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Vegetation-Associated Impacts on Arctic Tundra Bacterial and Microeukaryotic Communities

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The Arctic is experiencing rapid vegetation changes, such as shrub and tree line expansion, due to climate warming, as well as increased wetland variability due to hydrological changes associated with permafrost thawing. These changes are of global concern because changes in vegetation may increase tundra soil biogeochemical processes that would significantly enhance atmospheric CO_2 concentrations. Predicting the latter will at least partly depend on knowing the structure, functional activities, and distributions of soil microbes among the vegetation types across Arctic landscapes. Here we investigated the bacterial and microeukaryotic community structures in soils from the four principal low Arctic tundra vegetation types: wet sedge, birch hummock, tall birch, and dry heath. Sequencing of rRNA gene fragments indicated that the wet sedge and tall birch communities differed significantly from each other and from those associated with the other two dominant vegetation types. Distinct microbial communities were associated with soil pH, ammonium concentration, carbon/nitrogen (C/N) ratio, and moisture content. In soils with similar moisture contents and pHs (excluding wet sedge), bacterial, fungal, and total eukaryotic communities were correlated with the ammonium concentration, dissolved organic nitrogen (DON) content, and C/N ratio. Operational taxonomic unit (OTU) richness, Faith's phylogenetic diversity, and the Shannon species-level index (H') were generally lower in the tall birch soil than in soil from the other vegetation types, with pH being strongly correlated with bacterial richness and Faith's phylogenetic diversity. Together, these results suggest that Arctic soil feedback responses to climate change will be vegetation specific not just because of distinctive substrates and environmental characteristics but also, potentially, because of inherent differences in microbial community structure.

The rate of increase in Arctic surface air temperatures has been twice as rapid as the average global rate (~0.10°C per decade) (1, 2) over the last few decades (3); this augmented warming has impacted Arctic species and ecosystems (4). Arctic shrub cover and density have increased, the tree line has migrated in some places, and the distribution of wetland vegetation is changing in association with permafrost thawing (5, 6). Because plants supply organic matter to soils and impact belowground soil microbial communities (7, 8), changes in soil vegetation cover could result in substantial bacterial, fungal, and metazoan community composition changes (9–12). These changes could alter overall biogeochemical functioning, such as the release of carbon via soil decomposition, in these ecosystems (13), which would be expected to further exacerbate climate change. A better understanding of the vegetation-associated soil microbial structures could facilitate predictions of the effects of climate change on tundra ecosystems and determine if Arctic soil feedback responses to climate change are vegetation specific.

Bacterial consortia display spatial patterns linked to soil pH and carbon/nitrogen (C/N) ratios (14–18), soil temperature (19), soil organic carbon (SOC) (20), moisture content (21), and nutrient availability (22). The diversity and composition of nematode, testate amoeba, and fungal communities can also be influenced by soil properties (23–25), including acidity, although this may not be true of all microbial metazoans (26). Notwithstanding our appreciation that soil characteristics can affect both bacterial and microeukaryotic communities, the key factors that structure communities of different microbial groups among vegetation types in Arctic tundra are poorly understood.

The highly heterogeneous soil conditions in the Arctic tundra mirror the variation in plant community composition over short distances (27, 28), and because of this, plant community distributions have proven to be indicators of spatial patterns in underlying microbial communities. Despite important insights, research to date has been based on techniques with relatively low taxonomic resolution and on sampling within rather than between replicate sites of the selected vegetation types. For example, Wallenstein et al. (2007) observed different fungal and bacterial communities in acidic tussock tundra and birch shrub tundra soils using clone libraries (11). However, the communities associated with birch hummock and tall birch tundra soils, analogous to those of the study of Wallenstein and colleagues (11), did not differ when analyzed using denaturing gel gradient electrophoresis (DGGE) (29). With terminal restriction fragment length polymorphism

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(T-RFLP) analysis, tundra shrub bacterial communities appeared to differ seasonally, but the same pattern was not found in tussock soils (30). Further DGGE analysis on each of the principal low Arctic tundra vegetation types (i.e., wet sedge, birch hummock, tall birch, and dry heath) revealed that the relative abundance of dominant bacterial community members changed, but overall distinct bacterial community compositions were not uniquely associated with each vegetation type (31). DGGE assessments of soil fungi and archaea from the same vegetation types also did not appear to show a correlation with plant cover (31). In summary, tundra studies to date have not shown a strong association between plant and soil microbial communities. However, this conclusion may be constrained by the type of analyses; high-taxonomic-resolution techniques can provide detailed phylogenetic-level data.

The influence of vegetation type on soil bacterial communities is better characterized in other ecosystems, such as the Tibetan Plateau, where a combination of phospholipid fatty acid analysis, community-level physiological profiles, and pyrosequencing has been employed (32–34). In particular, the pyrosequencing profiles of rRNA genes indicated that both bacterial and fungal communities clearly differed in three plateau habitats. In a montane elevation gradient study, vegetation-type zonations were associated with distinct microeukaryotic, fungal, and protistan microbial communities (26). Microscopic analysis showed that the bacterial and microeukaryotic structure changed with plant species in *Sphagnum* peatlands when perturbed by warming (35). Taken together, these reports reinforce the hypothesis that plant communities and belowground microbial communities are highly interdependent, at least at lower latitudes. Because climate change may have a more substantial initial impact on Arctic ecosystems than other ecosystems and may lead to changes in ecosystem function, it is both crucial and timely to determine if this dependency is also true for tundra soils. This can be achieved only by using high-resolution techniques and by sampling soils appropriately (i.e., sampling once in replicate independent noncontiguous patches of each vegetation type, rather than collecting multiple samples within a single patch of each vegetation type) so that correlations can be extrapolated to a landscape scale.

