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ORIGINAL ARTICLE

# Long-term experimental warming alters nitrogen-cycling communities but site factors remain the primary drivers of community structure in high arctic tundra soils

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**Arctic air temperatures are expected to rise significantly over the next century. Experimental warming of arctic tundra has been shown to increase plant productivity and cause community shifts and may also alter microbial community structure. Hence, the objective of this study was to determine whether experimental warming caused shifts in soil microbial communities by measuring changes in the frequency, relative abundance and/or richness of *nosZ* and *nif*H genotypes. Five sites at a high arctic coastal lowland were subjected to a 13-year warming experiment using open-top chambers (OTCs). Sites differed by dominant plant community, soil parent material and/or moisture regimen. Six soil cores were collected from each of four replicate OTC and ambient plots at each site and subdivided into upper and lower samples. Differences in frequency and relative abundance of terminal restriction fragments were assessed graphically by two-way cluster analysis and tested statistically with permutational multivariate analysis of variance (ANOVA). Genotypic richness was compared using factorial ANOVA. The genotype frequency, relative abundance and genotype richness of both *nosZ* and *nif*H communities differed significantly by site, and by OTC treatment and/or depth at some sites. The site that showed the most pronounced treatment effect was a wet sedge meadow, where community structure and genotype richness of both *nosZ* and *nif*H were significantly affected by warming. Although warming was an important factor affecting these communities at some sites at this high arctic lowland, overall, site factors were the main determinants of community structure.**
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## Introduction

Climate change is expected to disproportionately affect Arctic and Antarctic latitudes (Maxwell, 1992; ACIA, 2004). The predicted effects of a doubling of atmospheric $CO_2$ will be dramatic: within 100 years, annual temperatures in the Arctic may be 3–5 °C higher, with winter temperatures as much as 4–7 °C higher over land than currently (Maxwell, 1992; ACIA, 2004). Most models predict that overall annual global precipitation will increase, and, like temperature, will be greater at the poles. Whether net soil moisture will increase or decrease is not clear; some models suggest an increase due to melting of ice-rich permafrost and earlier snowmelt, while others predict a decrease due to better drainage and increased evaporative losses (Kane *et al.*, 1992; Maxwell, 1992; Shaver *et al.*, 2000; ACIA 2004).

Arctic tundra ecosystems are considered nutrient (especially nitrogen) limited (Henry *et al.*, 1986; Chapin *et al.*, 1995). Even slight increases in temperature and precipitation could lead to deeper active layers, higher rates of chemical transformations, and ultimately, greater nutrient availability

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(Berendse and Jonasson, 1992; Shaver *et al.*, 2000; Rolph, 2003). Changes in soil moisture status will impact microbial processes such as decomposition in dry, moist and wet arctic ecosystems; the resulting interaction between moisture and temperature will dictate organic matter turnover rates, and ammonium ($NH_4^+$) availability via mineralization (Nadelhoffer *et al.*, 1992; Paul and Clark, 1996).

It is unclear how climate warming will affect nitrogen fixation, with some predicting that future arctic environments will experience increased nitrogen fixation due to heightened enzyme activity and increased concentration of carbon dioxide (Chapin and Bledsoe, 1992) and others that nitrogen fixation will be inhibited by increased available nitrogen (N) due to increased mineralization acting as a negative feedback upon this process (Paul and Clark, 1996). Nitrification and denitrification are closely associated in soils, especially at aerobic–anaerobic interfaces where the nitrification product, nitrate ($NO_3^-$), is readily available for reduction (Nicolaisen *et al.*, 2004; Seitzinger *et al.*, 2006), although recent evidence indicates that this relationship may exhibit chaotic behavior due to the dynamics of the mutualistic relationship between ammonia-oxidizing and nitrite-oxidizing bacteria (Graham *et al.*, 2007). If mineralization promotes nitrification, this may in turn stimulate denitrification and the interaction between these processes could increase nitrous oxide ($N_2O$) production at many stages in this complex cycle (Paul and Clark, 1996). Although changes in nitrate availability often exert only short-term effects on the denitrifier community, an increase in this substrate has been shown to decrease denitrifier abundance (Wallenstein *et al.*, 2006). It is known that experimental nutrient amendments increase the rates of $N_2O$ production (Avrahami *et al.*, 2002; Seitzinger *et al.*, 2006), but it still has not been confirmed if denitrifying enzyme activity is correlated with the genetic structure of denitrifier communities (Rich and Myrold, 2004; Boyle *et al.*, 2006; Sharma *et al.*, 2006).

Techniques such as terminal restriction fragment length polymorphism (T-RFLP), which can be used to measure the presence and/or relative abundance of functional genes, are ideal for assessing microbial community structure (Tiedje *et al.*, 1999; Zehr *et al.*, 2003; Deslippe *et al.*, 2005; Wallenstein *et al.*, 2006). Assessments of microbial community structure based upon functional genes may track differences in potential gene function based upon larger scale environmental change, although T-RFLP is most effective where species diversity is low or moderate (Engebretson and Moyer, 2003). However, the link between shifts in microbial community structure (measured by changes in gene frequency, abundance and richness) and corresponding changes in the processes mediated by these communities has not been satisfactorily demonstrated. Deslippe *et al.* (2005) found little relationship between *nif*H genotype diversity and nitrogen-fixation rates. In addition, although excess nitrogen should negatively affect nitrogen fixation (Zehr *et al.*, 2003), the diversity of the nitrogen-fixing community assessed using the functional gene *nif*H has not been shown to diminish in such cases (Piceno and Lovell, 2000). Although changes in the distribution of functional gene markers may not reliably predict changes in physiological function, they remain useful markers for delineating changes in specific functional communities.

The objective of this study was to investigate changes in the frequency, relative abundance and richness of the functional genes nitrous oxide reductase (*nos*Z) and dinitrogenase reductase (*nif*H). *Nos*Z catalyzes the reduction of $N_2O$ to nitrogen gas, and thus targets those organisms that are able to complete the denitrification cycle. Although *nos*Z diversity captures only a small portion of the total denitrifying community, it is consistently detected in soils and increases in density with progressing soil development, suggesting that it has biological relevance (Kandler *et al.*, 2006). *Nif*H, which encodes the dinitrogenase reductase component of nitrogenase, is a commonly used marker for studying nitrogen-fixing communities (Zehr *et al.*, 2003). We examined shifts in the *nos*Z and *nif*H soil microbial communities at sites with different dominant plant communities, on different soil parent material and across different moisture regimens after a 13-year warming experiment in high arctic tundra.

## Methods

*Site description*

The research site at Alexandra Fiord is located on the northern side of Johan Peninsula, on the East coast of Ellesmere Island, Nunavut (78°53′ N, 75°55′ W) (Freedman *et al.*, 1994). The study area is an 8 $km^2$ lowland outwash plain, bordered by ocean to the north and glaciers to the south, and by upland plateau (up to 750 m), on the east and west. Alexandra Fiord is one of the study locations for the International Tundra Experiment (ITEX) and has contributed to ITEX studies over the past two decades (Arft *et al.*, 1999; Rustad *et al.*, 2001; Walker *et al.*, 2006).

