RESEARCH ARTICLE

Long-term warming alters the composition of Arctic soil microbial communities

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Abstract

Despite the importance of Arctic soils in the global carbon cycle, we know very little of the impacts of warming on the soil microbial communities that drive carbon and nutrient cycling in these ecosystems. Over a 2-year period, we monitored the structure of soil fungal and bacterial communities in organic and mineral soil horizons in plots warmed by greenhouses for 18 years and in control plots. We found that microbial communities were stable over time but strongly structured by warming. Warming led to significant reductions in the evenness of bacterial communities, while the evenness of fungal communities increased significantly. These patterns were strongest in the organic horizon, where temperature change was greatest and were associated with a significant increase in the dominance of the *Actinobacteria* and significant reductions in the *Gemmatimonadaceae* and the *Proteobacteria*. Greater evenness of the fungal community with warming was associated with significant increases in the ectomycorrhizal fungi, *Russula* spp., *Cortinarius* spp., and members of the Helotiales suggesting that increased growth of the shrub *Betula nana* was an important mechanism *driving this change. The shifts in soil microbial community structure appear sufficient* to account for warming-induced changes in nutrient cycling in Arctic tundra as climate warms.

Introduction

The western North American Arctic is currently experiencing the fastest rate of warming on earth. Here, the rate of temperature increase has reached 0.1 °C per year over the last 35 years (Anisimov *et al.*, 2007), highlighting the relevance of Polar Regions as sensitive indicators of global climate trends. Of particular concern are the globally significant C-stores that have accumulated in tundra soils in response to cold and short growing seasons and the presence of permafrost, which restricts drainage. Current Arctic warming is associated with increased microbial activity leading to higher plant N availability (Chapin, 1983; Nadelhoffer *et al.*, 1992; Aerts, 2006) and faster C turnover in soils (Hobbie & Chapin, 1998; Shaver *et al.*, 2006; Rinnan *et al.*, 2007b; Biasi *et al.*, 2008). These findings have led to a growing concern that warming threatens the stability of Arctic C-stores, which upon release are likely to result in significant, additional, positive climate forcing (Cox *et al.*, 2000; Schuur *et al.*, 2009).

Soil bacteria and fungi are central to the C balance of tundra ecosystems because of their dual role as decomposers of soil organic matter and as determinants of plant community diversity (Van der Heijden *et al.*, 1998, 2008), which in turn controls the quality and quantity of C inputs to soils (De Deyn *et al.*, 2008). Currently, Arctic soil microorganisms experience temperatures substantially below their apparent physiological optima. While significant bacterial growth has been observed in frozen soils (McMahon *et al.*, 2009) and in soils as cold as −6 °C (Rinnan *et al.*, 2011), the optimal temperature for bacterial growth in a sub-Arctic heath soil was 25 °C (Rinnan

et al., 2011), and measured Q_{10} values for respiration in Arctic tundra soils are similar (Mano *et al.*, 2003) or equivalent (Anderson, 2010) to the median global Q_{10} of 2.4 (Raich & Schlesinger, 1992). These findings suggest that warmer Arctic soils should lead to substantially greater microbial activity and growth and to greater CO_2 losses to the atmosphere. Evidence to suggest that this happens, is however, somewhat lacking. For example, experimental warming by open-top greenhouses for 7 or 17 years led to 28% and 73% reductions in bacterial growth in a sub-Arctic heath, which was interpreted to reflect decreased availabilities of labile substrates with warming (Rinnan *et al.*, 2011). Likewise, the very few studies that have measured the response of soil fungi to warming have found small or no changes in the concentrations of their lipid or sterol biomarkers (Rinnan *et al.*, 2007a, 2008, 2009).

Warming-induced changes in the composition of soil microbial communities have the potential to cause sustained changes in microbial activity (Schimel & Gulledge, 1998; Bardgett *et al.*, 2008), yet despite their importance to the stability of Arctic C-stores we lack a sound understanding of the response of soil microbial communities to warming. Progress is hampered by: (1) the many interacting biotic and abiotic factors that structure microbial communities, which may in themselves be altered by warming; (2) the response time of belowground communities to experimental warming is often slow (Rinnan *et al.*, 2007a; Biasi *et al.*, 2008; Lamb *et al.*, 2011); and (3) the vast diversity in physiology and function of soil microorganisms means that their response to warming is unlikely to be monotonic. This last point is well illustrated in a series of studies that sought to examine the response of N-cycling soil bacteria to warming treatment on Ellesmere Island, Canada. Warming treatment had strong impacts on the structure of nitrogen-fixing bacterial communities as characterized by analysis of *nif*H genes (Deslippe *et al.*, 2005), but similar analysis of *nos*Z communities found site factors to be more important than warming in structuring denitrifier communities (Walker *et al.*, 2008) and no significant effect of warming was found on ammonia oxidizing bacterial *amo*A communities (Lamb *et al.*, 2011). The diversity of responses of soil microorganisms to warming treatment suggests that it may be necessary to employ methods that allow for high taxonomic resolution of microorganisms to gain a comprehensive understanding of the response of the community as a whole.