We have investigated both soil bacterial and microeukaryotic communities among the four major tundra vegetation types to test the following hypotheses: (i) the distributions of bacterial and microeukaryotic communities, including fungi, protists, and metazoans, differ among the principal vegetation types of the low Arctic, (ii) these distinct bacterial and microeukaryotic communities are the result of different environmental factors, and (iii) under conditions of similar pH, nitrogen-related factors play important roles in bacterial and eukaryotic community composition.

MATERIALS AND METHODS

The study site was located near the Daring Lake Tundra Ecological Research Station (TERS; 64°52′N, 111°35′W), which is 300 km north of Yellowknife, NWT, Canada. Briefly, this region is locally referred to as "the barrens" and is ~75 km north of the tree line (*Picea mariana*). The site is underlain by continuous permafrost to a depth of >160 m (36) and has a shallow active layer that develops during seasonal thaws and that reaches a maximum depth of 0.3 to 1.2 m, depending upon the soil and the plant cover (37). Detailed vegetation mapping (Ecological Monitoring and Assessment Network) (9) shows a hydrologically driven mosaic of tundra vegetation types, including dry heath, dwarf birch hummock, and inundated wet sedge (37). Tall birch vegetation is found in local patches near seeps and streams or in topographic depressions that are protected from winds and where snow preferentially accumulates (38).

Soil sampling and biogeochemical analysis. Soil samples were collected (23 to 26 June 2007) at TERS from active layers from four well-separated replicate patches (300 to 3,000 m apart; minimum patch size, ~100 m^2) of each vegetation type. This study design avoided pseudoreplication by using replicate patches of similar vegetation across the landscape, rather than replicate plots within a single vegetation patch. We note that our study represents a snapshot during a time of active vegetative growth of these communities, which have been found to vary seasonally (9, 30). However, the purpose of this research was to compare microbial communities between ecosystems rather than to assess seasonal microbial community dynamics. In each patch, surface soil samples (~10 cm by 10 cm) were collected from the top 5 cm at six representative locations using a sterile blade and then mixed together to form a single replicate sample (i.e., four composite replicates for each vegetation type). Soil samples were transported to the laboratory in insulated boxes fitted with ice packs and chilled until processed (within 7 days of collection). Plant litter and roots (diameter, >2 mm) were removed prior to processing. Soil biogeochemical analyses were conducted as described previously (38). Briefly, soil pH was determined using a fresh soil-to-water ratio of 1:5 (AB15 pH meter; Accumet; Fisher Scientific), and total soil C and N content was determined by combustion (CNS-2000; LECO, St. Joseph, MI, USA). Dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) were extracted by adding 50 ml of 0.5 M K_2SO_4 to 10 g fresh soil, shaking for 1 h, and then vacuum filtering through glass fiber filters (pore space, 1.2 μm; G4 filters; Fisher). DOC and DTN were determined using a total organic carbon-total nitrogen (TOC-TN) analyzer (Shimadzu, Kyoto, Japan). The ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in the extracts were assessed colorimetrically by automated segmented flow analysis (AAIII; Bran+Luebbe, Germany) using the salicylate/dichloroisocyanuric acid and cadmium column/sulfanilamide reduction methods, respectively. N mineralization potentials were determined after incubation of moist soil samples (i.e., four replicates of samples of 30 g each for each vegetation type) in 120-ml sampling cups, which were covered by polyvinyl chloride cling film in the darkness at 22°C for up to 10 weeks.

DNA extraction and pyrosequencing. Soil DNA was extracted and purified using a PowerSoil DNA kit (MO BIO, Carlsbad, CA, USA), followed by an UltraClean 15 DNA purification kit (MO BIO, Carlsbad, CA, USA). DNA concentrations were estimated by electrophoresis on 1% agarose gels, and DNA was diluted to 1 to 10 ng μl^{-1} before use as the template in the PCR.

Bacterial 16S rRNA and microeukaryotic 18S rRNA genes were amplified using primer set F519 and R907 and primer set Euk1F and Euk516R, respectively (39). The forward primer was attached to the Roche 454 B pyrosequencing adapter and a unique 7-bp bar code; the reverse primer was modified with the Roche 454 A sequencing adapter. PCR amplifications were conducted with 25 μl 2× premix (TaKaRa), 0.5 μl 20 mM each forward and reverse primer, and 50 ng of DNA, and the volume was completed to 50 μl with double-distilled water. Each sample was amplified in triplicate using 30 cycles (94°C for 30 s, 55°C for 30 s, and 72°C for 30 s) with a final extension at 72°C for 10 min for bacterial DNA and 35 cycles (95°C for 45 s, 56°C for 45 s, and 72°C for 1 min) with a final extension at 72°C for 7 min for eukaryotic DNA. The three reaction products were pooled and purified using a QIAquick PCR purification kit (Qiagen) and then quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA). All bar-coded PCR products were combined in equal DNA amounts before pyrosequencing using the 454 GS-FLX platform.

