

The Influence of Vegetation Type on the Dominant Soil Bacteria, Archaea, and Fungi in a Low Arctic Tundra Landscape

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Arctic vegetation communities vary greatly over short distances due to landscape heterogeneities in topography and hydrological conditions, but corresponding patterns and controls for soil microbial communities are not well understood. We characterized and compared the most abundant phylotypes within replicate soil microbial communities ($n = 4$) underlying the four principal vegetation types in Canadian low Arctic tundra (dry heath, birch hummock, tall birch, and wet sedge) using denaturing gradient gel electrophoresis (DGGE) of small subunit rRNA genes. We identified 10 major bacterial phylotypes. Although most were present in all soil samples, their relative abundances differed significantly and consistently according to vegetation type. By contrast, the fungal communities of all vegetation types were dominated by two common phylotypes. The communities of major archaea (11 identified) differed substantially among some of the vegetation types and even among replicate patches of the same vegetation type, indicating large spatial heterogeneities that could not be attributed to the influence of vegetation type. Bacterial and fungal communities in all vegetation types were dominated by *Acidobacteria* and *Zygomycota*, respectively. Archaeal communities were dominated by *Euryarchaeota* in tall birch and wet sedge although both *Euryarchaeota* and *Thaumarchaeota* were abundant in the birch hummock and dry heath soils. We conclude that vegetation type exerts a strong influence on soil bacterial community structure, and a relatively small and varying influence on archaeal and fungal communities in low Arctic tundra. Finally, variation in bacterial community structure among the vegetation types was correlated with soil soluble N and N mineralization potential, suggesting a close association between the relative abundances of dominant soil bacteria and N availability across low Arctic tundra.

Abbreviations: DGGE, denaturing gradient gel electrophoresis; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DTN, dissolved total nitrogen; PCR, polymerase chain reaction.

Global warming is widely predicted to be largest and most rapid in the Arctic (ACIA, 2005; IPCC, 2007). Thus, the large amount of C stored in Arctic tundra soils (Billings et al., 1982; Oechel and Vourlitis, 1994; Ping et al., 2008) is at a risk of loss due to accelerated soil organic matter decomposition in a warmer climate (Nadelhoffer et al., 1992). Recent evidence suggests that there have been substantial changes in plant community structure across the circumpolar tundra over the past three decades, with an increase in cover and density of shrubs that may be a response to climate warming (Devi et al., 2008; Sturm et al., 2001; Tape et al., 2006). The potential significance of these vegetation changes to the community structure and functioning of associated microorganisms is not clear because we do not yet understand the extent of influence of vegetation type on soil microbial communities in Arctic tundra. Microorganisms play a critical role in decomposition and mineralization processes in soil, transferring substantial C from soils to the atmosphere, which in turn could provide a positive feedback to climate warming if their activities increase (ACIA, 2005; IPCC, 2007). Soil microbial communities may also change in response to Arctic warming and it is particularly important to

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collect “baseline” information on the structural composition of these communities and the factors that influence their distribution, before potential changes occur.

Arctic tundra plant community composition varies greatly across short distances due to heterogeneities in topography and hydrological conditions across the landscape (Bjork et al., 2007; Walker, 2000). Soil biogeochemical qualities and microbiological activities also vary substantially among different vegetation types in Arctic tundra (Bjork et al., 2007; Buckeridge et al., 2010; Cheng et al., 1998; Christensen et al., 1999; Chu and Grogan, 2010; Grogan and Jonasson, 2006; Nadelhoffer et al., 1991; Neff and Hooper, 2002; Schmidt et al., 2002). However, the influence of vegetation type on the composition and structure of Arctic soil microbial communities has not been resolved. For example, phylum-level investigations of bacterial and fungal communities indicated large differences in composition between shrub and tussock tundra soils on the Alaskan north slope (Wallenstein et al., 2007). In contrast, shrub- and grass-dominated soils in a Finnish Arctic tundra site had very similar bacterial and fungal communities at the phylum level (Männistö et al., 2007). Recently, Buckeridge et al. (2010) observed similar bacterial and fungal communities between birch hummock and tall birch soils in a Canadian low Arctic tundra site. In summary, the relationships between tundra vegetation types and microbial community structure are not well understood, despite their fundamental importance to understanding the biogeochemistry and functioning of Arctic ecosystems.

Previously, we observed large differences in soil biogeochemical variables among the four principal vegetation types within a Canadian low Arctic tundra landscape (Chu and Grogan, 2010). Furthermore, soil N mineralization potentials in those vegetation types were significantly and closely correlated with soil soluble C and N pools, and with soil total C/N ratios (Chu and Grogan, 2010). We hypothesized that these biogeochemical differences among vegetation types may correspond to differences in soil microbial communities. Here, we identify the dominant members of the bacterial, archaeal, and fungal communities in soils of those vegetation types at the same site. Our study is novel because it is a simultaneous investigation of all three principal soil microbial taxonomic groups, with a design based on replicate soil samples from well separated patches of each vegetation type that is appropriate to landscape-level extrapolation. Furthermore, it is one of very few molecular ecology studies that relate soil microbial community structure to biogeochemical cycling. We used our data to test the following research hypotheses: (i) distributions of the dominant bacterial, archaeal, and fungal phylotypes differ among vegetation types in low Arctic tundra and (ii) variation in community structure within these microbial groups corresponds to landscape-level variation in soil biogeochemical properties.