Five distinct sites were investigated in this study: three in the lowland and two on the surrounding upland. They differed primarily by dominant plant community, and each corresponded to a particular soil moisture gradient and/or soil parent material. All soils at the three lowland sites are derived from granitic parent material (Muc *et al.*, 1994). The Sedge Meadow (SM) site is hydric with a thick organic layer over mineral soil and a pH range of 6.6 in surface soils to 5.9 in deeper layers. It is dominated by sedge, cushion plant and dwarf shrub species that include *Carex stans*, *C. membranacea*, *Eriophorum angustifolium* spp.

*triste* and *Polygonum viviparum* with hummocks of *Salix arctica* and *Dryas integrifolia.* The Cassiope Heath (CH) site has an average of 3–5 cm of organic soil over coarse mineral soil with a pH range of 4.9–5.4; the site is hydric–mesic. Dwarf shrub and cushion plant species at this location include *Cassiope tetragona* with *Salix arctica*, *D. integrifolia* and *Saxifraga oppositifolia.* The driest (mesic–xeric) lowland site is Riverside Willow (RW). Sandy-silt mineral soils at this site range in pH from 5.2 to 4.6, and support the greatest plant diversity of all sites. *Salix arctica* was the dominant dwarf shrub with prominent graminoids (*Festuca brachyphylla*, *Arctagrostis latifolia* and *Luzula confusa*) and forbs (*Papaver radicatum*, *Draba* spp.). The Upland Granitic (UG) and Upland Dolomitic (UD) sites are distinguished by the origin of their mineral soils, which is reflected in their soil pH. The UG site has an acidic pH range of 4.9–5.5, while the alkaline UD site has an average pH of 7.9. Both sites are xeric, and are dominated by the deciduous dwarf shrub *Salix arctica* and *D. integrifolia* (Muc *et al.*, 1989; Klady, 2006); *C. tetragona* is absent from the UD site.

*Experimental design*

All lowland treatment plots had 1.8 $m^2$ open-top chambers (OTCs) (hexagonal transparent fiberglass chambers with 0.5 m high inclined sides) established in 1992 over randomly chosen areas. OTCs were installed at the upland sites in the following year and all OTCs remained in place throughout the year. Limited, mainly non-destructive, sampling has occurred since their installation (Marion *et al.*, 1997; Rolph, 2003; Klady, 2006). OTCs did not cause a significant difference in soil water content (Rolph, 2003). On the basis of data averaged over the 2001 growing season, OTCs at the lowland sites increased air temperatures by 0.3–1.5 °C and soil temperatures by up to 0.7 °C at −10 cm. These are similar to the long-term averages for the experimental sites (Marion *et al.*, 1997; GHR Henry, unpublished data).

For all lowland sites, adjacent control plots were 25 paces from their associated OTC in a direction perpendicular to the overall site layout to preserve the original random location of each treatment plot. For both upland sites, the same method was used, but the distance was limited to 10 paces due to size constraints of the area.

Random sampling was accomplished with a random numbers table plus a 50 cm × 50 cm quadrat. The quadrat was always placed as close to the center of each OTC as was practical, or was dropped at 25 or 10 paces from the OTC for every control plot. The quadrat was divided into 5 cm × 5 cm squares that easily accommodated the soil corer, and the numbers table was used to choose rows and columns for sampling.

Four treatment pairs (one OTC plus one adjacent control plot) were sampled at each of the five sites. Sampling occurred once at each site over the period of peak plant growth from 18 to 27 July 2004. Six 2 cm diameter soil cores were taken from each OTC and from each of the corresponding control plots. A 45 cm long soil corer was used, and samples were recovered from the top 5 cm (upper) and the bottom 5 cm (lower) layers based upon the depth of the core. This corer adequately sampled all sites, as the active layer never extended beyond its reach. Upper samples were always taken from the top 0–5 cm of the soil core, while the average depths of lower samples were as follows: SM 39–44 cm, CH 38–43 cm, RW 34–39 cm, UG 30–35 cm and UD 8–13 cm. Cores that were not at least 10 cm deep were rejected so that separation could always be maintained between the top and bottom 5 cm. Approximately 1 g of soil was taken from each of the six replicate cores at the two different depths. This yielded 12 × 1 g soil samples from each OTC and each control plot, resulting in 480 × 1 g soil samples that were frozen for transport back to the laboratory for DNA extraction. The 1 g samples remained frozen on site in an underground 'permafrost freezer' at approximately −1 °C in 2 ml microcentrifuge tubes in sealed airtight bags until transport to the laboratory at University of Northern British Columbia (UNBC). Every attempt was made to keep the samples frozen during transport and upon return to the laboratory they were stored at −20 °C until ready for DNA extraction.

*DNA extraction and PCR*

DNA extractions were performed with a MoBio UltraClean Soil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) using the 'Alternative protocol for maximum yields.' Spectrophotometric analysis of extractions revealed final DNA concentrations of 50–150 ng/μl.

Functional genes targeted for amplification were nitrous oxide reductase (*nos*Z), and dinitrogenase reductase (*nif*H). Degenerate primer pairs were previously designed, tested and optimized for *nos*Z (Throbäck *et al.*, 2004), and *nif*H (Deslippe, 2004; Deslippe *et al.*, 2005).

The half-nested *nos*Z amplification protocol utilized the primers nosZ-F (5′-CG(C/T)TGTTC(A/C)TCGACAGCCAG-3′) (Kloos *et al.*, 2001 in Throbäck *et al.*, 2004) and nosZ1622R (5′-CGC(G/A)A(C/G)GGCAA(G/C)AAGGT(G/C)CG-3′) (Throbäck *et al.*, 2004) for the primary amplification. The secondary reverse primer Nos1773R (5′-AACGA(A/C/G)CAG(T/C)TGATCGA(T/C)AT-3′) (Throbäck *et al.*, 2004) was labeled with Light Sabre Blue (D4) dye (Integrated DNA Technologies, Coralville, IA, USA). Each 30 μl PCR reaction contained 3 μl 1:10 dilutions of genomic DNA, 1 × PCR buffer, 0.2 mM dNTPs, 2.0 mM $MgCl_2$, 0.04 μM of each primer and 0.75 U Platinum *Taq* DNA Polymerase (Invitrogen, Burlington, Ontario, Canada). The secondary PCR mix differed only in $MgCl_2$ concentration (2.125 mM). Thermocycler conditions were the same

for both reactions: a 2 min denaturation step at 94 °C was followed by 35 cycles of denaturing, annealing and extension at 94 °C for 30 s, 55 °C for 1 min and 72 °C for 1 min, respectively. The final extension required 10 min at 72 °C.