Bioinformatics and data deposition. Small-subunit (SSU) rRNA gene data were processed and analyzed as previously described (40), using the QIIME software package (41). Poor-quality sequences (i.e., sequences of <200 bp with an average quality score of <25 and ambiguous characters) were discarded (42). Filtering of the sequences to remove erroneous

operational taxonomic units (OTUs) due to sequence errors and chimeras was conducted using the USEARCH tool in QIIME, version 1.8.0 (43). Because we relied on filtering rather than denoising of the data, we also deleted singleton OTUs and set a threshold for a high-quality score (i.e., 30) when running the command split_libraries.py, similar to practices from other studies (40, 44, 45). The remaining high-quality sequences were assigned to OTUs on the basis of 97% similarity with the sequence determined with the UCLUST algorithm (46). The most highly connected sequence (i.e., the sequence with the highest similarity to all other sequences in the cluster) was chosen to represent each OTU (40). All selected representative sequences were aligned by use of the PyNAST tool (47). Each phylotype was classified using the Greengenes database (http://greengenes.lbl.gov/), with sequences with no hits designated "unclassified." A total of 2,000 bacterial sequences were randomly selected from each sample, and sample dissimilarities were calculated from the derived OTU table using the Bray-Curtis dissimilarity metric (48). Microeukaryotic sequences were classified into OTUs *de novo* at a 97% similarity level using the UCLUST algorithm (46). Taxonomy was assigned to eukaryotic OTUs of the Silva 104 database (http://www.arb-silva.de/download/archive/qiime/). Microeukaryotic sequences included those of fungal, protistan, and metazoan microorganisms. To rarify all the data sets to the same level of sampling effort, 1,300, 390, 120, and 160 sequences were randomly selected for total eukaryotes, fungi, protists, and metazoans, respectively. Nonmetric multidimensional scaling (NMDS) ordinations were generated using the vegan tool of R, version 2.3.0 (49), on the basis of Bray-Curtis dissimilarities. In order to describe biodiversity, which incorporates the phylogenetic difference between species, Faith's index (50), which has been extensively used in the literature (14, 26), was used to calculate phylogenetic diversity. Species-level measurement was assessed by use of the Shannon index (H'). In summary, rarefied OTU collections were used to calculate richness (i.e., the number of phylotypes), Faith's phylogenetic diversity (PD), and the Shannon index of species-level diversity.

In order to identify environmental and biogeochemical factors associated with diversity, correlations between diversity metrics and soil metadata were conducted by use of SPSS software (version 20.0) and Mantel tests and carried out by comparing the Bray-Curtis distances for the community data and for the associated sample metadata. Permutational multivariate analyses of variance were conducted, using the ADONIS function within the vegan package (51), to determine whether vegetation type contributed to microbial community composition. Distance-based multivariate analysis for a linear model using forward selection (DISTLM forward) was applied to evaluate the cumulative effect of each environmental variable on the composition of bacterial, eukaryotic, fungal, protistan, and metazoan communities (52).

The 454 pyrosequencing data set was deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (http://trace.ncbi.nlm.nih.gov/Traces/sra/) under study SRP047020 with accession numbers SRX699300 and SRX699338.

RESULTS

Soil microbial community composition. We obtained between 2,018 and 8,103 high-quality bacterial sequences per sample, and 99.3% were classified into a total of 2,286 distinct OTUs. *Acidobacteria* and *Alphaproteobacteria* dominated the assigned bacterial phyla and accounted for ~35% and 23% of all OTU sequences, respectively (see Tables S1 and S2 in the supplemental material). The relative abundance of each bacterial taxonomic group varied among vegetation types and differed the most for the wet sedge soils compared to the others (Fig. 1A). For example, *Acidobacteria* phylotypes were slightly less frequent in the wet sedge and tall birch sites than in the sites with the two drier vegetation types (31% versus 36%; see Table S1 in the supplemental material), and *Gammaproteobacteria* were more abundant in tall birch soils than in soils from the other three vegetation types (13% versus 7%).

A wider range of eukaryotic sequences (1,361 to 10,799 per sample) than bacterial sequences was recovered from the soils, with 99.9% being categorized into 1,304 OTUs. These were dominated by fungal sequences (48%; representing 398 to 6,869 fungal sequences per sample), and within this grouping, the classification with the largest number of sequences was by far the *Basidiomycota*, with over 56% of all fungal sequences consisting of those from members of the *Basidiomycota* (see Table S3 in the supplemental material). Protists constituted 11% of the eukaryotes (128 to 3,052 sequences in each sample; see Table S3 in the supplemental material), and metazoans constituted 17% (162 to 1,935 sequences per sample; see Table S3 in the supplemental material). Overall, there were strong differences in the eukaryotic community structure of wet sedge soils from the structure of soils from the other vegetation types (Fig. 1B). Although fungi were generally well represented in all soils, their relative abundance was less in the wet sedge samples than in samples of the other vegetation types (Fig. 1B; see Table S3 in the supplemental material). For example, members of the *Basidiomycota* (7%), *Chytridiomycota* (6%), and *Ascomycota* (3%) were less frequent in wet sedge samples, where they represented an average of 26%, 11%, and 9% of the sequences, respectively, than in samples of the other three vegetation types (see Table S3 in the supplemental material). The wet sedge soils did, however, contain more than twice as many sequences representing protists (i.e., *Alveolata*, *Rhizaria*, and *Amoebozoa*) than soils from the three other vegetation types (19% versus 7%) (see Table S3 in the supplemental material).