In this study, we tested the hypothesis that long-term warming alters the composition and structure of soil bacterial and fungal communities in Low-Arctic tundra. We utilized warming treatments maintained as part of the Arctic Long-Term Ecological Research (LTER) experiment at Toolik Lake Alaska, which had been in place for 18 years, allowing us insight to longer-term responses. Despite good evidence for winter-time bacterial activity and growth in frozen Arctic tundra soils (McMahon *et al.*, 2009, 2011), studies that have included a temporal component to their sampling report fairly stable bacterial community assemblages across seasons (Männistö *et al.*, 2007; Wallenstein *et al.*, 2007; McMahon *et al.*, 2011). Thus, we predicted that microbial community change resulting from warming would be greater than that because of normal seasonal succession during the thawed-soil seasons (spring–autumn). Given that no field experiment could completely partition all interacting factors that influence soil microbial community structure, one advantage of the LTER experiment is that it allows for the combined effects of warming on biota to be evaluated, including the nonlinear responses that may have been difficult to predict. In moist acidic tundra (MAT), the LTER warming treatments lead to the increasing dominance of the deciduous ectomycorrhizal (ECM) shrub *Betula nana* (Chapin *et al.*, 1995). This change is associated with increased ECM fungal biomass (Clemmensen *et al.*, 2006) and strong changes in the composition of ECM fungal communities (Deslippe *et al.*, 2011). The strong responses of plants and their symbionts to warming are likely to influence the physical and chemical environments experienced by soil microorganisms. These factors, which are indirect effects of warming, have in combination the potential to be important proximal drivers of soil microbial communities. Our study design allowed us to test the combined effects of warming treatment on microbial community composition and structure over time. We used a molecular approach to achieve the high taxonomic resolution necessary to detect changes in microbial community composition and structure with warming treatment.

Materials and methods

Study site and treatments

The study site was located on a gentle (< 5°), north-facing slope in MAT near Toolik Lake, Alaska, USA (68° 38′N, 149°34′W, elevation 780 m). For a description of the landform and plant community composition at the study site see Deslippe *et al.* (2011). Characteristics of the organic and mineral soil horizons are shown in Table 1.

The study treatments were established in 1988 and are maintained as part of the Arctic Long Term Ecological Research (LTER) experiment. Summer warming is accomplished passively with greenhouses constructed of 0.15 mm polyethylene that are fixed during the summer months onto permanent wooden frames, which are

Table 1. Soil characteristics of moist acidic tundra at Toolik Lake, Alaska

Soil properties	Organic	Mineral	Source
Depth (cm)	7–15	15–40	This study
Texture			
Gravel (%)		16	Marion *et al.* (1997)
Sand (%)		46	
Silt (%)		26	
Clay (%)		28	
Bulk density (g cm^{-3})	0.12	0.60	Marion *et al.* (1997)
pH	4.4	4.5–4.7	Nadelhoffer *et al.* (1991); Marion *et al.* (1997)
Organic matter (%)	72	6.5	Marion *et al.* (1997)
Soil C (g m^{-2})	5655	ND	Schmidt *et al.* (2002)
Soil N (g m^{-2})	182	ND	Schmidt *et al.* (2002)
Soil P (g m^{-2})	19.5	ND	Schmidt *et al.* (2002)
CEC (cmol kg^{-1})	29.0	6.9–7.9	Nadelhoffer *et al.* (1991); Marion *et al.* (1997)
Exchangable Ca (cmol kg^{-1})	11.1	1.7	Marion *et al.* (1997)
Exchangeable Mg (cmol kg^{-1})	4.8	0.68	Marion *et al.* (1997)
Exchangeable K (cmol kg^{-1})	1.7	0.14	Marion *et al.* (1997)

2.5 × 5 m and 1.5 m in height. The greenhouses are replicated four times in a randomized block design. The uneven microtopography of the study site allows for air circulation from the base of the greenhouse walls. Despite a significant increase in the annual maximum depth of thaw, similar greenhouses have had no effect on soil water content at the study site, but led to significant increases in soil extractable ammonium in the organic horizon (Chapin *et al.*, 1995).

Mean soil temperature increased because of warming treatments over the 2-year study period (2006 and 2007) by values of 1.33 °C at 10 cm depth; 1.21 °C at 20 cm depth; and 0.96 °C at 40 cm depth. Mean daily ambient air temperature and soil temperatures at 10 cm depth in warming and control plots, during the sampling periods, are shown in Supporting Information, Fig. S1 (Shaver & Laundre, 2007). Warming treatment leads to strong shifts in the plant community composition at this and other MAT sites, whereby vascular plant biomass increases at the expense of mosses and lichens (Chapin *et al.*, 1995). In time, the deciduous shrub *B. nana* dominates plots treated with warming. *Betula nana* increasingly allocates resources aboveground with warming, resulting in a 55% reduction in its belowground relative to above-ground biomass (see Deslippe *et al.*, 2011). This investment in leaf and stem biomass tends to shade-out or smother (when leaves abscise) cryptograms in warming plots. Figure S2 shows the dramatic dominance of *B. nana* in warming treatment plots at the time of sampling in 2007.

Soil sampling and nucleic acid extraction

Soil core samples were collected from three random locations in each of the four replicate warming and control plots at each sampling date. Soil cores were 2 cm in diameter and extended the entire depth of thaw. Soils were sampled on July 9 and August 17 in 2006, and on June 20, July 13, and August 14 in 2007. The months of June, July, and August correspond closely to the seasons of spring, summer, and autumn, in terms of snow-melt dynamics and plant phenological stages. Unfortunately, it was not possible to sample frozen soils. Each soil core was divided into organic and mineral horizons, packaged separately in clean, airtight, plastic bags, and placed immediately on ice. All soil cores were frozen at −80 °C within 2 h of sampling and remained frozen during transport.

Under sterile laboratory conditions, the outer surface of each core was removed with a sharp knife. We then selected the central portion of each horizon, where soil of uniform color and texture could be obtained. One gram of frozen soil was weighed into a beaker containing 200 μL of CTAB buffer and mixed by hand until a thick slurry formed. For organic horizons, a 0.5-g portion of this slurry was subjected to nucleic acid extraction using the protocol described by Griffiths *et al.* (2000). Mineral horizons had high clay content (Table 1), requiring modification of the protocol to reliably yield high quality nucleic acids, whereby 0.1% pyrophosphate and 200 μg poly A were added to the CTAB buffer-soil slurry and incubated at room temperature for 5 min prior to application of the Griffiths protocol. A 5-μL aliquot of each soil extract was analyzed on a 1% agarose gel, stained with ethidium bromide and visualized under UV light to check extraction efficiency. The three nucleic acid extracts per sampling plot were then pooled, resulting in 80 DNA extracts (5 dates × 2 treatments × 4 plot replicates × 2 soil horizons) for downstream analysis.