MATERIALS AND METHODS

Study Site Characteristics

The study site was located near the Tundra Ecological Research Station (TERS) at Daring Lake (64°52' N, 111°35' W), in central

Northwest Territories, Canada, approximately 300 km northeast of Yellowknife. Although designated as a southern Arctic ecozone, this region is locally referred to as “the barrens”, and lies approximately ~25 km north of the nearest substantial patches of trees (*Picea mariana*). The region is underlain by continuous permafrost to a depth of >160 m (Dredge et al., 1999) and has a shallow active layer developing during the thaw season, reaching a maximum of 0.3 to 1.2 m depending on soil type and vegetation cover (Nobrega and Grogan, 2008). The landscape is characterized by numerous Canadian Shield outcrops and occasional eskers that were formed toward the end of the most recent glacial period (Rampton, 2000), and a large number of lakes covering ~30% of the surface (Lafleur and Humphreys, 2007). Climate records from the Daring Lake Weather Station (Bob Reid, Indian and Northern Affairs Canada, unpublished data, 1996–2008) indicate daily average temperatures as low as −41.8°C in the winter and as high as 22.2°C in the summer. An average of 123 d a year is above 0°C (May–September), and annual precipitation ranges between 200 and 300 mm.

Detailed vegetation mapping of the area as part of the Ecological Monitoring and Assessment Network indicates a hydrologically driven mosaic of tundra vegetation types including dry heath, dwarf birch hummock, and inundated wet sedge (Nobrega and Grogan, 2008). Tall birch is generally in local patches near seeps and streams, or in topographic depressions that are protected from winds and where snow preferentially accumulates. Detailed plant community compositions have been previously reported (Chu and Grogan, 2010).

Experimental Design and Soil Sampling

We surveyed the landscape at the TERS site, and located four similar, well-separated (300–3000 m apart) patches (minimum size ~100 m²) of each vegetation type (23–26 June 2007). This study design with replicate patches (rather than replicate plots within a patch) avoids pseudoreplication and makes our results appropriate to landscape-level extrapolation. In each patch, subsamples (~10 by 10 cm) of the soil organic layer (to 5-cm depth from the soil surface measured directly below the litter layer or from the green-brown moss transition) were collected at six representative points using a sterile blade and composited together as a single replicate sample. Four replicate samples for each vegetation type were stored in a cooler with ice packs during transfer to the lab in Kingston, ON and processed within 7 d of collection. Any aboveground plant material and living roots that were >2 mm in diameter were removed before processing.

Soil Biogeochemical Analyses

Soil biogeochemical analyses including N mineralization potential (Table 1) were determined as described previously (Chu and Grogan, 2010). Briefly, soil pH was determined using a fresh soil to water ratio of 1:5. Total soil C and N contents were determined by combustion (CNS-2000, LECO, St. Joseph, MI). Soil mineral N, dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) were extracted by adding 50 mL of 0.5 M K₂SO₄ to 10 g fresh soil, shaking for 1 h and then vacuum filtering through glass fiber filters (Fisher G4, 1.2 µm pore space). Mineral N contents in the extracts were determined colorimetrically by automated segmented flow analysis (Bran+Luebbe AAIII, Germany), and DOC and DTN were determined using a TOC-TN analyzer (Shimadzu, Kyoto, Japan). Dissolved organic nitrogen

(DON) was calculated as follows: (DON = DTN - NH₄⁺-N - NO₃⁻-N). Soil microbial biomass C and N was determined by the chloroform fumigation-direct extraction method (Brookes et al., 1985). Soil N mineralization potential was determined by incubating moist soil in darkness at 22°C for 10 wk, and then extracted with 0.5 M K₂SO₄ before N determination as described above (Chu and Grogan, 2010).

Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (DGGE)

Total DNA was extracted using a PowerSoil DNA kit (MO BIO, Carlsbad, CA) according to the manufacturer's instructions. The extracted soil DNA was further purified using an UltraClean 15 DNA Purification Kit (MO BIO, Carlsbad, CA), and stored at -20°C. DNA concentrations were estimated on 1% agarose gels and typically diluted 20-fold before use in polymerase chain reactions (PCR) to 1 to 10 ng µL⁻¹.

Bacterial 16S rRNA gene sequences were amplified using the primer pairs 338f (5'-ACTCCTACGGGAGGCAGCAG-3') with GC clamp (Ovreas et al., 1997) and the primer 907r (5'-CCGTCAATTCCTTTRAGTTT-3') (Lane et al., 1985). Fungal 18S rRNA gene sequences were amplified using primer pairs FF390 (5'-CGATAACGAACGAGACCT-3') and FR1 (5'AICCATTCGAATCGGTAIT-3'), and re-amplified with FF390 and FR1-GC (Vainio and Hantula, 2000). Sufficient fungal PCR products were obtained in a single PCR amplification from tall birch and wet sedge, but amplification products were too low from the birch hummock and dry heath sites for DGGE analysis. Efforts were made to pool amplification products from several parallel reactions, but these were again insufficient. To ascertain the impact of re-amplification, fungal DGGE patterns derived from first round and second round PCR for tall birch and wet sedge samples were compared. The two sets of two patterns were almost identical, suggesting that at least for these two sites, re-amplification did not have obvious effects on the predominant DGGE patterns. To keep fungal PCR conditions consistent for all the vegetation types for comparative purposes, the re-amplification protocol was done for all the vegetation types. Archaeal 16S rRNA sequences were amplified using nested PCR with the primer pair PRARCH112F (5'-GCTCAITAWCACGTGG -3') (Hoj et al., 2005) and PREA1100R (5'-YGGGTCTCGCTCGTT RCC-3') (Ovreas et al., 1997), followed by the amplification of 1 µL of the initial product with the primer pair PARCH340F (5'-CCCTACGGGGYGCASCAG-3') with GC clamp and PARCH519R (5'-TTACCGCGGCKGCTG-3') (Ovreas et al., 1997).