The *nif*H protocol used Nh21F (5′-GCIWTI TAYGGNAARGGNGG-3′) and WidNhR (5′-GCRT AIABNGCCATCATYTC-3′) for the primary PCR reaction (Widmer *et al.*, 1999) and Nh428R (5′-C CRCCRCANACMACGTC-3′) for the second amplification (Deslippe 2004; Deslippe *et al.*, 2005) (sequences follow standard IUPAC notation for mixed bases). The reverse primer Nh428R was labeled with Light Sabre Green (D3) dye. Each 31.2 µl PCR reaction contained 4.5 µl 1:10 dilutions of genomic DNA, 1 × PCR buffer, 0.2 mM dNTPs, 2.0 mM $MgCl_2$, 0.04 µM of each primer and 0.75 U Platinum *Taq* DNA Polymerase (Invitrogen, Burlington, Ontario, Canada). Thermocycler conditions were the same for both reactions: a 1 min denaturation step at 94 °C was followed by 35 cycles of denaturing, annealing and extension at 94 °C for 45 s, 53 °C for 45 s and 72 °C for 1 min 30 s, respectively. The final extension required 10 min at 72 °C.

Positive and negative controls were included for all PCR steps. PCR product quality was assessed by ethidium bromide staining on a 1% agarose gel. If bands of the expected size (approximately 250 bp for *nos*Z and approximately 400 bp for *nif*H) were identified from a soil sample, the PCR product was cleaned via ethanol precipitation, dissolved in pH 8.0 Tris-EDTA buffer and stored at −20 °C until used. If no PCR product was generated, the reaction was attempted at least one more time with higher dilution of the template before it was concluded that the target gene was below detectable limits for that sample or alternately that PCR amplification failed due to inhibitors co-extracted with the DNA sample.

*T-RFLP*

A number of restriction endonucleases were tested on replicate samples, and selected based upon reproducible fragment generation from a range of different samples. Restriction enzymes used were *Hha*I for the *nos*Z amplicon and *Mbo*I for the *nif*H amplicon (Invitrogen, Burlington, Ontario, Canada). For each reaction, 6 µl of PCR product was digested with 2.5 U enzyme and 1 × buffer. Digests were incubated at 37 °C for at least 5 h and the reactions were terminated at 65 °C for 10 min. Digested fragments were desalted by ethanol precipitation and resuspended in sample loading solution (Beckman-Coulter Inc., Mississauga, Ontario, Canada). Fragments were prepared for analysis as suggested by the manufacturer for the Beckman-Coulter CEQ 8000 Fragment Analysis System (Beckman-Coulter Inc., Mississauga, Ontario, Canada) for 40 µl non-multiplexed samples, although resuspended fragments were not diluted 1:10 prior to loading. For each reaction, 2.5 µl of dye-labeled, digested, desalted product was combined with 37.0 µl of SLS and 0.5 µl of 400-bp size standard.

Fragments were binned and analyzed in the AFLP program of the CEQ 8000 Fragment Analyzer (Beckman-Coulter Inc.). Analysis parameters were as per the 400 size standard cubic model with minimum relative peak height set at 1% and a bin width of 3 bp. Samples from each of two soil depths were analyzed individually for all four treatments and all four control plots from all five sites. Gene community profiles were constructed using only peak heights generated by the sequencer that passed analysis. These peaks represented distinct terminal restriction fragments (TRFs), which in turn corresponded to unique genotypes. Samples that failed due to contamination during fragment analysis or undetected size standard were deleted so that they were not tallied along with samples that did not produce peaks because the target was below detectable limits (that is, true negatives).

TRF frequency was determined for each plot (OTC or control) by summing the presence data for all successful sample replicates. For example, if a TRF was present in three of six samples, its frequency was designated as 0.50 for that plot. This made it possible to compare plots with variable numbers of successfully amplified replicates (after PCR and fragment analysis). To establish the relative abundance of TRFs, the fluorescent signal strength of each peak was relativized to total peak area for each successful sample. Once the relative abundance of TRFs was determined for each sample, the average was calculated to determine the average relative abundance of TRFs for that plot. For genotype richness, much like frequency, the average number of genotypes from all of the successfully amplified sample replicates was determined per plot before the data were analyzed.

Theoretical TRFs were determined for 48 *nos*Z sequences downloaded from GenBank. These sequences represent genes from a wide range of bacterial genomes. TRFs were also calculated for 42 cloned *nif*H sequences from Alexandra Fiord (Deslippe and Egger, 2006), and from 26 select *nif*H and *nif*H-like sequences from the GenBank database. The restriction site for *Hha*I (5′GCG/C3′) or *Mbo*I (5′/GATC3′) was located on the reverse complement DNA strand of *nos*Z or *nif*H sequences, respectively. In both cases, TRF length was calculated by counting the base pairs between the restriction site cleavage point and the 5′ end of the labeled reverse primer.

*Statistical analysis*

Data visualization using non-metric multidimensional scaling (NMS) ordination was performed initially to gauge differences among sites and

treatments. Permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2005) was then used to statistically test the effects of site, treatment and depth on community composition. A large number of unique permutable units allowed us to establish significance using permutation *P*-values versus Monte Carlo *P*-values. The distribution of major TRFs among plots was examined with two-way cluster analysis using the relative Sorensen index and the flexible beta ($\beta = -0.25$) method for linking groups as implemented in PC-ORD 5.0 (McCune and Mefford, 1999). Genotypic richness was assessed by comparing differences in the average number of TRFs per sample by site, depth and treatment. Theoretically, each TRF represents a unique genotype so that an increase or decrease in these numbers reflects an increase or decrease in genotype richness (Tiedje *et al.*, 1999). Factorial ANOVA (Statistica 6.0) was used to detect overall significant differences in mean number of TRFs per sample between sites and due to depth or treatment over all sites. Where significant interaction terms were apparent, one-way ANOVA was then used to investigate differences due to depth or treatment unique to each site. For all statistical tests, a significance level of $P<0.05$ was used.

## Results

*Community composition*

*nos*Z. The *nos*Z TRF frequency data set showed the clearest patterns of differentiation in NMS ordinations, and demonstrated that site was the most important factor distinguishing the communities (see Supplementary Information). The importance of site was confirmed by PERMANOVA, which showed site ($P=0.0001$) and treatment ($P=0.0419$) to be significant factors differentiating the communities. However, PERMANOVA indicated a significant interaction between site and depth ($P=0.0.0434$) and a strong interaction between site and treatment ($P=0.0544$), so the analysis was run separately for each site. Individual site comparisons showed that treatment and depth were significant factors differentiating the *nos*Z communities only at SM and UD (Table 1). There were no significant interactions between treatment and depth at any site.

Two-way cluster analysis (Figure 1) confirmed that common TRFs were shared between CH and RW, that upland sites differed from lowland sites and from each other and that SM had fewer genotypes than are otherwise present at adjacent lowland sites. This figure also shows which of 44 distinct *nosZ* TRFs were important for structuring each site. The SM site was defined by the presence of a few dominant TRFs; only four *nos*Z TRFs showed an average frequency over all plots greater than 0.50. This site was strongly structured by TRF 198 (0.943), 196 (0.749), 252 (0.634) and 200 (0.565).