PCR and automated ribosomal intergenic spacer analysis (ARISA)

Details of PCR primers and conditions are given in the Appendix S1. PCRs for ARISA analysis of bacteria and fungi yielded products that ranged in size from 300 to 1000 bp.

ARISA (Fisher & Triplett, 1999) of bacteria and fungal communities was accomplished using an Applied Biosystems 3730S, 48-capillary sequencer. An injection voltage of 1.6 kV was applied for 15 s. One microlitre of each sample plus 0.15 μL of a GeneScanTM 1200 LIZ® size

standard (Applied Biosystems Carlsbad, CA) were loaded onto each capillary. Resulting ARISA profiles were analyzed using GeneMarker® v1.70 software (Software Genetics, State College, PA). ARISA fragment lengths were determined using the internal size standard and the local Southern algorithm. To avoid binning errors and subsequent data loss that result from applying binning procedures to highly diverse ARISA community profiles, we compared the topographies of each profile's trace data (Hartmann *et al.*, 2009). Trace data were sized in GeneMarker and exported in bins of 0.5 relative migration units. A threshold of 300 relative fluorescence units was applied. Dividing each peak by the sum signal of each profile and multiplying by the mean sum of signals from all profiles normalized profiles. Threshold and normalization steps were iterated until the data set was stable (Dunbar *et al.*, 2001). Normalized fungal and bacterial ARISA profile data were used for subsequent statistical analyses.

Cloning and sequencing

In bacteria, we targeted the 3′ end of the 16S ribosomal RNA gene adjacent to the ribosomal intergenic spacer (RIS) for sequencing, while we targeted the internal transcribed spacer (ITS) for fungal sequencing. PCR conditions and chemistries for the cloning and sequencing of bacterial and fungal amplicons are given in the Data S1. PCR products were purified using MinElute purification columns (Qiagen) and quantified using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Amplicons from all 80 samples were pooled by treatment, combining 100 ng of DNA from each PCR. Thus, eight cloning reactions were performed: bacteria/fungi * warming/control * organic/mineral horizon. Pooled templates were cloned using a TOPO TA Cloning® kit with pCR®II-TOPO® vector suited for blue/white screening and DH5α-T1TM competent cells (Invitrogen, Burlington, Canada). Clones were plated, picked, and bidirectionally sequenced at the Genome Sciences Centre (Vancouver, Canada) using the vector specific primers M13for and M13rev.

Phylogenetic analyses

Bidirectional sequence reads were assembled using the ContigExpress function of Vector NTI 10.3 (Invitrogen), manually checked for base-calling errors, and trimmed of the vector sequence. The Bellerophon server (Huber *et al.*, 2004) was used to detect chimeric sequences in the bacterial 16S-ITS rRNA clone libraries, while a BLAST-based, open-source software package was used to detect chimeric sequences in the fungal ITS clone libraries (Nilsson *et al.*, 2010). Chimeric sequences were discarded. Single alignments for all bacterial and fungal sequences were computed using MAFFT v6 (Katoh, 2008). Alignments were visually checked for accuracy and manually edited in BIOEDIT version 7 (Hall, 1999). Bacterial sequences were trimmed manually to retain only the 16S rRNA gene fragment. Phylogenetic affiliations of all fungal ITS and 16S rRNA gene bacterial sequences were determined using a naïve Bayesian classifier (Wang *et al.*, 2007) implemented in Mothur (Schloss *et al.*, 2009). Bacterial 16S rRNA gene sequences were queried against the SILVA database release 102 (Pruesse *et al.*, 2007), while fungal ITS sequences were queried against the Emerencia-curated BLAST database (Nilsson *et al.*, 2009). Sequences were hierarchically classified to the highest taxonomic resolution that received at least 80% bootstrap support in 100 iterations. The 871 fungal and 1421 bacterial ITS and rRNA gene sequences generated in this study were deposited in GenBank under the accession numbers HQ211490–HQ213783.

Pielou's evenness and statistical analyses

Pielou's evenness (J') was calculated for each ARISA profile. J' is derived from the Shannon diversity index (H') and measures the evenness of the community relative to the maximum and minimum evenness possible for a given richness (Jost, 2010). J' was a measure of the evenness of the distribution of ARISA phylotypes relative to the total number of ARISA phylotypes per sample. Two-way factorial General Linear Models (GLM) were used to assess the effects of warming treatment and sampling date on ARISA profile evenness (J'). Barlett's and Levene's tests were used to test for heteroscedascity among groups. Where data violated the assumptions of the GLM, an equivalent nonparametric test was used. For treatment, which had only two categories, a Mann–Whitney U-test was applied. For sampling date, with five categories, a Kruskal–Wallis ANOVA was applied. Fisher's LSD test was applied to the GLM *post hoc* to test for significant differences among treatment across all sampling dates. All means are reported ± one standard error.

Nonmetric multidimensional scaling (NMS) was used to visualize samples in phylotype space. Ordinations were run using PC-ORD version 5.0 in the 'auto-pilot' mode, which performed 500 iterations (250 runs each of real and randomized data) with random starting configurations, and assessed dimensionality by minimizing stress. Sørensen distance (Bray & Curtis) was selected as the distance measure for each initial matrix (McCune & Mefford, 1999). NMS ordination does not rely on a normal distribution of the species data or the assumption of linear relationships among variables (McCune & Grace, 2002). We tested the effect of warming treatment and sampling date on pairwise differences in community

structure by calculating chance-corrected within-group agreement (A) using nonparametric multiple response permutation procedure (MRPP) with Sørensen (Bray & Curtis) distances in PC-ORD. The chance-corrected within-group agreement (A) is a measure of within-group homogeneity compared with that expected by chance, where $A = 1$ corresponds to identical members within each given group (maximum effect of factor), and where $A \leq 0$ corresponds to within-group heterogeneity equal to or larger than that expected by chance (no effect of factor). In community ecology, values for A are often below 0.1 (McCune & Grace, 2002).