All amplifications were performed with a Techne TC-3000 thermocycler (Barloworld Scientific, Burlington, NJ). Polymerase chain reaction mixtures were prepared with 0.2 mM of each dNTP, 5 µL of 10×PCR buffer, 2.0 mM MgCl₂, 0.4 µM each primer, 1.25 U (1.0 U for fungal PCR) of Taq polymerase (Fermentas, York, United Kingdom), 0.1% bovine serum albumin (for archaeal DNA) and 1 µL DNA template, in a final volume of 50 µL. For bacteria and fungi PCRs, amplification was started at 95°C for 10 min and followed by 35 cycles of 1 min at 95°C, 1 min at 50°C, and 1 min at 72°C (or 1.5 min for fungal PCR); followed by 5 min at 72°C (or 10 min for fungal PCR). For archaeal nested PCR, the first amplification used 35 cycles of 45 s at 95°C, 45 s at 51°C, and 1 min at 72°C; followed by 10 min at 72°C, and the second used 30 cycles of 30 s at 95°C, 30 s at 58°C, and 1 min at 72°C; followed by 10 min at 72°C. The PCR products were electrophoresed on 1% agarose, and were then separated using DGGE with a D-Code universal mutation detection system (Bio-Rad, Hercules, CA) according to manufacturer's recommendations. Note that DGGE distinguishes only the higher frequency DNA sequences, and therefore indicates the major (i.e., abundant) members of the microbial community, but not the overall microbial community richness or composition. Bacterial amplified DNAs were separated on 6% (w/v) polyacrylamide gels with a denaturing gradient of 40 to 60% (100% denaturant contains 7 M urea and 40% formamide) and the fungal and archaeal PCR products were separated on 8% (w/v) gels with denaturing gradients of 25 to 55% and 45 to 65%, respectively. Equal concentrations of amplified DNA (300 ng) were loaded onto DGGE gels and electrophoresed in 1x Tris-acetate-EDTA buffer at 60°C and 65 V for 20 h (16 h for the fungal and archaeal samples), stained with 1:10,000 SYBR Green I (Invitrogen, Molecular Probes, Eugene, OR) and scanned using a ChemiGenius scanner (Syngene, Cambridge, UK). Note that the DGGE patterns for all samples were obtained from a single gel for each microbial group to avoid gel-to-gel variations and staining efficiencies. Reproducibility was assessed by repeating the PCR-DGGE several times for each sample to ensure that bacterial, archaeal, and fungal DGGE patterns were consistent.

Table 1. Soil moisture, soil pH, nutrient contents, microbial biomass, and N mineralization potential under the principal vegetation types near Daring Lake in June 2007 (n = 4). Values within the same column that do not share the same letter differ significantly (P < 0.05). Standard deviations are shown in parentheses (n = 4).

Vegetation type	Gravimetric moisture %	Soil pH (H ₂ O)	Soil C/N ratio		DOC†	DON mg kg ⁻¹	NH ₄ ⁺ -N mg kg ⁻¹	Biomass C g kg ⁻¹	Biomass N	N mineral mg N kg ⁻¹
			Soil C	Soil N						
Dry heath	183 (46)a	4.5 (0.2)a	34.5 (7.8)a	1.11 (0.19)a	353 (73)a	20.6 (6.7)a	0.15 (0.10)a	8.6 (0.9)ab	0.77 (0.07)a	7.6 (1.2)a
Birch hummock	309 (15)bc	4.3 (0.3)a	46.5 (1.7)b	1.58 (0.20)b	577 (136)ab	35.4 (14.0)a	0.42 (0.53)a	12.4 (1.3)c	0.96 (0.06)a	45 (23)b
Tall birch	275 (79)b	4.6 (0.2)a	36.2 (5.5)a	1.79 (0.29)b	789 (133)b	65.0 (11.9)b	9.04 (3.64)b	7.4 (0.5)a	0.91 (0.19)a	419 (274)c
Wet sedge	382 (41)c	5.2 (0.1)b	35.0 (1.2)a	1.97 (0.17)b	532 (99)a	61.8 (8.6)b	12.5 (6.94)b	11.3 (2.4)bc	0.83 (0.24)a	359 (144)c

† DOC: dissolved organic carbon; DON: dissolved organic nitrogen; Biomass N: microbial biomass nitrogen; N mineral: nitrogen mineralization potential over a 10-wk incubation.

The more intense DGGE bands (i.e., relatively abundant phylotypes) were excised, washed with sterile distilled water and a small portion (<1 mm³) was used as a template for PCR (as described above). Amplified DNAs from these bands were cloned using the QIAGEN PCR cloning kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. We re-amplified the clones and repeated the DGGE analysis to make sure that each clone represented a single band and migrated to the same gel position compared to the DGGE pattern from original soil DNA. Four to eight clones per DGGE band containing inserts of the appropriate size were sequenced commercially (BigDye terminator version 3.1 kit, Applied Biosystems, Foster City and an ABI 3730 genetic analyzer, Applied Biosystems) to generate a consensus sequence for each band. Sequences were edited (FinchTV), aligned (CLC Sequencing View 6) and neighbor-joining (NJ) trees were constructed (MEGA Version 4.0.2; <http://www.megasoftware.net>). The sequences generated in this study have been submitted to GenBank and assigned accession numbers: HM803120 to HM803166.

Statistical Analyses

To identify similarities among the DGGE community fingerprints for each microbial group, individual fingerprints were extracted from the gel images and their backgrounds were subtracted using Gelcompar II (Applied Maths, Sint-Martens-Latem, Belgium). Comparisons between fingerprints were based on the shared presence of individual bands as well as their relative intensities. Dendrograms of relative similarity (unweighted pair group method using average linkages: UPGMA) for each microbial group were generated using Pearson correlations of background-subtracted densitometric curves. The output of the clustering analysis was independent of the input order of the DGGE fingerprints.

