Šabacká and Priscu 2012). Cyanobacterial endemicity to cold desert environments has been demonstrated before using other cyanobacterial taxa (Bahl *et al.* 2011), so it is not surprising that this OTU from the Synechococcophycideae may be native to glacial environments. Certain algae from the Ulvophyceae are also commonly found in cold and dry environments, although the presence of these algae on the Middle Fork Toklat Glacier was already known (Schmidt and Darcy 2015).

Similar to the Ulvophyceae, previous research showed that *Polaromonas* bacteria were abundant atop the Middle Fork Toklat Glacier (Darcy *et al.* 2011) and on other glaciers and cryospheric locations as well (Ambrosini *et al.* 2016; Rime, Hartmann and Frey 2016; Kim *et al.* 2017). With the spatial transect we present here (Fig. 1), it is now apparent that these bacteria have a biogeographic pattern within the glacier. *Polaromonas* were abundant high atop the glacier, but were less abundant near the terminus. This pattern is similar to that of the cyanobacteria and algae we detected, suggesting that perhaps similar forces structure the relative abundance of both. If *Polaromonas* are undergoing selection across the transect, they are not random 'propagule rain' appearing in otherwise low biomass environments (Darcy *et al.* 2011; Schmidt *et al.* 2014) but are instead active members of the glacier's microbial community.

The existence of a spatially structured chronosequence of microbial communities on the surface of debris-covered glaciers may indicate that soil formation begins before the depositing of the debris at the melting terminus. The buildup of N and the weathering of P from the debris may allow for faster ecological succession after the melting of the glacier at the terminus in cases where glaciers are debris covered. In contrast, debris-free glaciers may have less nutrient input from the melt out because microbial activity is almost entirely restricted to cryoconite holes (Bagshaw *et al.* 2013, 2016). Also, some glacial recession chronosequences have high rates of ecological succession, and nutrient inputs from melt-out are thought to be an important driver (Sattin *et al.* 2009). Thus, active microbial communities on top of debris-covered glaciers likely influence the course of ecological succession in the foreland after glacial melt (Rime, Hartmann and Frey 2016).

Cumulatively, our findings inform a broader understanding of life atop debris-covered glaciers, from a biogeochemical and biogeographic perspective. The strong spatial autocorrelation we observed for both bacteria and eukaryote communities is evidence against the hypothesis that supraglacial communities consist of randomly deposited, non-functioning transients. Instead, the spatial patterns in beta-diversity and in the relative abundances of microbial phototrophs suggest that the surface of the glacier is actually a chronosequence. This conclusion is further supported by our biogeochemical analysis, which revealed that supraglacial biogeochemistry mirrors that of the adjacent glacier forefield chronosequence. The biogeochemical and biogeographic patterns we observed atop the glacier also suggest that ecosystem succession in the glacier forefield gets its start on the glacier's surface. Our findings represent a large but early step toward understanding life on (and near) debris-covered glaciers.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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