OTU richness and diversity. The richness, Faith's phylogenetic diversity (PD), and the Shannon index (H') of OTUs followed similar patterns in each vegetation type, with relatively high values being found for bacteria and low values being found for the eukaryotic groupings (Table 1). Among the vegetation types, bacterial OTU richness was the highest in wet sedge, protistan OTU richness was the lowest in tall birch, and there were no significant effects of vegetation type on the OTU richness of fungi and metazoans or pooled total eukaryotes, except for protists. Faith's phylogenetic diversity was significantly higher in the wet sedge, birch hummock, and dry heath bacterial communities. In addition, similar to the OTU richness and Faith's phylogenetic diversity, H' values were significantly lower in the tall birch bacterial communities (Table 1). In general, bacterial OTU richness and Faith's phylogenetic diversity were positively correlated with soil pH (Fig. 2) and H' was significantly related to the DOC (see Table S4 in the supplemental material). Overall, there was no significant correlation between OTU richness, PD, H', and soil variables for total eukaryotic microbes and their subgroups. However, if the data from the wet sedge soil were removed from these analyses, OTU richness, PD, and H' were negatively correlated with DOC, DON, NH_4^+, and N mineralization potential across the dry heath, birch hummock, and tall birch soils (see Table S5 in the supplemental material). Bacterial OTU richness and the Shannon index correlated positively with the soil C/N ratio, and metazoan OTU richness was negatively correlated with the soil C/N ratio (see Table S5 in the supplemental material).

Influence of biogeochemical properties on soil microbial communities across all vegetation types. Comparisons of soil microbial community structures among all soil samples demonstrated robust ordinations for bacteria, total eukaryotes, fungi,

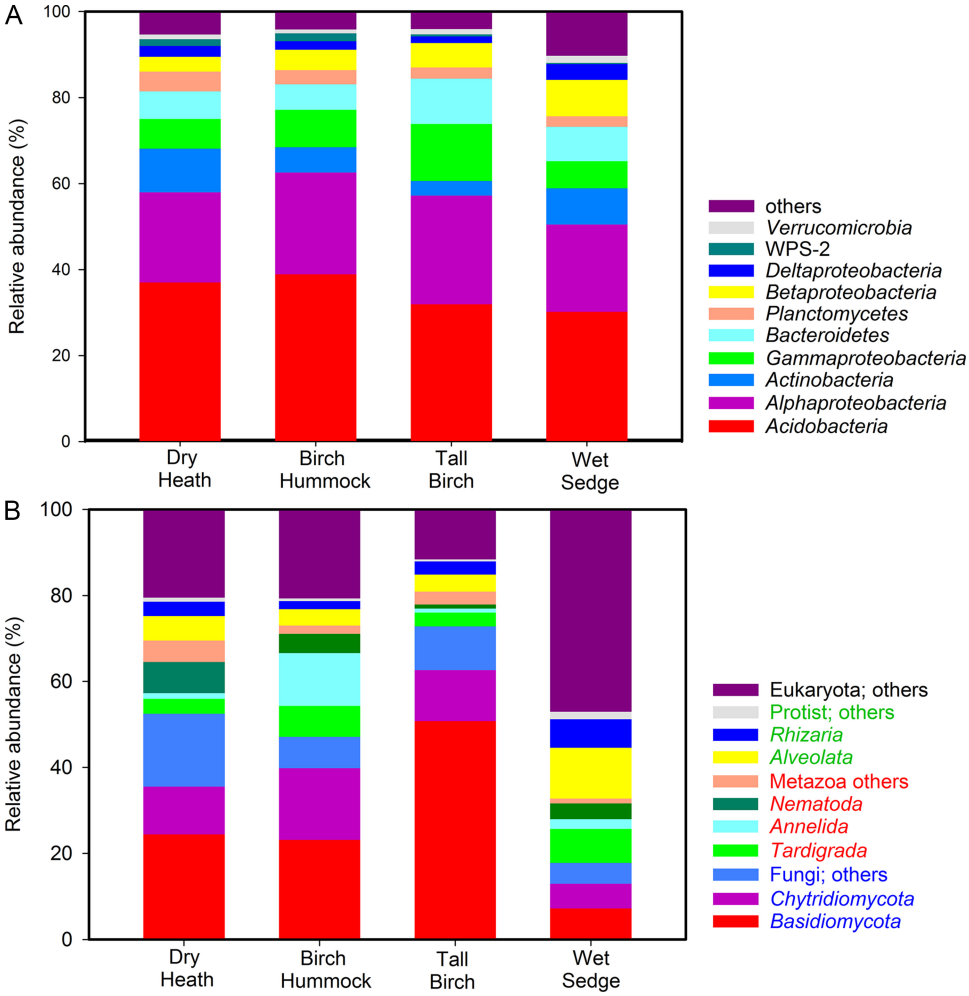

FIG 1 Relative abundance of the dominant bacterial phyla (A) and eukaryotic soil microbial groups (B) in soils from the four principal low Arctic vegetation types at Daring Lake, NWT, Canada. Relative abundance is based on the frequencies of those DNA sequences that could be classified to the phylum level. "Others" represent sequences that were unclassified and sequences which were present in amounts less than 1% of the total. Blue, red, and green typefaces, fungal, metazoan, and protistan groups, respectively.