The effects of vegetation type on each of the biogeochemical variables were determined by separate one-way ANOVA (JMP 7.0.2, 2008, SAS Institute, Cary, NC), and Tukey Kramer HSD tests. All significant results are reported ($p < 0.05$). Nonmetric multidimensional scaling (NMDS) analyses were conducted to identify possible correlations between DGGE band profiles and corresponding soil biogeochemical variables using PC-ORD (MJM Software Design, Gleneden Beach, OR). For each gel (bacterial, fungal, archaeal), DGGE band classes were identified within Gelcompar II and the relative abundances of each band were used as inputs for multivariate statistics within PC-ORD. However, first, any differences in overall fingerprint intensities between lanes were normalized. Thus, the DGGE pattern in this study were treated not just in the form of presence/absence data, but semi-quantitatively to assess shifts in relative abundance of individual bands within and among the vegetation types. Sørensen (Bray–Curtis) distance measures were used to generate the NMDS plots, and significances were determined by Monte Carlo tests with 1000 iterations comparing real data to those from randomized data (all resulting axis p values were < 0.05). Correlation vectors between DGGE band profiles and biogeochemical data that had R^2 values > 0.3 ($p < 0.05$) were superimposed on the NMDS plots. A multi-response permutation procedures (MRPP) analysis using PC-ORD and a Sørensen distance measure was conducted to identify the strength with which vegetation type correlated with the DGGE fingerprint heterogeneity. For the MRPP analysis, the chance-corrected within-group agreement statistic (A) can be interpreted as follows: $A = 1$ when all samples within each of the vegetation types possess identical DGGE fingerprints, $A = 0$ when DGGE fingerprint heterogeneity within the vegetation types is the same as that expected by chance, and $A < 0$ when fingerprint heterogeneity within the vegetation types is greater than expected by chance.

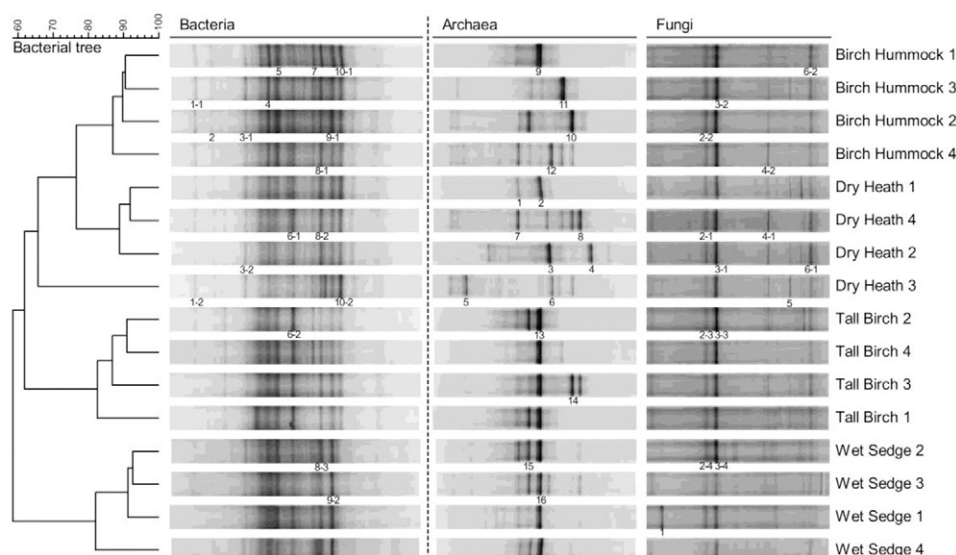


Fig. 1. Denaturing gradient gel electrophoresis (DGGE) fingerprints of bacteria, archaea, and fungi from soils under the principal vegetation types near Daring Lake, NWT. All samples within each microbial group were electrophoresed on the same gel, and separate gels were run for each group. The dendrogram illustrates relative similarities among the soil bacterial communities based on the patterns and intensities of fingerprint bands (unweighted pair group method with arithmetic means [UPGMA] of the Pearson correlations between densitometric curves). Dendrogram analyses of the densitometric curves for the archaea and fungi were ineffective because of extremely low and high similarities between samples, respectively. The scale bar indicates difference (%) in Pearson correlations based on the UPGMA tree.

RESULTS

Community Structures of the Dominant Soil Microbes among the Vegetation Types

The DGGE analyses indicated that vegetation type had differing influences on the community structures of the major (i.e., relatively abundant) bacterial, archaeal, and fungal phylotypes (Fig. 1). Although most (8/10) of the more numerous bacterial phylotypes were present in all patches of all vegetation types, bacterial community structure clustered according to vegetation type (Fig. 1), because of differences in relative abundances, and differences in composition of the less numerous phylotypes. Bacterial communities in the wet sedge and tall birch vegetation types were clearly different from each other, and from the birch hummock and dry heath vegetation

Table 2. Phylogenetic affiliations of fungal 18S rRNA gene sequences obtained from soils under the principal vegetation types near Daring Lake. Closest GenBank sequences are included and accession numbers are shown in parentheses.

DGGE bands	Closest matches	Phylum designations
band 1 (HM 803120)	<i>Candida santjacobensis</i> strain JCM 8924 (AB018150)	Ascoycota
band 2-1 (HM803121)	<i>Chytridiomyces</i> sp. JEL378 (DQ536483)	Chytridiomycota
band 2-2 (HM803122)	<i>Chytridiomyces</i> sp. JEL378 (DQ536483)	Chytridiomycota
band 2-3 (HM803123)	<i>Chytridiomyces</i> sp. JEL378 (DQ536484)	Chytridiomycota
band 2-4 (HM803124)	<i>Chytridiomyces</i> sp. JEL378 (DQ536485)	Chytridiomycota
band 3-1 (HM803125)	<i>Mortierella</i> sp. 20006 (EU710842)	Zygomycota
band 3-2 (HM803126)	<i>Mortierella</i> sp. 20006 (EU710843)	Zygomycota
band 3-3 (HM803127)	<i>Zygomycete</i> sp. AM-2008a (EU428773)	Zygomycota
band 3-4 (HM803128)	<i>Zygomycete</i> sp. AM-2008a (EU428774)	Zygomycota
band 4-1 (HM803129)	<i>Camarophyllopsis hymenocephala</i> isolate 1892 (DQ4444862)	Basidiomycota
band 4-2 (HM803130)	<i>Camarophyllopsis hymenocephala</i> isolate 1892 (DQ4444862)	Basidiomycota
band 5 (HM803131)	<i>Cystostereum murraii</i> strain CBS 257.73 (AF082850)	Basidiomycota
band 6-1 (HM803132)	<i>Byssosascus striatosporus</i> strain CBS 642.66 (AF222535)	Ascoycota
band 6-2 (HM803133)	<i>Byssosascus striatosporus</i> strain CBS 642.66 (AF222535)	Ascoycota