**Table 1** Statistical test results for structure (PERMANOVA) and average richness per core sample (ANOVA) for *nos*Z and *nif*H communities in response to warming and with depth of sample

| | | | *SM* | *CH* | *RW* | *UG* | *UD* |
|---|---|---|---|---|---|---|---|
| *nosZ* | Structure | Warming | **0.030** | 0.783 | *0.098* | 0.171 | **0.012** |
| | | Depth | **0.016** | 0.353 | 0.904 | 0.881 | **0.001** |
| | Richness | Warming | **0.021** | 0.927 | 0.392 | *0.080* | *0.052* |
| | | Depth | **0.017** | 0.948 | 0.653 | 0.465 | **0.013** |
| *Nif*H | Structure | Warming | **0.037** | 0.179 | **0.004** | **0.010** | 0.807 |
| | | Depth | **0.001** | **0.003** | 0.166 | **0.001** | **0.001** |
| | Richness | Warming | **0.007** | 0.611 | **0.008** | *0.054* | 0.720 |
| | | Depth | **0.002** | 0.351 | **0.028** | 0.918 | 0.438 |

Abbreviations: ANOVA, analysis of variance; CH, Cassiope Heath; PERMANOVA, permutational multivariate analysis of variance; RW, Riverside Willow; SM, Sedge Meadow; UD, Upland Dolomitic; UG, Upland Granitic.
Statistically significant results ($\alpha=0.05$) are indicated in bold. Additional results that would be significant at a less conservative $\alpha=0.10$ are indicated in underlined italics; although not significant at the standard accepted 95% confidence level, these results may have biological significance.

Important TRFs at the SM site also include 123 (0.408), 100 (0.273) and 177 (0.219). A greater number of distinct TRFs were present at CH and RW and many were shared between sites. The most frequent CH TRFs were 183 (0.866), 200 (0.802), 198 (0.795), 243 (0.793), 223 (0.788) and 193 (0.773). These TRFs were also important at RW, although with slightly different frequencies; this site was dominated by 193 (0.780), 183, 198 (both 0.734), 243 (0.699), 200 (0.622) and 100 (0.620). Common TRFs from other sites were detected at UG, but all were present with a frequency less than 0.50: 123 (0.455), 252 (0.423), 198 (0.310), 223 (0.306), 200 (0.300) and 183 (0.233). UD also contained TRFs 123 (0.700), 252 (0.698), 196 (0.651), 198, 223 (both 0.643) and 103 (0.571).

Nine important *nos*Z TRFs from this study were matched to those derived from a selection of relevant GenBank sequences (Table 2). Many of these fragments represent more than one putative genotype.

*nif*H

The *nif*H TRF relative abundance data set showed the clearest differentiation among plots in NMS ordinations (see Supplementary Information). PERMANOVA tests of *nif*H relative abundance data indicated that site ($P=0.0001$) and depth ($P=0.0001$) were important factors structuring the community. However, there were significant interactions between site and treatment ($P=0.0004$) and site and depth ($P=0.0001$), so analyses were run individually by site. Treatment was a significant factor at SM, RW and UG (Table 1). Depth was a significant factor at SM, CH, UG and UD (Table 1). There was no significant interaction between treatment and depth at any site.

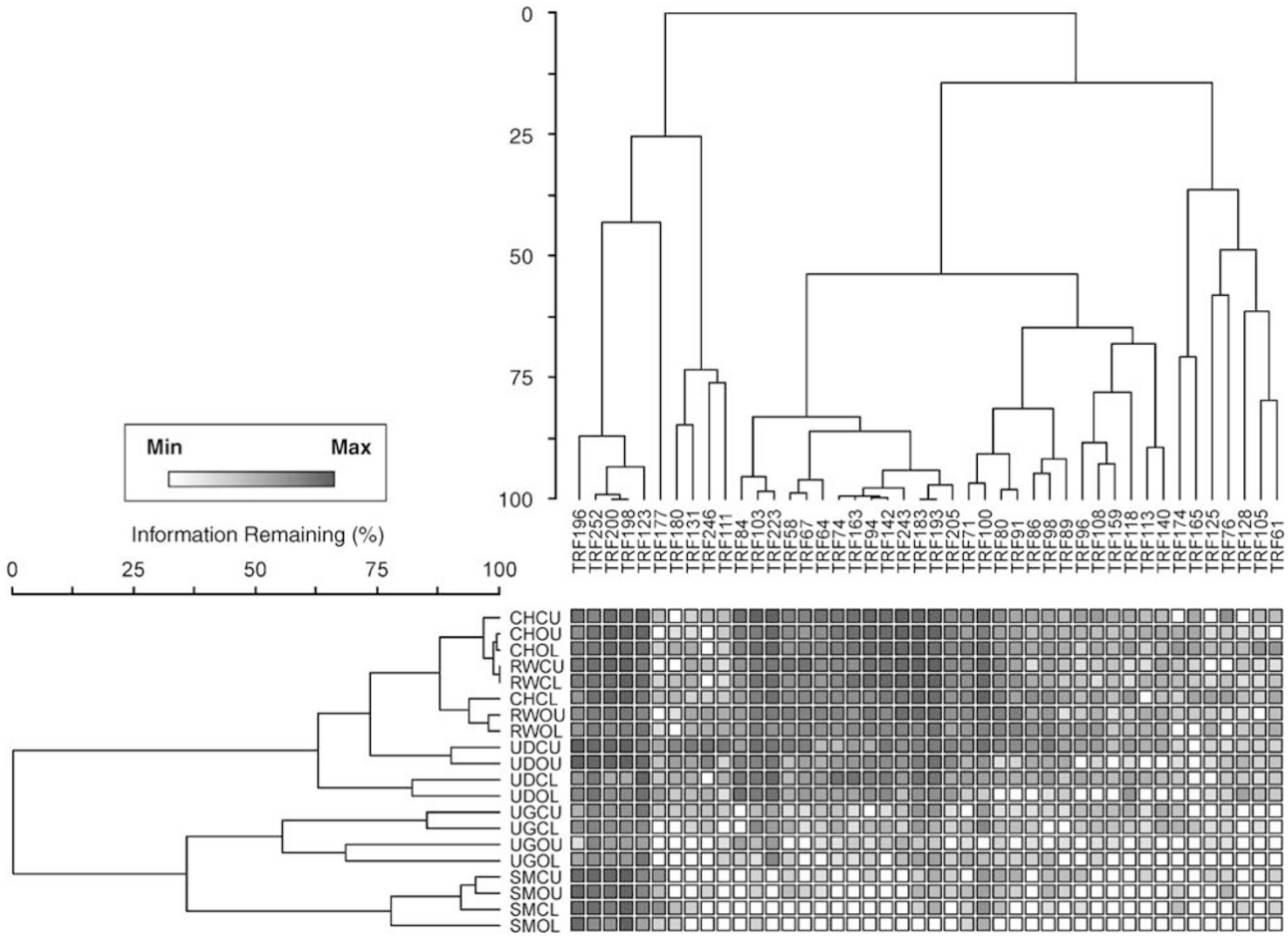


**Figure 1** Two-way cluster analysis of *nos*Z terminal restriction fragments (TRFs) using the relative Sorensen similarity index and the flexible beta (−0.25) method for tree construction. The first two letters of the horizontal tree labels indicate site (CH, Cassiope Heath; RW, Riverside Willow; SM, sedge meadow; UD, Upland Dolomitic; UG, Upland Granitic), the third letter indicates warming treatment (C, ambient control; O, open-top chamber (OTC)), and the last letter indicates depth (L, lower; U, upper). The vertical tree labels indicate the terminal restriction fragment genotypes. The darker the intensity of the square the higher the percentile of the total data matrix represented by that TRF genotype (that is, the lighter the square, the rarer the genotype).