Mean ARISA profiles by treatment and soil horizon were calculated in Excel® and plotted with SIGMAPLOT 11.0 (Systat Software, Inc. San Jose, CA). Significant differences in the abundance of each RIS-phylotype between control and warming treatments were tested using Student's t-tests that accounted for heteroscedasticity in Excel®. Additionally, we used the IndVal method of indicator species analysis (Dufrene & Legendre, 1997) to identify RIS-phylotypes associated with warming and control treatments. The IndVal score combines the relative abundance of a phylotype in each treatment (specificity) with its relative frequency of occurrence (fidelity) in that treatment and varies between zero and 100%, with 100% representing perfect indication of a RIS size for the treatment. RIS-phylotypes that differed significantly between treatments using both methods of analysis, at $\alpha = 0.05$ for fungi and $\alpha = 0.01$ for bacteria, were plotted beneath their respective ARISA profiles using SIGMAPLOT 11.0.

We wished to link phylogenetic information from the clone libraries to the replicated ARISA data sets so that quantitative changes in the structure of microbial communities with warming could be evaluated. Thus, a simulated ARISA profile based on amplicon length of all bacterial and fungal clones was plotted in SIGMAPLOT 11.0. Where RIS-phylotypes that changed significantly with warming treatment (as assessed by t-tests and the IndVal method) could be affiliated with phylogenetic groups, significant differences in the frequency of clones in libraries between the control and warming treatment soils were also compared using chi-squared tests with Yates correction. Clone library size was normalized to that of the control. Chi-squared tests were performed using a purpose-made Excel® spreadsheet obtained from http://udel.edu/~mcdonald/statchigof.html.

Results

ARISA profile evenness

Two-way analyses of variance (ANOVA) to assess the response of RIS profile evenness (J') to warming treatments and sampling dates revealed different responses of bacteria and fungi to warming that were consistent among the organic and mineral soil horizons (Fig. 1). For bacteria in the organic horizon, profile evenness (J') significantly decreased with warming treatment ($F = 7.02$, $P = 0.01$) but did not differ among sampling dates ($F = 0.19$, $P = 0.94$). In the mineral horizon, bacterial RIS profile J' also tended to decrease with warming, although this change was only statistically significant at $\alpha = 0.1$ ($F = 3.78$, $P = 0.059$), and J' did not differ among sampling dates ($F = 1.02$, $P = 0.41$). Conversely, RIS profile J' of fungi in both horizons significantly increased with warming treatment (organic horizon Mann–Whitney U-test, $z = 2.93$, $P < 0.01$; mineral horizon Mann–Whitney U-test = 119; $z = 2.18$, $P = 0.03$) and also did not differ among sampling dates (organic horizon $F = 0.75$, $P = 0.57$; mineral horizon Kruskal–Wallis ANOVA $H = 3.39$, $P = 0.49$). Table S1 summarizes a variety of diversity indices for the ARISA data.

NMS ordinations of ARISA profiles

NMS ordinations of ARISA profiles for bacteria and fungi revealed that the structures of both communities were strongly changed by warming treatments. Two- or three-dimensional NMS solutions described high proportions (0.70–0.90) of the total variance in microbial community structure observed in this study (Fig. 2). MRPP tests confirmed that the effect of warming on microbial community structure was highly significant and revealed that in both soil horizons, its effect was greater on bacterial communities than on fungal ones, as illustrated by the greater chance-corrected within-group agreement values (A) for bacteria (Fig. 2). By contrast, the NMS ordinations indicated that strong seasonal succession did not occur in these microbial communities (Fig. 2). Accordingly, MRPP failed to show that sampling date was a significant factor in structuring bacterial and fungal communities in organic and mineral horizons. These results suggest that the composition of MAT microbial communities was stable over the three thawed-soil seasons.

Composition of clone libraries

In this study, 93% (1328/1421) of bacterial 16S rRNA gene clones could be identified minimally at the phylum level. Clone libraries of bacterial 16S rRNA genes from the organic and mineral horizons in control and warming treatments were dominated by sequences affiliated with the *Actinobacteria* (Fig. 3i), with this group representing nearly two-thirds of each of the four bacterial clone libraries. Also abundant were members of the *Gemmatimonadetes* and *Proteobacteria*, which comprised about