types, which also differed substantially from each other (Fig. 1). By contrast, the archaeal communities in the wet sedge and tall birch soils were very similar, but there were strong differences among and within the dry heath and birch hummock vegetation types (Fig. 1). Fungal communities differed markedly from the bacterial and archaeal groups in that all vegetation types were dominated by only two common phylotypes, although there was substantial variation in the faint, low density bands both among and within vegetation types (Fig. 1).

within the bands that we sequenced from the tall birch and wet sedge soils (Fig. 3). The *Euryarchaeota* were closely related to known methanogens (*Methanosphaerula*, *Methanosaeta*, *Methanosarcina*, *Methanobacterium*) (Fig. 3). The most intense fungal bands (2 and 3) originated from the phyla *Zygomycota* and *Chytridiomycota*, respectively, while the minor bands (which were more intense in the birch hummock and dry heath plant communities than in the other two vegetation types) derived from the *Ascomycota* and *Basidiomycota* (Table 2).

Phylogenetic analyses showed that bacterial bands 5–10 and bands 3–4 belonged to the *Acidobacteria* subgroup 1 and 3, respectively (Fig. 2). Bacterial bands 1 and 2 which were more intense in birch hummock and dry heath than the other two vegetation types derived from the phylum *Firmicutes* (Fig. 2). *Euryarchaeota* and *Thaumarchaeota* were detected within the archaeal communities in birch hummock and dry heath soils but we did not detect *Euryarchaeota*

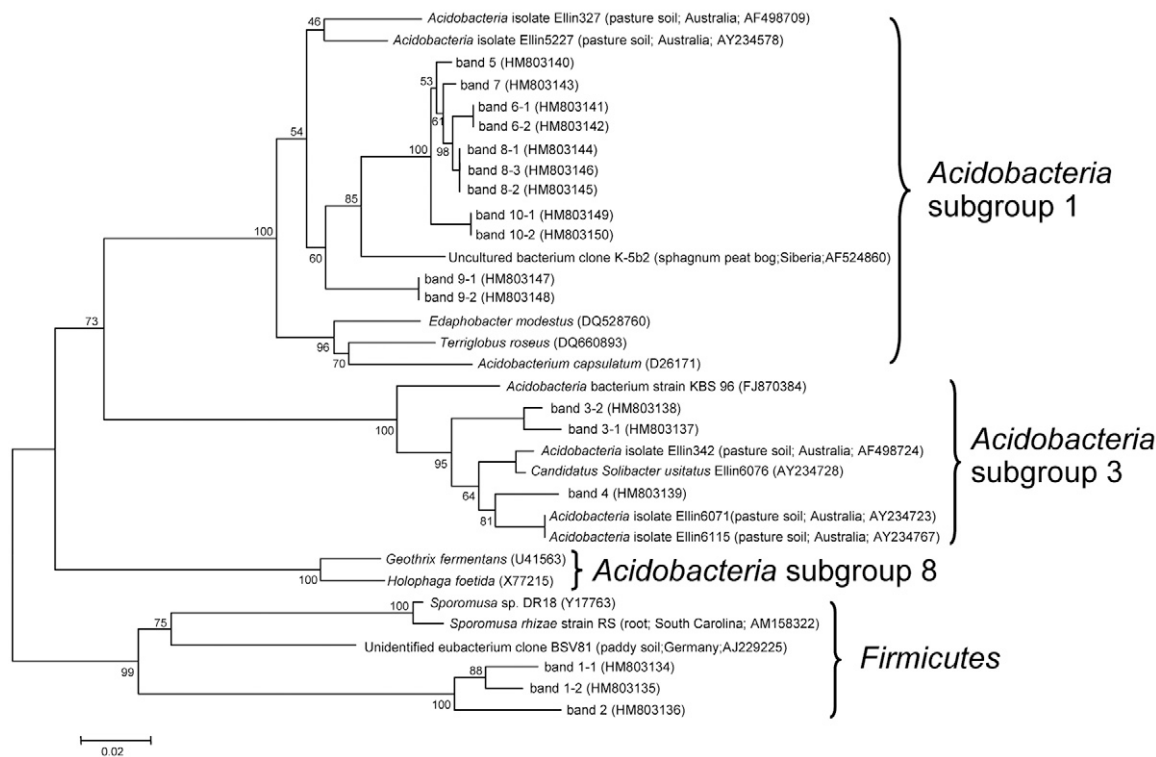


Fig. 2. Phylogenetic affiliations of bacterial 16S rRNA gene sequences obtained from soils under the principal vegetation types near Daring Lake. Selected GenBank sequences from uncultivated clones and reference strains are included for comparison. Bootstrap values are included for main branch points on this neighbor-joining tree. GenBank accession numbers are shown in parentheses. The scale bar represents 1% sequence divergence.