protists, and metazoans (Fig. 3). Combined with additional multivariate statistics (ADONIS; see Table S6 in the supplemental material), we found that taxonomic representations in wet sedge soil samples were similar among replicate sites across the landscape and clearly distinct from those in soils sampled beneath other vegetation types. Except for protists, the community compositions of bacteria, total eukaryotes, fungi, and metazoans were also similar among replicate tall birch sites and distinct from those at sites with other vegetation types. In contrast, there was relatively little difference in the community composition between the dry heath and birch hummock soils for any of the major taxonomic groups, save for the total eukaryotes and metazoans. Together, these results show that vegetation type has a strong influence on the community composition of microorganisms in the underlying surface soils.

Biogeochemical controls on soil bacterial and eukaryotic community structure. Correlations between community composition dissimilarities and soil biogeochemical properties across vegetation types suggested that the soil pH, moisture content, NH_4^+ concentration, and C/N ratio explained much of the distribution of the major soil taxonomic groups (Table 2). Bacterial community composition was most strongly correlated with soil pH and was correlated to a lesser extent with the C/N ratio, NH_4^+ concentration, moisture content, N mineralization potential, and DON. Indeed, the highest correlation was with soil pH in every community except metazoans (Table 2). Additional calculations of cumulative proportions confirmed that pH and moisture properties consistently contributed to the variation in composition within each of the major taxonomic groups (Table 3; see Tables S8 to S12 in the supplemental material for details). In particular, soil pH was the primary explanatory variable in all models (Table 3).

To test if pH remained an important factor in explaining the bacterial community composition in soils of similar acidity, the wet sedge soil data were removed and the analysis was repeated. Soil pH remained significantly correlated with bacterial consortia, as did DOC, DON, N mineralization potential, NH_4^+ concentration, and the soil C/N ratio; however, the main driver for bacterial distributions was the C/N ratio (Table 4). Soil pH did not influence the total eukaryotic, fungal, protistan, and metazoan community distributions significantly. When eukaryotes were exam-

TABLE 1 Richness and diversity of OTUs in the four principal vegetation types of low Arctic tundra near Daring Lake, NWT, Canada[a]

Vegetation type	OTU richness					Faith's PD					*H'*				
	Bacteria	Total eukaryotes	Fungi	Protists	Metazoans	Bacteria	Total eukaryotes	Fungi	Protists	Metazoans	Bacteria	Total eukaryotes	Fungi	Protists	Metazoans
Dry heath	502 (29)^{AB}	213 (31)^{A}	81 (7.0)^{A}	49 (6.9)^{B}	23 (3.8)^{A}	57.5 (2.4)^{AB}	39.8 (3.9)^{A}	15.8 (1.2)^{A}	11.9 (2.8)^{A}	8.8 (2.0)^{A}	7.8 (0.2)^{A}	6.3 (0.5)^{A}	5.1 (0.1)^{A}	5.0 (0.3)^{A}	4.7 (0.3)^{A}
Birch hummock	492 (32)^{AB}	215 (36)^{A}	75 (8.5)^{A}	60 (4.2)^{A}	25 (6.4)^{A}	56.7 (3.2)^{AB}	38.0 (5.7)^{A}	15.3 (2.1)^{A}	13.4 (1.3)^{A}	9.0 (1.0)^{A}	7.7 (0.1)^{AB}	5.8 (1.0)^{A}	4.8 (0.6)^{A}	5.5 (0.3)^{A}	5.0 (0.2)^{A}
Tall birch	444 (21)^{B}	215 (53)^{A}	72 (17)^{A}	55 (5.7)^{AB}	31 (6.4)^{A}	53.4 (2.2)^{B}	38.5 (7.3)^{A}	14.7 (3.6)^{A}	12.4 (0.7)^{A}	8.9 (0.3)^{A}	7.4 (0.2)^{B}	5.8 (1.3)^{A}	4.5 (0.3)^{A}	5.2 (0.2)^{A}	4.8 (0.2)^{A}
Wet sedge	564 (52)^{A}	230 (75)^{A}	83 (11)^{A}	54 (2.4)^{AB}	24 (7.2)^{A}	61.8 (3.1)^{A}	40.3 (12)^{A}	16.6 (1.9)^{A}	12.7 (0.3)^{A}	8.9 (0.6)^{A}	8.0 (0.2)^{A}	6.0 (1.4)^{A}	4.9 (0.6)^{A}	5.1 (0.2)^{A}	4.8 (0.2)^{A}

[a] For the bacteria, total eukaryotes, fungi, protists, and metazoa, 2,000, 1,300, 390, 120, and 160 sequences were randomly selected, respectively. Values within the same column that do not share the same superscript letter differ significantly ($P < 0.05$). Standard deviations are in parentheses and were calculated for samples taken from the four replicate sites of each vegetation type.