Two-way cluster analysis of *nif*H relative abundance data (Figure 2) confirmed that a unique distribution of TRFs was represented at SM and that important TRFs differ between CH and RW plots. Figure 2 also shows which TRFs are important for structuring each site. The SM site was composed of a number of different genotypes in low abundance: TRFs 92 (10.6%), 222 (9.5%), 296 (5.9%), 299 (5.4%) and 384 (5.2%). CH was structured instead by TRF 83 (16.2%), 80 (10.7%), 299 (5.7%), 366 (4.3%) and 78 (3.6%). The most abundant TRFs at RW were 278 (14.4%), 80 (9.4%), 384 (8.7%), 280 (7.6%) and 275 (5.4%). UG was represented by TRFs 384 (21.1%), 83 (12.1%), 299 (9.7%), 257 (8.1%) and 296 (6.9%), while at UD TRF 384 accounted for 52.1% of total genotype abundance, whereas only a few other TRFs were represented in low abundance: 282 (3.8%), 260 (3.4%), 83 (3.3%) and 290 (3.0%).

Twelve important *nif*H TRFs from this study closely matched TRFs derived from the *nif*H sequences of putatively identified clones in Deslippe and Egger (2006) (Table 3). In addition, 19 TRFs from this study matched hypothetical TRFs derived from GenBank sequences. These TRF genotypes encompass most, but not all, of the sequences examined because some hypothetical TRFs were less than the 50 bp minimum size threshold used when collecting the fragment data.

*Genotype richness*

*Nos*Z genotype richness per sample varied significantly by site ($P<0.0001$) as determined by ANOVA. SM and UG had fewer genotypes per sample than CH, RW and UD (Figure 3). Treatment was also a significant factor overall ($P=0.0168$), although ANOVA tests by site revealed that warmed plots had significantly fewer genotypes only at the SM site, with reduced numbers at UG and UD (Table 1). Although depth was not a significant factor overall ($P=0.0951$), individual comparisons showed there were significantly fewer genotypes in deeper

**Table 2** Putative identification of important *nos*Z TRFs at Alexandra Fiord based on TRFs derived from a selection of *nos*Z gene sequences obtained from GenBank

| *GenBank accession number* | *Organism name* | *Calculated TRF* | *TRF match from this study* |
|---|---|---|---|
| X65277 | *Pseudomonas aeruginosa* strain DSM 50071 | 73 | 74 |
| AF361795 | *Alcaligenes faecalis* | | |
| AF125260 | *Rhodobacter sphaeroides* f. sp. denitrificans | | |
| AF315434 | Uncultured temperate forest soil bacterium CZ0X04 | | |
| AF016055 | Uncultured eubacterium S321195A | 83 | 84 |
| DQ289947 | Uncultured α-Proteobacterium clone LC1N-21 | | |
| RMU47133 | *Rhizobium meliloti* | | |
| AY072227 | *Achromobacter xylosoxidans* | 101 | 100 |
| AF315440 | Temperate forest soil bacterium YC08 | 174 | 174 |
| AY072230 | *Azospirillum irakense* | | |
| AF016059 | *Pseudomonas denitrificans* | 192 | 193 |
| DQ377783 | *Pseudomonas lini* PD 11 | | |
| DQ377773 | *Pseudomonas migulae* PD 1 | | |
| AF056319 | *Pseudomonas fluorescens* | | |
| AB054991 | *Pseudomonas* sp. MT-1 | | |
| AF016058 | *Paracoccus denitrificans* | | |
| EU192075 | *Paracoccus* sp. BW001 | | |
| AJ010260 | *Paracoccus denitrificans* | | |
| EU346731 | *Shinella zoogloeoides* strain BC026 | | |
| AF047429 | *Achromobacter cycloclastes* | | |
| DQ377784 | *Sinorhizobium* sp. PD 12 | | |
| AF361791 | *Azospirillum brasilense* strain Sp7 | | |
| AF361793 | *Azospirillum lipoferum* | | |
| AF361794 | *Azospirillum halopraeferens* | | |
| AY072231 | Uncultured temperate forest soil bacterium CZ1496 | | |
| AY074762 | *Azospirillum* sp. A1-3 | 199 | 198/200 |
| AJ002531 | *Bradyrhizobium japonicum* | 246 | 246 |
| DQ504302 | *Marinobacter hydrocarbonoclasticus* strain 617 | | |
| AB088344 | *Marinobacter* sp. HS7 | 254 | 252 |
| AY912909 | Uncultured forest soil bacterium clone | | |
| DQ179250 | DUNnos001 | | |
| DQ377781 | Antarctic soil bacterium 2-1 | | |
| DQ179249 | *Pseudomonas grimontii* PD 9 | | |
| AJ704213 | *Pseudomonas* sp. 3-13 | | |
| DQ377775 | *Pseudomonas* sp. ED3 | | |
| DQ377776 | *Pseudomonas* sp. PD 3 | | |
| DQ377803 | *Pseudomonas brassicacearum* PD 4 | | |
| DQ377774 | *Pseudomonas kilonensis* PD 31 | | |
| DQ377779 | *Pseudomonas mandelii* PD 2 | | |
| DQ377801 | *Achromobacter* sp. PD 7 | | |
| DQ377790 | *Ensifer adhaerens* PD 29 | | |
| | *Bosea* sp. PD 18 | | |

Abbreviation: TRF, terminal restriction fragment.
In the case of theoretical TRF 199, experimental TRFs 198 and 200 are equally relevant and reasonable matches.

samples compared to upper samples at the SM and the UD sites (Table 1).

*Nif*H genotype richness per sample also varied significantly by site ($P<0.0001$); richness per sample was comparable at the SM, RW and UD sites, but lower at CH and UG (Figure 4). Significant interaction terms between site and treatment ($P=0.0011$) and site and depth ($P=0.0081$) indicated that treatment and depth only played a role at some sites. Warmed plots and deeper samples had significantly fewer genotypes at SM and RW (Table 1).