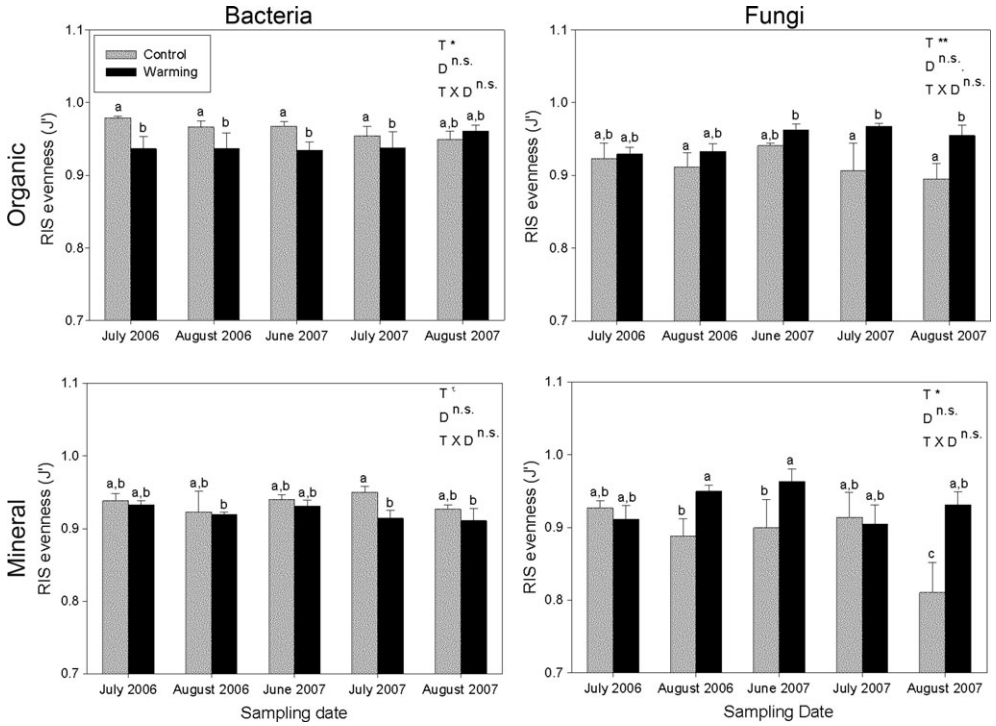

Fig. 1. Mean RIS profile evenness (*J'*) of bacteria and fungi in organic and mineral soil horizons in warmed or control treatment plots at the five sampling dates. Whiskers represent the standard error of the mean. Different letters above bars indicate significant differences among means as determined by a *post hoc* Fisher's LSD test. Results of two-way ANOVAS or the equivalent nonparametric test are shown in the top right-hand corner of each bar-chart; T, treatment (control, warmed); D, sampling date. Levels of significance are denoted by superscripts; τ, $P < 0.1$; *$P < 0.05$; **$P < 0.01$; n.s., nonsignificant.

20% and 10%, respectively, of each library. The *Chloroflexi* and the *Acidobacteria* were present in low numbers, both comprising less than 3% of clones overall. Several phyla, including the *Firmicutes, Bacteroidetes, Spirochaetes,* and Candidate Division TG-1 and OP10, were rare in all libraries, each comprising < 1% of clones overall.

Of the fungal ITS clones generated in this study, 96% (836/873) could be identified minimally at the phylum level. Fungal clone libraries were dominated by members of the *Ascomycota* and *Basidiomycota*, with 59% and 28% of clones, respectively. Fungal clones affiliated with the basal fungal lineages comprised 8% of the libraries (Fig. 3j). Among the ascomycetous clones, the *Saccharomyceta* were vastly dominant, comprising 96% all clones; the remaining 4% were members of the mitosporic *Ascomycota*. For clones that could be identified to order, the *Helotiales* were dominant (88%), while members of the *Capnodiales* (6%) and *Chaetosphaeriales* (4%) were less common. Members of the orders *Chaetotyriales, Eurotiales, Hypocreales, Onygenales, Pezizales, Pleosporales,* and *Rhytismatales* occurred only rarely in the four clone libraries, and each constituted less than 2% of clones overall. Among the basidiomycetous clones, the Agaricales (68%), *Sebacinales* (13%), *Russulales* (10%), and *Thelephorales* (7%) made up the majority of each library. Other orders were rare; with members of the *Cantharellales, Corticiales, Cystofilobasidiales, Exobasidiales, Filobasidiales*, and *Tremellales* combined, representing < 2% of clones overall.

Linking clone library data to ARISA profiles

Indicator analyses and *t*-tests comparing individual RIS-phylotypes between the control and warming treatments also suggest that bacteria in the organic horizon showed the greatest response to warming. Forty-three RIS-phylotypes declined significantly with warming treatment, while less than half this number (20) increased with warming. Many of the phylotypes that declined were affiliated with *Actinobacteria*, including those in the sub-orders *Micrococcineae* and *Acidomicrobineae* and sub-class *Rubrobacteridae*, and with the families *Coriobacteriaceae*, and *Intersporangiaceae* (Fig. 3a, Table S2). The significant decline in *Micrococcineae* with warming was consistent with clone library results, where we observed a strong decline in the proportion of *Micrococcineae* in the organic horizon ($\chi^2 = 10.41$; $P = 0.0013$). Dominant RIS-phylotypes affiliated with the family *Gemmatimonadaceae* also

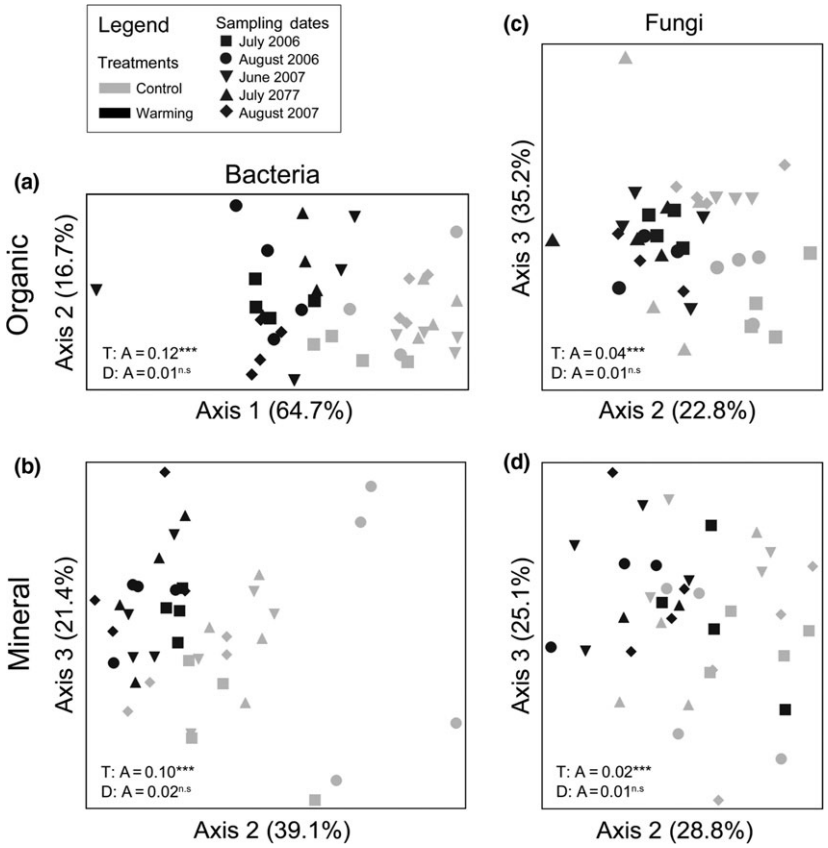

Fig. 2. NMS ordinations of soil samples in bacterial 16S rRNA gene and fungal ITS space; (a) bacteria organic horizon; (b) bacteria mineral horizon; (c) fungi organic horizon; (d) fungi mineral horizon. The chance-corrected within-group agreement value (*A*) of the MRPP results for factors warming treatment (T) and sampling dates (D) are shown in the bottom left-hand corner of each panel; ***$P < 0.00001$, n.s., nonsignificant.