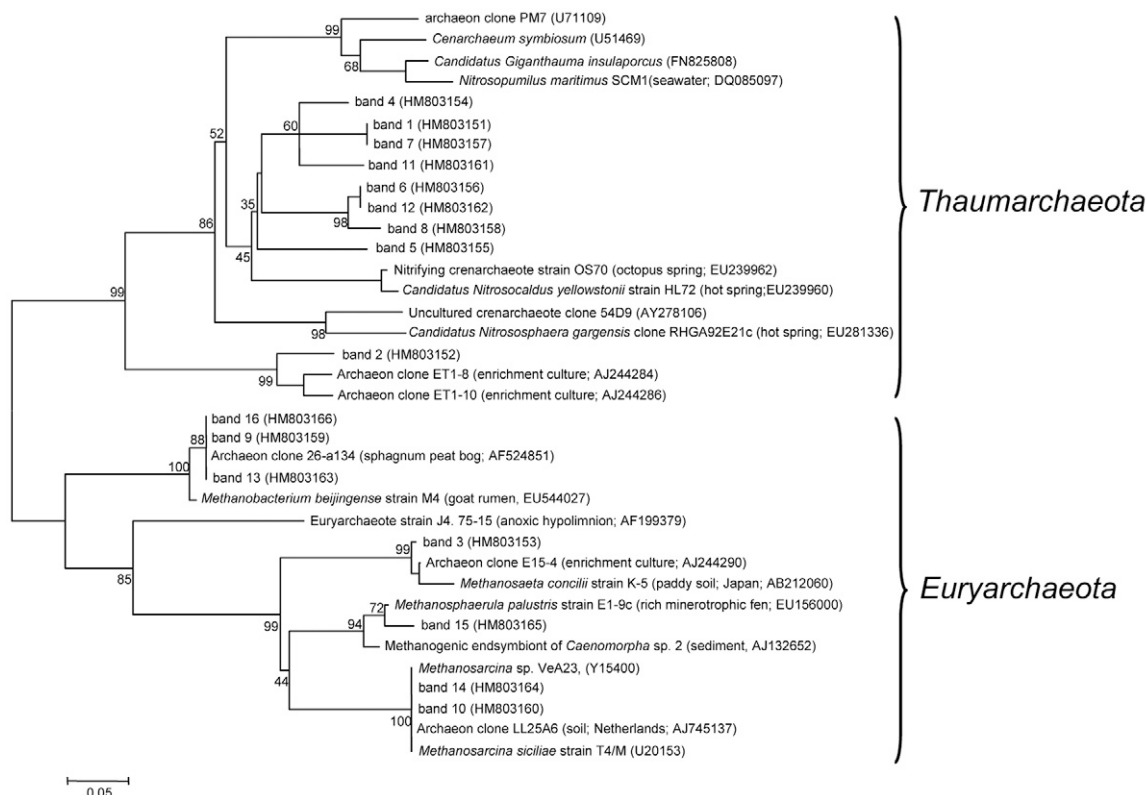


Fig. 3. Phylogenetic affiliations of archaeal 16S rRNA gene sequences obtained from soils under the principal vegetation types near Daring Lake. Selected GenBank sequences from uncultivated clones and reference strains are included for comparison. Bootstrap values are included for main branch points on this neighbor-joining tree. GenBank accession numbers are shown in parentheses. The scale bar represents 5% sequence divergence.

Community Structures of the Dominant Microbes in Relation to Biogeochemical Variables

Correlations between microbial community structures and soil biogeochemical variables were analyzed using nonmetric multidimensional scaling plots (NMDS; Fig. 4). Variation in bacterial community fingerprints was positively correlated with soil moisture, soil pH, and soil N along the second NMDS axis, which explained ~62% of the variation in DGGE profiles. Soil DOC, DON, and N mineralization potential were positively correlated with variation along the first axis, whereas soil C/N ratio was negatively correlated with variation along the first axis (Fig. 4A). Overall, the wet sedge soil community cluster was associated with relatively high soil moisture and pH levels, the dry heath soil community cluster was associated with relatively high soil total C/N, and the tall birch community cluster was associated with relatively high soluble C and N and high N mineralization potential (Fig. 4A). Similar neat clustering was not apparent in either the archaeal or fungal communities except for the dry heath samples of the latter microbial group which clustered discretely away from the others in association with relatively high soil C/N ratio (Fig. 4B, 4C). Nevertheless, soil ammonium, DON, and soil N were positively correlated, and soil C/N ratio was negatively correlated with the variation in the archaeal communities along the first NMS axis (Fig. 4B). By contrast, soil C/N ratio was positively correlated with the variation along the second axis for the fungal communities

(Fig. 4C), while soil moisture, DON, and soil N were negatively correlated with the variation along the second axis.

As anticipated from the dendrogram analysis of densitometric curves (Fig. 1), the bacterial community fingerprints clustered together according to vegetation type (Fig. 4A). Clustering of replicate samples according to vegetation type using MRPP analyses (see Materials and Methods) was strongest for bacteria ($A = 0.36$; $p < 0.0001$), weak for the fungi except in the dry heath samples ($A = 0.13$; $p < 0.001$) and absent for the archaea ($A = 0.07$; $p = 0.1$).

DISCUSSION

The Influence of Vegetation Type on Dominant Bacterial, Archaeal, and Fungal Communities

Our study of the full range of ecosystems along a typical low Arctic tundra moisture gradient demonstrates that although the composition of the major soil bacteria was similar, their relative abundances differed substantially and consistently among vegetation types (Fig. 1, Fig. 4A). By contrast, the dominant archaea differed among some vegetation types but not others (e.g., tall birch and wet sedge), and the dominant fungi were common across all of the vegetation types. Our simultaneous investigation of these microbial groups in replicate, well-separated patches of a variety of ecosystems suggests that low Arctic tundra vegetation types have a strong influence on distributions of the major soil bacteria, and relatively little effect on the distributions of the principal archaea or fungi.