## Discussion

Site was the dominant factor differentiating communities for both *nos*Z and *nif*H, reflecting the variation in plant community composition, soil characteristics, moisture and temperature that combine to create the unique characteristics of each site. Although site was the dominant driver of community structure, differences between sites were not as clearly defined for nitrogen fixers, especially when assessed by genotype frequency. Although frequency provided the best results for the *nos*Z gene,

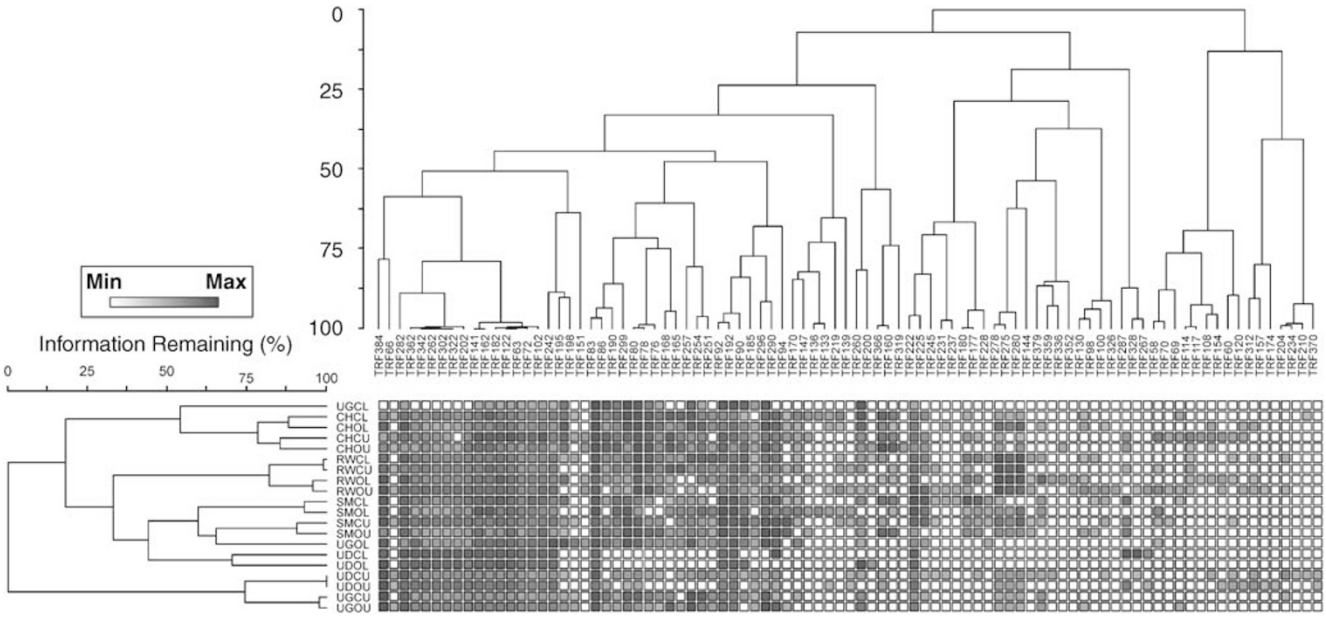


**Figure 2** Two-way cluster analysis of nif*H* terminal restriction fragment (TRFs) using the relative Sorensen similarity index and the flexible beta (−0.25) method for tree construction. The first two letters of the horizontal tree labels indicate site (CH, Cassiope Heath; RW, Riverside Willow; SM, Sedge Meadow; UD, Upland Dolomitic; UG, Upland Granitic), the third letter indicates warming treatment (C, ambient control; O, open-top chamber (OTC)) and the last letter indicates depth (L, lower; U, upper). The vertical tree labels indicate the TRF genotypes. The darker the intensity of the square the higher the percentile of the total data matrix represented by that TRF genotype (that is, the lighter the square, the rarer the genotype).

relative abundance was best for the *nif*H gene. This is consistent with observations that *nos*Z genotypes were more distinct between sites, while *nif*H genotypes were more likely to be shared across sites, differing mainly in their abundance.

*Nos*Z genotypes are known to exhibit high habitat specificity (Rösch *et al.*, 2002; Rich *et al.*, 2003; Rich and Myrold, 2004; Stres *et al.*, 2004), with few genotypes shared between sites with different C:N, pH, dominant vegetation and N availability (Rich *et al.*, 2003). Experiments using other denitrification gene markers confirm that denitrifier gene communities tend to be unique to the environment from which they are sampled, with few genotypes shared between locations (Braker *et al.*, 2000, 2001). Large variations in *nos*Z TRF distribution at distances of centimeters, meters and especially kilometers has also been found in marine studies, which is consistent with observations that terrestrial denitrifier genotypes are strongly structured by site (Scala and Kerkhof, 2000). It is also important to note that large seasonal shifts have been detected in both terrestrial (Wolsing and Priemé, 2004) and marine (Scala and Kerkhof, 2000) denitrifying communities and that this study examined samples taken only once during the summer growing season. Detection of seasonal variation in denitrifying gene community structure (due directly or indirectly to temperature manipulations) depends to some extent on the timing and frequency of sampling, plus the molecular technique and gene marker employed. Instead, this study sought to detect persistent changes brought about by factors that affect the long-term community structure of denitrifiers, such as carbon availability, pH, moisture and temperature (Wallenstein *et al.*, 2006).

We putatively identified several *nos*Z TRFs based upon theoretical TRFs derived from GenBank sequences. Although most *nos*Z genotypes did appear to be unique to a few sites, there were some TRFs that were detected at most or all sites. These TRFs may represent numerous genotypes, for example TRF 193, and the uncut fragment 252, may include up to 6 different genera (Table 2), however, *nos*Z TRF 100 was frequently found at almost all sites even though it appears to delineate only one species, *Achromobacter xylosoxidans.*

*Nif*H gene communities are also known to vary over large and small spatial scales (Poly *et al.*, 2001; Rösch *et al.*, 2002) but the factors that control this variation are complex. *Nif*H communities tend to be habitat specific (Zehr *et al.*, 1998; Shaffer *et al.*, 2000), being most similar when their site characteristics are comparable. Similarly, *nif*H TRFs that were found to be dominant at one site were often less frequent, or less abundant, or not present at all at adjacent sites with different plant and soil characteristics (Shaffer *et al.*, 2000). Genotype distribution has been related to plant cover, soil chemistry, soil management and soil texture, all of which influence inorganic N availability but none of these factors can completely explain the observed distributions (Poly *et al.*, 2001). Disturbance of marsh soils by long-term fertilization led to changes in *nif*H community composition and a reduction in genotype richness due to the loss of competitive

**Table 3** Putative identification of important *nif*H TRFs at Alexandra Fiord based upon a selection of *nif*H gene sequences obtained from GenBank and cloned *nif*H sequences from soils at the same site collected by Deslippe and Egger (2006)