declined significantly with warming, as did RIS-phylotypes associated with unidentified bacteria. RIS-phylotypes affiliated with different *Actinobacteria*, including those in the order *Actinomycetales* and sub-order *Acidomicrobineae*, increased significantly with warming (Fig. 3a and b, Table S2). The significant increase in *Acidomicrobineae*, which were the dominant sub-order of *Actinobacteria* in both treatments, was also a feature of the clone library analysis ($\chi^2 = 6.13$; $P = 0.013$). The significant increase in this dominant group is consistent with the reduction in bacterial evenness in the organic horizon with warming, which was revealed by the ANOVA for ARISA profile evenness.

In the mineral horizon, similar numbers of bacterial RIS-phylotypes significantly increased (16) or decreased (19) with warming treatment. Many of the RIS-phylotypes adversely affected by warming were affiliated with the same groups of *Actinobacteria* as those in the organic soil horizons (Fig. 3c and d, Table S2). Also similar to the response of bacteria in the organic horizon was the significant decline of RIS-phylotypes affiliated with the family *Gemmatimonadaceae*, although this group was less dominant in the mineral horizon. A small group of RIS-phylotypes affiliated with the *Rhodospirillales* declined significantly with warming and were the only group of *Proteobacteria* to respond to the treatment. The significant reduction in the *Proteobacteria* with warming treatment was also supported by the clone library analysis ($\chi^2 = 6.08$; $P = 0.014$). The RIS-phylotype affiliated with the *Acidomicrobineae* was remarkable in its dominance in the mineral horizon, and it increased significantly with warming treatment, again agreeing with the results of the clone library analysis. Other RIS-phylotypes that increased with warming included those affiliated with the *Chloroflexi*, *Actinomycetales*, and unidentified bacteria (Fig. 3c and d, Table S2).

In stark contrast to the pattern for bacterial RIS-phylotypes, indicator analyses and *t*-tests revealed that twice as many fungal phylotypes increased with warming (17) treatment as those that declined (8) in the organic horizon.

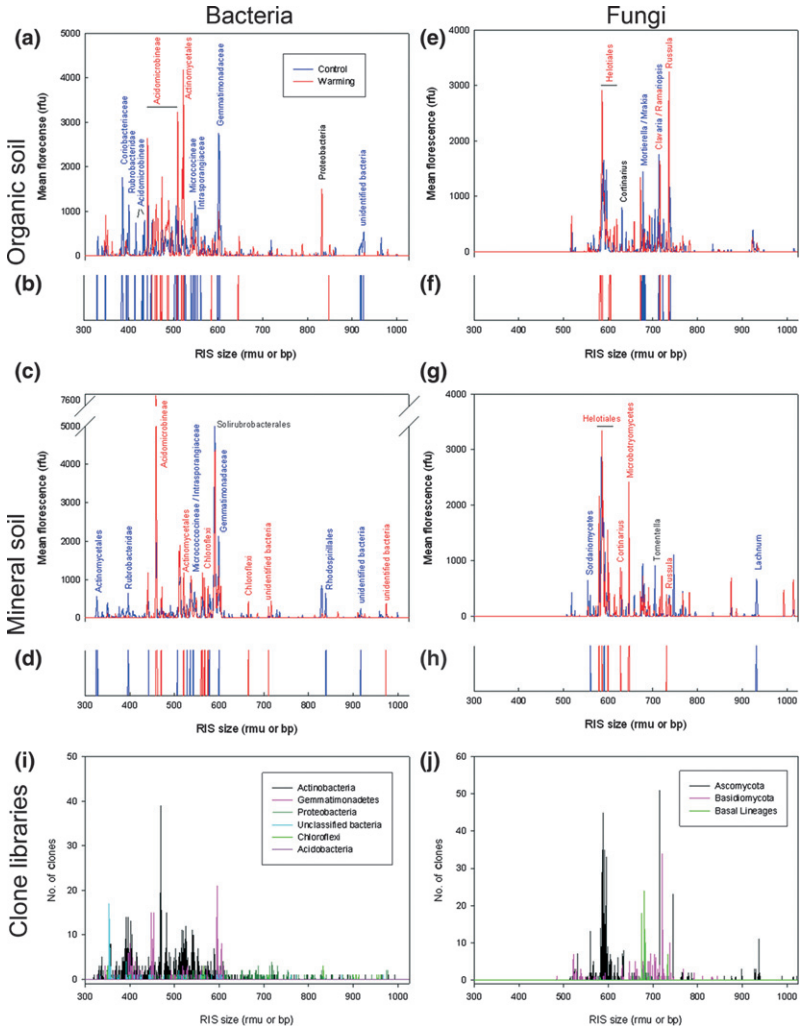

Fig. 3. ARISA profiles for soil samples. Mean ARISA profiles by treatment (a, c, e, g). RIS sizes that were significantly different among treatments ($\alpha = 0.01$ for bacteria; $\alpha = 0.05$ for fungi) (b, d, f, h). Those RIS-phylotypes depicted in red were significantly greater in the warming treatment; those RIS-phylotypes depicted in blue were significantly greater in the control, as determined by paired *t*-tests and the IndVal method of indicator species analysis (Dufrene & Legendre, 1997). Phylogenetic affiliations of RIS-phylotypes are shown in blue if they declined significantly with warming treatment and in red if they increased significantly. Phylogenetic affiliations of RIS-phylotypes that were dominant in the profiles were unaffected by warming treatment are shown in black. Simulated ARISA profiles for clone libraries used for taxonomic identification of RIS-phylotypes above (i, j).