FIG 2 Relationship between bacterial OTU richness (A) or Faith's phylogenetic diversity (B) and soil pH. Symbols: circles, dry heath; triangles, birch hummock; squares, tall birch; stars, wet sedge.

ined as a whole, they were distributed along a soil NH_4^+ concentration gradient in the soils under dry heath, birch hummock, and tall birch (Table 4). In soils from these three vegetation types, the distributions of fungi were explained by the NH_4^+ concentration, C/N ratio, and DON, with the metazoans being associated with the soil C/N ratio. Additional calculations of cumulative proportions further suggested that NH_4^+ availability was a consistent and significant control on the variation in composition within total eukaryotes and fungal groups (see Table S7 in the supplemental material; see also Tables S13 to S17 in the supplemental material for details).

DISCUSSION

Potential for microbial community changes in low Arctic tundra landscapes. Our high-taxonomic-resolution-pyrosequencing results, combined with our landscape-level sampling design, demonstrate conclusively that soil bacterial and microeukaryotic communities differed significantly among the principal low Arctic vegetation types represented in the Daring Lake region. The test of our first hypothesis showed that there are substantial differences in the bacterial, total eukaryotic, fungal, protistan, and metazoan communities at a landscape scale between tall birch sites, birch hummock sites, and sites with the other two vegetation types. Although the data have not always been consistent, other studies suggest that different bacterial or fungal communities associate with distinct tundra vegetation cover. These have been based on comparisons of fewer vegetation types than the number compared in this study and on smaller-scale sampling within vegetation

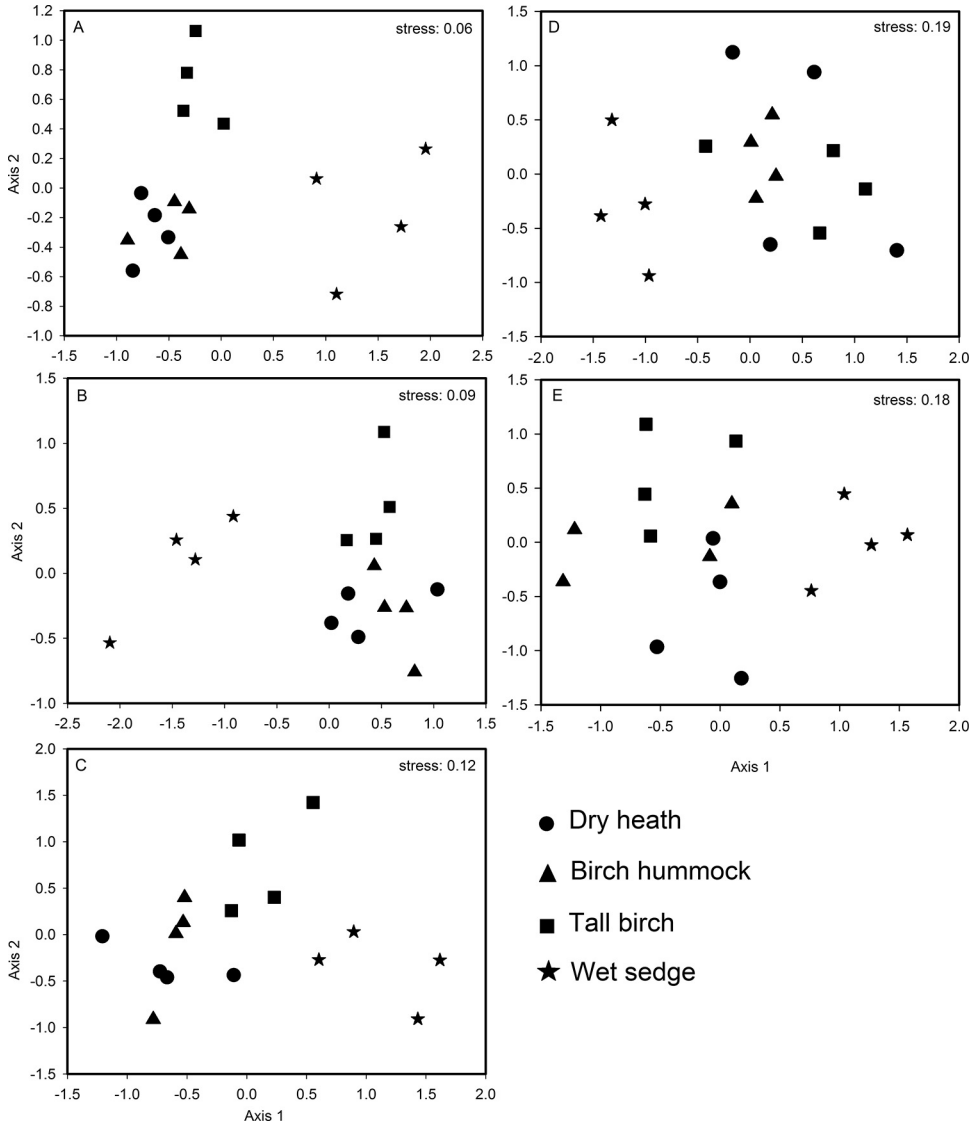

FIG 3 Nonmetric multidimensional scaling (NMDS) ordinations of the community compositional dissimilarities among the four replicate sites of each of the four vegetation types for bacterial (A), total eukaryotic (B), fungal (C), protistan (D), and metazoan (E) communities.