| *GenBank accession number* | *Clone host and designation[a] or organism name[b]* | *Phylogenetic placement in Deslippe and Egger (2006)* | *Calculated TRF* | *TRF match from this study* |
|---|---|---|---|---|
| X07474[b] | *Methanococcus voltae* | | 58 | 58 |
| U73134[b] | *Stanieria cyanosphaera* PCC 7437 | | 67 | 66 |
| DQ059362[a] | *Salix* root clone IC-4 | Type I nitrogenases: clade | 69 | 69 |
| DQ059339[a] | *Dryas* bulk clone 12-3 | 1: α- and β-Proteobacteria[a] | | |
| DQ059364[a] | *Salix* root clone 2C-4 | | | |
| DQ059352[a] | *Rhizobium* sp. K00487 | | | |
| DQ059342[a] | *Bradyrhizobium* sp. AB079616 | | | |
| DQ059357[a] | *Bradyrhizobium* sp. AB079617 | | | |
| DQ059347[a] | α-Proteobacterium AF099777 | | | |
| DQ059349[a] | *Dryas* bulk clone 12-7 | Type I: clade 4: posited | 78 | 78 |
| DQ059341[a] | *Dryas* root clone 2-1 | Novel clade[a] | | |
| DQ059358[a] | *Dryas* bulk clone 9-8 | | | |
| L04499[b] | *Rhodobacter capsulatus* | | 83 | 83 |
| DQ059377[a] | *Herbaspirillum* sp. AB196476 | | 85 | 86 |
| DQ059365[a] | *Dryas* aggregate clone 5-9 | Type I: clade 1: α- and β-Proteobacteria[a] | 153 | 154 |
| M16709[b] | *Azorhizobium caulinodans* | Type I: clade 1: α- and | 167 | 168 |
| DQ059374[a] | *Dryas* bulk clone J12-7 | β-Proteobacteria[a] | 168 | |
| U73133[b] | *Myxosarcina* sp. | | 177 | 177 |
| AY768416[b] | *Lyngbya aestuarii* PCC 7419 | | | |
| DQ059350[a] | *Azoarcus communis* strain S2 U97117 | | 182 | 182 |
| M73020[b] | *Rhodobacter sphaeroides* | | 185 | 185 |
| DQ291144[b] | *Aphanizomenon* sp. KAC15 | | 188 | 190 |
| Z11165[b] | *Rhodobacter capsulatus* | | 233 | 234 |
| AY221815[b] | *Oscillatoria sancta* PCC 7515 | | 241 | 242 |
| AJ010302[b] | *Rhodobacter capsulatus* | | 257 | 257 |
| DQ059369[a] | α-Proteobacterium AF099776 | | 258 | |
| U49515[b] | *Fischerella* sp. UTEX 'LB 1931' | | 267 | 267 |
| DQ059351[a] | *Paenibacillus polymyxa* AJ224428 | | 276 | 275 |
| DQ059363[a] | *Paenibacillus polymyxa* AJ224428 | | | |
| DQ059367[a] | *Paenibacillus polymyxa* AJ224428 | | | |
| U23507[b] | *Trichodesmium thiebautii* | | 280 | 280 |
| L04499[b] | *Anabaena* sp. L-31 | | | |
| X04465[b] | *Clostridium pasteurianum* | | | |
| DQ059370[a] | *Dryas* root clone 3-7 | Type I: clade 2: Firmicutes[a] | 284 | 284 |
| DQ059376[a] | *Dryas* aggregate clone J7-5 | | | |
| DQ059379[a] | *Dryas* aggregate clone J7-1 | | | |
| DQ059353[a] | *Dryas* aggregate clone 6-9 | Type I: clade 4: posited | 290 | 290 |
| DQ059359[a] | *Dryas* root clone 1-7 | Novel clade[a] | | |
| DQ059345[a] | *Dryas* aggregate clone 6-1 | | | |
| DQ059356[a] | *Dryas* aggregate clone 7-5 | | | |
| X07472[b] | *Anabaena* sp. (strain L31*)* | | 296 | 296 |
| DQ059375[a] | *Dryas* aggregate clone J6-2 | Type I: clade 4: posited | 300 | 302 |
| DQ059378[a] | *Dryas* root clone J3-8 | Novel clade[a] | | |
| X57006[b] | *Methanobacterium ivanovii* | | 351 | 352 |
| X07501[b] | *Frankia alni* | | 363 | 362 |
| DQ059338[a] | *Pseudomonas* sp. DC AF117976 | | 366 | 366 |
| DQ059373[a] | *Pseudomonas* sp. DC AF117976 | | | |
| X03777[b] | *Methanococcus thermolithotrophicus* | | | |
| Z31716[b] | *Nostoc* sp. PCC 6720 | | 386 | 384 |
| V0001[b] | *Nostoc* sp. PCC 7120 | | | |
| AF003336[b] | *Cyanothece* sp. ATCC 51142 | | | |
| M15270[b] | *Clostridium pasteurianum* | | | |

Abbreviation: TRF, terminal restriction fragment.
[a]Cloned sequences from Deslippe and Egger (2006).
[b]Sequences obtained from GenBank.

advantage over other N-limited microbes (Piceno and Lovell, 2000). This may mimic the impact of long-term soil warming on N mineralization and help explain why we observed a general loss of genotype richness with OTC treatments that was significant at two of the lowland sites. Long-term disturbance of forest soils due to clearcutting led to distinctly different *nif*H communities with new dominant TRFs and losses of previously dominant genotypes and additionally, these communities exhibited atypical seasonal variation (Shaffer *et al.*, 2000). Deslippe *et al.* (2005) also noted that *nif*H communities were strongly structured by OTC warming, with NMS ordinations showing differences between control plots and OTCs at lowland sites that developed over the growing season.

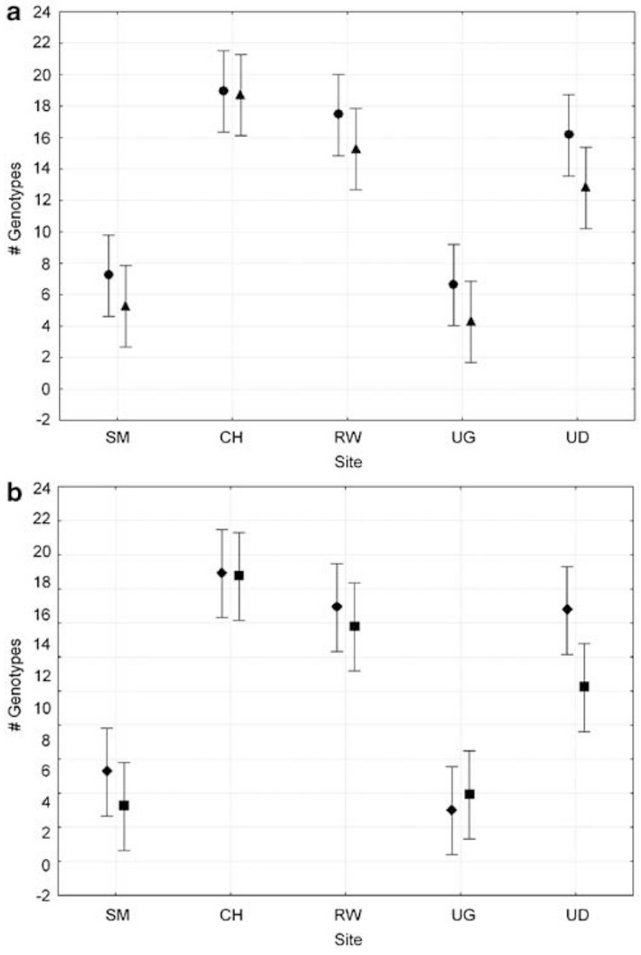


**Figure 3** (**a**) Average number of *nos*Z genotypes per sample by treatment with 95% confidence intervals. Circles indicate ambient controls; inverted triangles indicate OTC-warmed plots. (**b**) Average number of *nos*Z genotypes per sample by depth with 95% confidence intervals. Diamonds indicate upper samples; squares indicate lower samples.