A group of RIS-phylotypes affiliated with *Russula* spp. as well as those affiliated with the *Helotiales* dominated the ARISA profiles and increased significantly with warming treatment in the organic horizon (Fig. 3e and f, Table S2). These patterns were also reflected in the clone library composition. Members of the *Helotiales* comprised 88% of the ascomycetous clones in the libraries for the organic horizon and their proportional abundance increased significantly with warming ($\chi^2 = 16.41$, $P < 0.001$). Likewise, members of the *Russulales* increased significantly with warming treatment ($\chi^2 = 8.65$, $P = 0.0033$), while clones belonging to the genus *Russula* increased by a factor of three in clone libraries for the warmed organic horizon. Significant both in their increase and decrease were RIS-phylotypes affiliated with the club fungi *Clavaria* and *Ramariopsis*. In the clone library for the warmed organic horizon, these genera were surprisingly rare, though in the clone library for the control organic horizon, *Clavaria* and *Ramariopsis* were the dominant members of the Agaricales and comprised 65% of the basidiomycetous clones. Other fungal RIS-phylotypes to decline with warming treatments were affiliated with the yeast-like psychrophillic soil saprobes *Mortierella* and *Mrakia*.

As with the organic horizon, fungal RIS-phylotypes that increased significantly (12) with warming treatment in mineral soils outnumbered by twofold those that declined significantly (6). In the mineral horizon, RIS-phylotypes affiliated with the *Helotiales* and the *Microbotryomycetes* were dominant. The *Microbotryomycetes*-affiliated and most of the *Helotiales*-affiliated RIS-phylotypes increased significantly with warming, although a few RIS-phylotypes affiliated with the *Helotiales* declined significantly, including one affiliated with *Lachnum* spp. and those affiliated with the *Sordariomycetes* (Fig. 3g and h, Table S2). As in the organic horizon, RIS-phylotypes affiliated with *Russula* spp. increased significantly with warming treatment as did the RIS-phylotypes affiliated with *Cortinarius* spp.

Discussion

ARISA profiles

Analysis of ARISA profiles suggests that, while the evenness of soil bacterial and fungal communities were both seasonally stable, warming significantly reduced bacterial community evenness and increased fungal community evenness. More even microbial communities have relatively high functional stability and resistance to stress or disturbance (Wittebolle *et al.*, 2009; Wrighton *et al.*, 2010; Werner *et al.*, 2011). For example, in experimental microcosms of denitrifiers, microbial community evenness was a key factor in preserving net ecosystem denitrification under increasing salinity stress (Wittebolle *et al.*, 2009). Our finding that bacterial evenness declined with warming treatment may thus indicate a reduced capacity for Arctic soil bacteria to maintain bacterial ecosystem functions with warming. Conversely, the significant increase in fungal evenness with warming suggests that, in the future, warmer Arctic tundra fungal communities will have enhanced capacities to perform their functions.

NMS ordinations of ARISA profiles for bacteria and fungi, which take into account both richness (number of RIS-phylotypes) and evenness (relative abundances of RIS-phylotypes), confirmed that both communities were strongly changed by warming treatments, while neither was significantly affected by the date of sampling. These findings agree with results from other Arctic tundra climate change experiments showing a strong response of soil microbial communities to warming treatments (Deslippe *et al.*, 2005; Rinnan *et al.*, 2007a) but suggest that MAT microbial communities were stable over the three thawed-soil seasons, a finding that agrees with other Arctic tundra studies (Männistö *et al.*, 2007; Wallenstein *et al.*, 2007; McMahon *et al.*, 2011), but contrasts with alpine tundra microbial communities that undergo strong seasonal succession from spring to autumn (Nemergut *et al.*, 2005).

The effect of warming in structuring bacterial and fungal communities was greater in the organic than in the mineral horizon, as evidenced by the greater F scores for organic than mineral horizon ANOVAs for J', and by the greater chance-corrected within-group agreement values (A) for MRPP analyses of the NMS ordinations of RIS-phylotypes. These results suggest that the effect of warming treatment in structuring soil microbial communities declined with soil depth. This finding fits with the soil temperature data collected at the study site, which shows a declining effect of the warming treatment with increasing soil depth. Thus, our data suggest that the response of Arctic soil microbial communities to warming may be proportional to the degree of temperature change experienced. A declining effect of warming on Arctic soil microbial communities over depth has also been noted by others (Rinnan *et al.*, 2007a).

Composition of clone libraries

While the composition of our fungal clone libraries corresponded well with a previous report for MAT soils, the composition of our bacterial clone libraries were quite different from that study (Wallenstein *et al.*, 2007). Most striking was the difference in the relative abundances of *Actinobacteria* and *Acidobacteria*. *Actinobacteria* made up 64% of clones in our study, but < 5% in the previous study. Conversely, *Acidobacteria* comprised < 2% of our clones, while the previous study found 30% of clones belonged to this group. Differences in primer biases that occurred in each of these studies likely contributed to the differing views of soil bacterial community structure. When the bacterial primers we used were applied to forest soils, similar community structures were observed (Hartmann *et al.*, 2009). While all primer sets provide different biased views of microbial community structure, we acknowledge that the bacterial community composition reported in the former study of MAT soils (Wallenstein *et al.*, 2007) is more similar to those reported for other Arctic tundra active layer soils (e.g. Wilhelm *et al.*, 2011) than our own. Nonetheless, we suggest that the value of our study lies in the relative effects of warming treatments on different bacterial and fungal groups rather than in the composition of the clone libraries, *per se*. While several studies have used clone libraries to characterize bacterial community composition in Arctic tundra soils (Männistö *et al.*, 2007; Wallenstein *et al.*, 2007; Wilhelm *et al.*, 2011), our study is novel in illustrating the impacts of long-term warming treatment on specific bacterial and fungal groups.