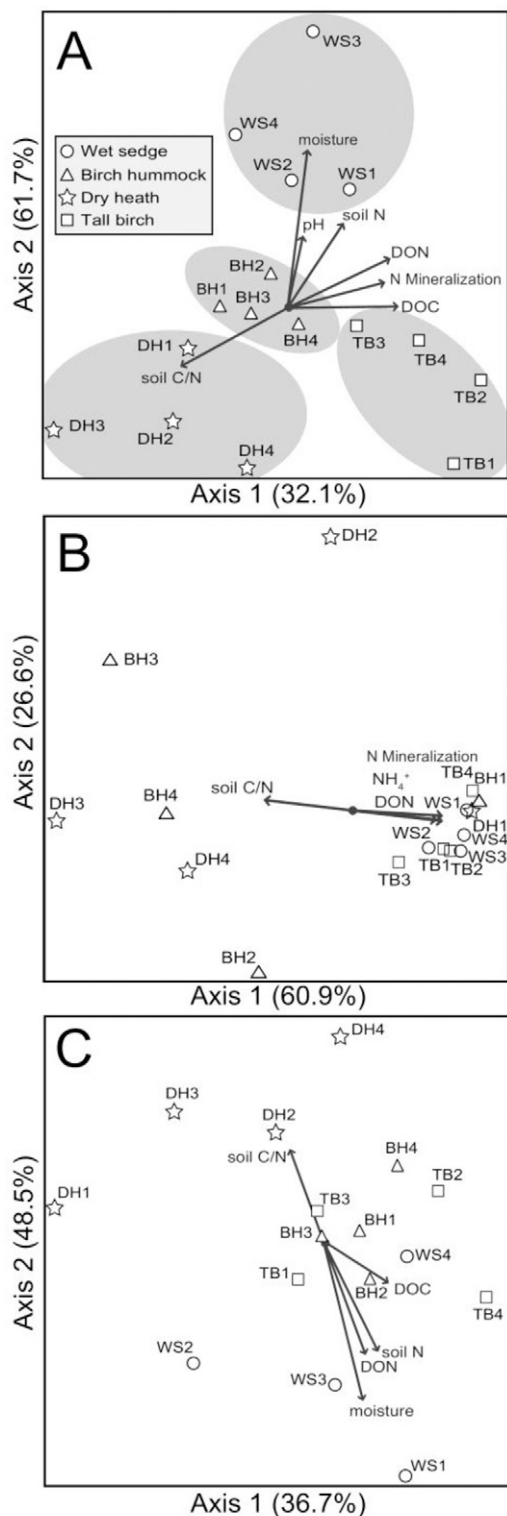


Fig. 4. Nonmetric multi-dimensional scaling (NMDS) plots showing the correlations between microbial denaturing gradient gel electrophoresis (DGGE) fingerprints (A: Bacteria, B: Archaea, and C: Fungi) and soil biogeochemical variables. Panel A inset indicates the symbols for each vegetation type in all three panels. The shaded ellipses in panel A illustrate clustering according to vegetation type for the bacterial DGGE fingerprints. Physicochemical correlates are shown with lines (for all correlates over $R^2 = 0.3$). The length of these lines indicated the relative importance of that variable in explaining the variation in DGGE profiles, while the angle between arrows indicates the degree to which they were correlated.

Accordingly, the data supported our first hypothesis (relating microbial community structure to vegetation type) for bacteria but refuted it for archaea and fungi.

Differences in bacterial communities between Alaskan tussock and shrub tundra vegetation have previously been observed using 16S rRNA gene clone libraries (Wallenstein et al., 2007). The authors suggested that plant communities regulate bacterial communities by determining the quantity and chemical quality of the litter substrate supply and by modifying the soil physical environment. Other studies have often failed to find substantial differences in bacterial communities among vegetation types. For example, shrub and grass-dominated soils in a Finnish Arctic tundra site had similar communities, as determined using DNA fingerprint analysis (terminal restriction fragment length polymorphisms; T-RFLP) (Männistö et al., 2007). A previous DGGE study at our site reported broadly similar compositions of the dominant bacteria and fungi between birch hummock and tall birch patches (Buckeridge et al., 2010), although differences in relative abundances were not investigated. At a much larger scale, no clear relationships between vegetation type and soil bacterial diversity were observed in a continental study that included tundra and temperate ecosystems using T-RFLP (Fierer and Jackson, 2006), or with a high taxonomic resolution pyrosequencing technique (Lauber et al., 2009). Although these conflicting results suggest that the influence of vegetation on bacterial communities may differ across disparate geographical locations in the Arctic, several other factors must also be considered in comparing studies. First, careful consideration of the sampling methods, site histories and ecological characteristics, and taxonomic resolution of the analytical techniques is necessary before determining that study comparisons are meaningful. Second, there may be an important temporal dimension. Seasonal microbial community shifts at subphylum taxonomic levels are now well documented in tundra soils (Lipson and Schmidt, 2004; Wallenstein et al., 2007), suggesting that at the species level, microorganisms may have specialized physiologies adapted to the environmental conditions associated with a particular season. If so, comparisons among studies (and between sites within a study) should be based on simultaneous sampling, and interpreted accordingly.

Our analysis of the soil fungal communities indicated substantial similarities but also some differences among vegetation types. The two fungal phylotypes that dominated in all soils of all vegetation types were members of the phylum *Zygomycota*. We also observed several members of the *Ascomycota* and *Basidiomycota* which were most abundant in the birch hummock and dry heath soils but also present in the tall birch soils (Fig. 1, 4). These differences in abundance are consistent with the differences in frequency and diversity of ericoid and to a lesser extent ectomycorrhizal host plants among the vegetation types. Large differences in fungal communities were detected among vegetation types in an Alaskan tundra site using the 18S rRNA gene clone library method: *Ascomycota* were dominant in tussock soils but *Zygomycota* dominated in shrub

soils (Wallenstein et al., 2007). Schadt et al. (2003) observed that <1% of the fungi were *Zygomycota* and more than 90% were *Ascomycota* in Colorado alpine tundra using a ribosomal gene library method. Taken together, these results indicate that the influence of plant communities on soil fungal community structure can differ substantially both within and among Arctic and alpine tundra sites.