patches rather than between separate independent replicate patches across the landscape, and furthermore, they have employed techniques with lower taxonomic resolution (11, 30, 31). In contrast, distinct microbial communities were obtained in this study, and the differences were apparent even within bacterial phyla or subphyla and within different eukaryotic groups (see Tables S1 and S3 in the supplemental material). For example, the relative abundance of *Acidobacteria* was the lowest in the higher-pH wet sedge soils, consistent with previous observations that several *Acidobacteria* groups predominate within acidic soils (18, 45, 53, 54). Members of the *Basidiomycota*, a major group of soil decomposers (33, 55), were very abundant in all sampled vegetation types, except for the wet sedge soils, where the lack of oxygen may limit their growth. Protists, on the other hand, were more numerous in wet sedge soils than in soils from the other three vegetation types (see Table S3 in the supplemental material), which is consistent with their known aquatic habitat distribution (56).

Results showing distinct consortia tied to biogeochemical constraints are important because as the climate changes, tall shrub (often birch) vegetation is increasing in many parts of the low Arctic, where it is replacing birch hummock and other similar vegetation types (57). Hydrological changes associated with thawing permafrost have degraded some wet sedge areas and established others (58–60). Because climate warming appears to alter interactions between the aboveground and belowground communities, it is predicted that a changing microbial food web may contribute positive feedbacks to global warming by destabilizing the carbon cycle (35). Our data suggest that vegetation changes will likely result in substantial perturbations to underlying soil microbial communities. Future research on the functional traits associated with these microbes would be particularly useful to provide an understanding of and predict the potential biogeochemical impacts of these changes.

Moisture and pH as drivers of microbial communities in wet sedge soils. On a circumpolar basis, wet sedge tundra covers

TABLE 2 Mantel test results for relationships between soil biogeochemical properties and bacterial, total eukaryotic, fungal, protistan, and metazoan community compositional dissimilarities across all four tundra vegetation types[a]

	Mantel test result				
Variable	Bacteria	Total eukaryotes	Fungi	Protists	Metazoans
Moisture content	**0.36**	**0.23**	**0.28**	**0.3**	0.15
Soil pH	**0.64**	**0.57**	**0.5**	**0.36**	**0.28**
Soil C	0	0	0.06	0	0
Soil N	0.19	0.03	0.13	0.15	0
DOC	0	0	0.09	0.04	0
DON	**0.21**	0.13	**0.32**	**0.19**	0.08
NH_4^+ concn	**0.47**	**0.57**	**0.46**	**0.26**	**0.26**
Avail. P	0	0	0	0.13	0.01
N Mineral.	**0.29**	0.18	0.12	**0.21**	0.12
C/N	**0.5**	**0.36**	**0.42**	**0.32**	**0.31**

[a] Data are for 4 replicate sites. Values in bold indicate statistically significant correlations ($P < 0.05$). DOC, dissolved organic carbon; DON, dissolved organic nitrogen; Avail. P, available phosphorus; N Mineral., N mineralization potential; C/N, carbon-to-nitrogen ratio.

TABLE 4 Mantel test correlation coefficients for relationships between soil biogeochemical properties and microbial community composition dissimilarities across birch hummock, dry heath, and tall birch[a]

	Mantel test correlation coefficient				
Variable	Bacteria	Total eukaryotes	Fungi	Protists	Metazoans
Moisture content	0	0.03	0.09	0.09	0
pH	**0.24**	0.09	0.19	0	0
C	0.2	0.14	0.16	0.07	0
N	0.18	0	0.05	0.2	0
DOC	**0.29**	0.17	0.34	0.2	0.01
DON	**0.44**	**0.28**	**0.44**	0.21	0.07
NH_4^+ concn	**0.69**	**0.49**	**0.6**	0.07	0.21
Avail. P	0	0.12	0.05	**0.33**	0.12
N Mineral.	**0.44**	0	0.07	**0.14**	0.04
C/N	**0.75**	**0.31**	**0.37**	**0.27**	**0.27**

[a] Data are for 4 replicate sites. Values in bold indicate statistically significant correlations ($P < 0.05$). DOC, dissolved organic carbon; DON, dissolved organic nitrogen; Avail. P, available phosphorus; N Mineral., N mineralization potential; C/N, carbon-to-nitrogen ratio.

880×10^6 ha (61). Wet sedge soils are characterized by a high soil carbon and water content and relatively high rates of net primary production compared to the findings for dry heath and birch hummock soils (37), despite low rates of decomposition (62, 63). Compared to the findings for soils from the other vegetation types, the richness and phylogenetic diversity of bacteria in the wet sedge soils were high and were correlated with high water saturation and moderate pH (pH 5.1 to 5.3) (Table 1; see also Tables S4, S5, and S12 in the supplemental material). Others have reported low levels of bacterial diversity in this pH range (45) and identified bacterial OTUs that preferentially associate with intermediate pH (17). As well, decomposition decreases at high water saturation values (>200% moisture) (64), where low oxygen availability becomes limiting to many fungi and probably also constrains surviving anaerobically tolerant fungal populations and their turnover. Indeed, the relative abundance of *Basidiomycota*, the classic decomposers (55), in wet sedge soils was reduced 4-fold compared to that in soils from the other vegetation types (Fig. 1; see also Table S3 in the supplemental material).