Effects of warming on the bacteria and fungi of an Arctic tundra soil

Bacteria in organic and mineral horizons generally declined with warming treatment, and those that increased in response to warming tended to be dominant in control soils, thus driving the reduction of bacterial evenness with warming treatment. The relatively slow-growing, gram-positive Actinobacteria increased in dominance with warming. These 'K-selected' recalcitrant C-recyclers are important chitin degraders in soils (Goodfellow & Wiliams, 1983), and their increased abundance fits well with the observed increased abundance of fungi with warming treatment. An increased dominance of slow-growing Actinobacteria, and the significant decline in at least one group of the relatively fast-growing Gram-negative *Proteobacteria*, the *Rhodospirillales*, agrees with the reduction in bacterial growth with long-term warming treatment, which was reported for a sub-Arctic heath (Rinnan *et al.*, 2011). Likewise, the increased dominance of these recalcitrant C-recyclers suggests a reduction in the availability of labile substrates with warming, as was suggested by those authors. While laboratory incubations and relatively short-term warming of MAT soils have shown that warming increases C turnover in soils (Hobbie & Chapin, 1998; Shaver *et al.*, 2006) and would suggest that r-selected bacteria may initially become more dominant with warming, our study suggests that over 18-years of warming, the niches occupied by these bacteria disappear as they are outcompeted by K-selected recalcitrant C-degraders such as the *Actinobacteria*.

Simultaneous with the decline in bacteria with warming treatment, was a significant increase in the abundance of fungi in Arctic tundra soils. This increase was almost exclusively driven by the dominant ECM fungi of the deciduous shrub *B. nana* (Deslippe *et al.*, 2011), which increasingly dominates MAT landscapes as climate warms (Sturm *et al.*, 2001). This result confirms previous studies reporting increased ECM fungal biomass with warming (Clemmensen *et al.*, 2006) and extends the observation that warming induces strong changes in the composition of the *B. nana* root tip ECM fungal community (Deslippe *et al.*, 2011) into the entire soil matrix. This finding also supports the idea that the influence of climate change on soil organisms will be largely plant-mediated (Johnson *et al.*, 2011; Chakraborty *et al.*, 2012).

As warming increases *B. nana*'s growth and nutrient demand from its mycorrhizal fungi, *Cortinarius* spp., *Russula* spp. and members of the *Helotiales*, it follows that fungal density and decomposition increase (Deslippe *et al.*, 2011). This activity leads a depletion of higher quality substrates (Rinnan *et al.*, 2011) and to the increased release of organic acids and ammonium (Chapin *et al.*, 1995; Read *et al.*, 2004), which act to acidify soils and likely further stimulates the growth of saprotrophic fungi (Bååth & Anderson, 2003; Rousk *et al.*, 2009), as we observed with the significant increase in the *Microbotryomycetes* in mineral soils. Increased acidity is also likely to select against bacteria that typically have higher pH optima than soil fungi (Paul & Clark, 1996). Indeed, decreasing soil pH has been associated with lower bacterial diversity in soils (Fierer & Jackson, 2006; Rousk *et al.*, 2010) while bacterial growth and role in C-mineralization declines relative to fungi over a declining soil pH gradient (Rousk *et al.*, 2009).

While a reduction in substrate quality caused by ECM mining of organic matter for nutrients was probably an important mechanism that altered the structure of soil bacterial and fungal communities over time, alterations in litter chemistry were also likely to have driven the shifts in microbial community composition and structure that we observed. Warming increases the photosynthetic yield of *B. nana* leading to significantly higher leaf C : N ratios as growth becomes increasingly N-limited (Welker *et al.*, 2005; Natali *et al.*, 2011), suggesting that warming acts directly to reduce litter substrate quality to soil microorganisms. Interestingly, studies of the response of soil microbial communities to elevated CO_2, where increased C/N ratios of plant litter also occur, find that soil food webs shift from bacterial to fungal dominated pathways (Chakraborty *et al.*, 2012 and references therein), suggesting that climate change will act to favor fungi in many ecosystems.

Thus, these data and those of our previous study (Deslippe *et al.*, 2011) suggest that long-term warming of MAT favors the mycorrhizal fungi of *B. nana*, which appear to play an important role in the spread of the shrub on tundra as climate warms (Deslippe & Simard, 2011). With their C supplies secured by their host, these fungi grow into new substrates mining soil organic matter for scarce N (Read *et al.*, 2004). As higher quality substrates are depleted, recalcitrant substrates become more important (Rinnan *et al.*, 2011), and soil pH likely declines. These conditions favor saprotrophic soil fungi, but reduce the niche breath occupied by bacteria, so that K-selected groups, which may even specialize in degrading fungal biomass, become increasingly dominant and the bacterial community declines in overall evenness. Thus, our results suggest that warming-induced changes in microbial community structure are sufficient to account for observed changes in C-cycling and N-cycling in Arctic tundra soils.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Mean daily air temperature at 1 m, and soil temperatures at 10 cm depth for warming and control plots in 2006 and 2007.

Fig. S2. LTER experimental warming treatment plots at Toolik Lake, Alaska, USA, showing (a) the dominance of *Betula nana* within greenhouse relative to surrounding vegetation, which is representative of the control treatment, (b) the dominance of *B. nana* relative to other plant species within the warming treatment plots.

Table S1. Mean diversity indices for RIS profiles generated for bacteria and fungi in organic and mineral horizons of soils treated with warming or in controls.

Table S2. Complete list and affiliations of bacterial and fungal RIS-phylotypes that declined or increased significantly with warming treatment in organic and mineral soils as determined by Student's *t*-tests and the IndVal method of indicator species analysis (Dufrene & Legendre, 1997).

Appendix S1. Materials and methods.

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