In sharp contrast to the bacterial and fungal DGGE fingerprints, we observed marked variation in the dominant members of the archaeal communities among replicate sampling sites of birch hummock and dry heath vegetation (Fig. 1), suggesting high spatial heterogeneity in soil archaeal communities across short distances that cannot be attributed to differences in plant composition. Similar small-scale heterogeneity and patchy distributions of soil archaeal populations have also been observed in more temperate ecosystems (Bomberg and Timonen, 2007; Sliwinski and Goodman, 2004; Timonen and Bomberg, 2009). Archaeal communities were also consistently less diverse than bacterial communities in a study of forest, shrubland, and prairie soils using metagenomic and small-subunit RNA based sequence analysis techniques (Fierer et al., 2007). We detected both *Euryarchaeota* and *Thaumarchaeota* in birch hummock and dry heath soils but only *Euryarchaeota* in tall birch and wet sedge soils from the dominant bands we sequenced, and the *Euryarchaeota* and *Thaumarchaeota* were mostly related to methanogenic and nitrifying archaea, respectively. In high Arctic tundra soils across a natural soil moisture gradient in Spitsbergen, Hoj et al. (2006) found that differences in soil water regime were correlated with major differences in archaeal community composition as determined by the DGGE fingerprinting technique. However, we found little impact of variation in soil hydrology among vegetation types along our low Arctic moisture gradient on archaeal community structure (Fig. 4). In our study, dry heath and birch hummock soils tended to have high spatial heterogeneity in archaeal community structure but relatively low N availability and N mineralization potential compared to tall birch and wet sedge soils (Fig. 4), suggesting that spatial heterogeneities in archaeal community structure may be inversely correlated with soil fertility in low Arctic tundra.

Relationships between Soil Biogeochemical Properties and Soil Microbial Communities

We observed several significant correlations ($p < 0.05$) between bacterial DGGE band phylotypes and soil moisture, soil pH, C/N ratio, DON, ammonium, and N mineralization potential (Fig. 4). These correlations along with the distinct clustering of dominant bacterial phylotypes according to vegetation type suggests that plant litter chemistry and microclimatic effects may influence biogeochemical cycling not just directly (i.e., as substrate and environmental controls), but also indirectly by influencing the community structure of soil bacteria. By contrast, we found no evidence for these inter-relationships in the archaeal or fungal microbial groups. Accordingly, the data supported our

second hypothesis (relating microbial community structure to biogeochemistry) for bacteria but refuted it for archaea and fungi.

Soil pH has long been recognized as having a profound influence on the structure and diversity of plant communities (Crawley et al., 2005; Partel, 2002; Virtanen et al., 2006; Walker et al., 1998). Soil pH also influences acidobacterial abundance, with the highest frequencies in soils with the lowest pH (Männistö et al., 2007; Lauber et al., 2009; Chu et al., 2010). Our bacterial communities were dominated by *Acidobacteria*, mainly from subgroup 1 (Fig. 2), which is consistent with the low pH of our soils (Table 1). Local small-scale variation in soil pH exerted a strong influence on the structure of soil bacterial communities in a Finnish Arctic tundra site even though there were few differences among the vegetation types studied (Männistö et al., 2007). A recent pan-Arctic study showed that soil bacterial community composition in dry heath vegetation was strongly related to soil pH and soil C/N ratio but not to other soil characteristics (Chu et al., 2010). Note that both of these studies illustrate an over-riding effect of soil pH on bacterial communities that is independent of vegetation type. This effect was evident in the present study even though soil pH varied only modestly (4.3–5.2). A unique cluster of bacterial community profiles was associated with the wet sedge soils, which had the highest pH (Fig. 1, Fig. 4A). Our results are consistent with the hypothesis that soil pH represents a crucial environmental controller for the development of vegetation-soil microbial community interactions in tundra (Eskelinen et al., 2009). Besides soil pH, soil moisture, soil C/N ratio and N availability may also significantly influence bacterial communities. Bacterial communities in dry heath vegetation types clustered separately from the other three vegetation types (Fig. 4A), which may be partly due to the lower moisture in dry heath soils than in other soils (Table 1). Furthermore, bacterial communities in the high C/N soils (dry heath and birch hummock vegetation types) were different from the low C/N soils (tall birch and wet sedge vegetation types). Finally, we observed significant correlations ($p < 0.05$) between bacterial community structure and soil N availability (DON, mineral N, and N mineralization potential (Fig. 4A). Since soil DON, mineral N and N mineralization differ considerably among vegetation types (Chu and Grogan, 2010), these results together suggest that differences in soil N availability associated with the vegetation types may be one of main drivers of bacterial community structure in low Arctic tundra landscapes.

Recent increases in shrub density and expansion in Arctic tundra have been associated with climate warming (Devi et al., 2008; Sturm et al., 2001; Tape et al., 2006). Shrub tundra soils contain a large pool of recalcitrant organic matter and a small labile N-rich pool that drives rapid net N mineralization, whereas tussock tundra soils have a very large pool of bioavailable C that promotes net immobilization of N (Weintraub and Schimel, 2005). In the present study, both the birch hummock and tall birch vegetation types contained the same shrub species (*B. glandulosa*), albeit at differing levels of abundance and stature,

and had similar vegetation composition, topography, soil pH, and moisture content. However, soil mineral N and DON pool sizes, and N mineralization rates are generally significantly larger in tall birch soils than in birch hummock soils (Table 1) (Buckeridge et al., 2010; Chu and Grogan, 2010). The strong differences in relative abundances of the dominant bacteria between these two vegetation types observed here (Fig. 4) correlates with these differences in soil N availability. Further research is now required to evaluate whether the biogeochemical differences are the cause or the effect of these microbial community differences, and therefore to improve our mechanistic understanding of the inter-relationships between shrub growth, nutrient availability, and microbial community structure.

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