TABLE 3 Cumulative relationships between soil biogeochemical properties and bacterial, total eukaryotic, fungal, protistan, and metazoan community composition across all four tundra vegetation types[a]

	% of variation in each data set attributable to each variable				
Variable	Bacteria	Total eukaryotes	Fungi	Protists	Metazoans
pH	**27.6**	**21.3**	**21.3**	**12.9**	5.14
DOC	**40.5**	**31.5**	27.9	20	**17.4**
Moisture content	**53.8**	**42.4**	**36.8**	**28.3**	**27.7**
DON	**59.5**	49.7	**52.4**	33.8	33.8
NH_4^+ concn	63.6	54.8	56.9	39.9	41.3
Soil N	67.3	58.5	61.2	45.9	47.1
Soil C	71.2	62.9	65.1	**51.2**	51.3
N Mineral.	75.1	67.5	69.5	60.3	56.1
C/N	78.8	72.7	74.7	66.2	**70.1**
Avail. P	82.6	77.7	78.9	74.2	75.1

[a] Data are for 4 replicate sites. Significant ($P < 0.05$) values are shown in bold. DOC, dissolved organic carbon; DON, dissolved organic nitrogen; N Mineral., N mineralization potential; C/N, carbon-to-nitrogen ratio; Avail. P, available phosphorus.

The influence of soil pH on bacterial distributions has been well characterized (14, 33, 45, 52, 53, 65, 66), but the data have been less compelling for fungi (26, 67, 68). In addition, few investigations have addressed the effect of soil pH on protists and metazoans (24, 26, 69). Here, the protistan and metazoan communities in the wet sedge soils were clearly distinct from those in the three other soil types (Fig. 3D and E), where the protistan and metazoan communities were significantly correlated with soil moisture, pH, DON, NH_4^+ concentration, N mineralization potential, and C/N ratio (for protists) and pH, NH_4^+ concentration, and C/N ratio (for metazoans) (Table 2). These results suggest that soil pH is also an important factor driving fungal, protistan, and metazoan distributions, in addition to the bacterial distribution. These observations do not support our second hypothesis that bacterial and microeukaryotic communities are structured on the basis of different factors among vegetation types because pH appears to be an overriding influence, irrespective of the specific taxa.

Nitrogen and pH influence soil microbial communities in mesic and dry vegetation types. When data for the wet sedge soils were removed from the data sets, the specific influences of biogeochemical variables within soils of similar acidity and moisture content on the community structure could be examined. Correlation coefficients for the three remaining vegetation types showed that bacterial communities were still significantly influenced by soil pH (Table 4) but that bacterial, fungal, and metazoan community variability was associated more closely with components of N availability (Table 4). In addition to soil pH, DOC, DON, C/N ratio, NH_4^+ concentration, and N mineralization also contributed to bacterial community variability. Strikingly, every other taxonomic grouping was also influenced by the C/N ratio and NH_4^+ concentration (except for the protists, where available phosphorus substituted for the C/N ratio as a major driver, and the metazoan community composition, which was related only to the C/N ratio). These results provide support for our third hypothesis. Together, our results indicate that variables associated with nitrogen transformations may be important determinants of the microbial

community structure in soils of broadly similar pH and moisture content.

Due to low temperatures and limited N availability, organic matter in Arctic tundra soils decomposes more slowly (70–72). Nevertheless, the enhanced leaf litter inputs associated with increased deciduous shrub growth and expansion accompanying Arctic warming may result in more fertile soils that enhance N availability (29, 38) and result in higher decomposition rates (72). Significantly, we found that the tall birch soils had microbial communities distinct from those in birch hummock soil samples (Fig. 3; see also Table S6 in the supplemental material) and higher mineral N and DON pool sizes and N mineralization rates than those in birch hummock soil samples (see Table S12 in the supplemental material). Because >85% of tundra plant N may be derived from fungi (73), the interdependence of plant cover and fungal communities is likely reflected by the correlation of fungi and N availability (Table 4). Together, these results suggest that shrub expansion and increased birch litter inputs could result in more rapid nitrogen transformation in soils, in turn affecting the microbial community structure in concert with climate change.

In summary, we have successfully addressed three important questions in tundra landscape ecology. Our results have demonstrated clear differences in the bacterial and microeukaryotic community structure among the four principal vegetation types of low Arctic tundra. We have also shown that gradients in soil pH and moisture structure influence bacterial and microeukaryotic distributions among vegetation types, but in soils with similar pHs and relative moisture contents, variables associated with nitrogen transformations are important determinants of the microbial community structure. Together, these results suggest that Arctic soil feedback responses to climate change will be vegetation specific not just because of distinctive substrate and environmental characteristics but also, potentially, because of inherent differences in microbial community structures.

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