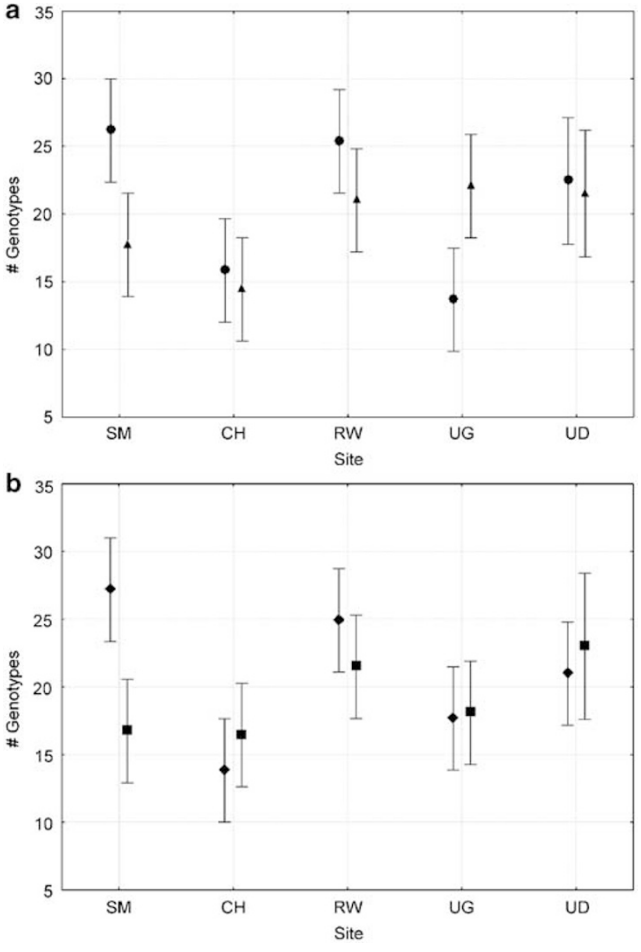


**Figure 4** (**a**) Average number of *nif*H genotypes per sample by treatment with 95% confidence intervals. Circles indicate ambient controls; inverted triangles indicate OTC-warmed plots. (**b**) Average number of *nif*H genotypes per sample by depth with 95% confidence intervals. Diamonds indicate upper samples; squares indicate lower samples.

Samples for this investigation were collected at approximately the same time of year (18–27 July) as Deslippe *et al*. (2005), but 2 years later, and at adjacent plots in the same study area.

We putatively identified *nif*H TRFs based upon theoretical TRFs derived from GenBank sequences, including cloned sequences from Deslippe and Egger (2006) that were collected at the same site (Table 3). Most of the genotypes identified correspond to Proteobacteria, particularly α-, β- and γ-Proteobacteria. Several TRFs corresponded to members of the Firmicutes. Putative cyanobacteria were also well represented. Three TRFs corresponded to a posited novel group of diazotrophs identified by Deslippe and Egger (2006). We did not identify any TRFs that corresponded to type IV nitrogenases, which represented a substantial component of the clones in Deslippe and Egger (2006). On the basis of this analysis, the taxonomic groups potentially represented by the TRFs in this study represent a wide taxonomic range, and include Proteobacteria, Firmicutes and Cyanobacteria, as well as a novel clade of unknown identify that was well represented in a previous study (Deslippe and Egger, 2006). Although most *nif*H TRFs were shared across all sites, some sites were strongly structured by one or a few genotypes. For example, the relative abundance of TRF 366 (which may represent *Methanococcus thermolithotrophicus*) was concentrated at Cassiope Heath. TRF 384 is the full-length, uncut *nif*H amplicon. It represents one or more genotypes without an *Mbo*I restriction site. This TRF was present at virtually all sites, treatments and depths but had particularly high relative abundance at the Upland Granite (>20%) and the Upland Dolomite (>50%) sites. It is difficult to speculate about the identity of all the species represented by this fragment, but TRF 384 may include *Clostridium pasteurianum* along with a number of cyanobacterial genera (see Table 3).

Treatment and depth were also significant factors structuring the communities according to PERMA-

NOVA, but not at all sites and often at different sites for each gene. Genotype richness provided additional insights into how warming and depth were affecting microbial communities. Again, sites differed significantly in their genotype richness for both *nos*Z and *nif*H and at most sites; for both genes, there tended to be lower genotype richness in warmed samples, but these differences varied by site. It is unclear how much the OTC response is due to increased soil temperatures. Hollister *et al.* (2006) found that OTC effects were variable at two different tundra sites, and differed greatly from day to day and from year to year. Differences between OTCs and control plots in average soil temperature (at −10 cm) in July ranged slightly from 0.3 to 0.6 °C at dry heath sites, and enormously from −0.8 to 0.7 °C at wet sedge sites. It is interesting to note that the SM site in our study was the one that was most consistently affected by warming, but we do not have other sites to compare to see if this is a general response of wet sedge meadow sites.

The SM site was unique within the study area in having the deepest active layer and the wettest (often flooded) conditions. It was at the SM site that we saw significant treatment and depth effects on community structure for both *nosZ* and *nif*H according to the PERMANOVA analysis, and a significant reduction in genotype richness of both genes with warming and depth according to the ANOVA. The SM site was also where Rolph (2003) found the greatest response to warming when nitrogen transformations were compared between control plots and OTCs. Specifically, both $NO_3^-$ and N immobilization increased in SM OTCs, suggesting that there was more substrate for denitrification and an increase in microbial activity in these plots.

*Nif*H genotype richness was relatively high at the wet SM site where diverse, aquatic cyanobacteria are typically dominant (Henry *et al.*, 1986; Chapin and Bledsoe, 1992; Zehr *et al.*, 1998; Callaghan *et al.*, 2004) and where there is a high C:N ratio due to a large amount of poorly decomposed plant material (Rolph, 2003). It is not uncommon to find diverse *nif*H communities in wet, anaerobic environments (Ueda *et al.*, 1995; Zehr *et al.*, 1998). The nitrogen-fixing community at SM experienced a large decrease in richness with warming, suggesting that either increased N suppressed N fixers, or that the site was dominated by an abundance of rare genotypes that were sensitive to disturbance. Richness of *nos*Z genotypes also declined significantly with warming. A marshy site such as SM might have been expected to have higher overall denitrifier richness (Priemé *et al.*, 2002), but the richness was comparable to the UG site, which also had a more acidic pH, so this may have limited *nos*Z diversity.

Depth was also a significant factor for both genes at the SM site, with deeper samples having significantly fewer genotypes. The SM site had a large depth range between upper and lower soil samples (0–5 and 39–44 cm deep, respectively). More importantly, the soil core itself was not as uniform as those from CH and RW; the upper section was wet organic peat, while the lower part of the core had a greater proportion of sandy mineral soil. Differences in gene frequency and abundance between upper and lower samples at SM may be partly explained by the vertical differentiation between upper and lower samples. Additionally, a greater proportion of cyanobacteria would be expected in surface samples due to their phototrophic mode of nutrition. At the same SM site, Henry and Svoboda (1986) found that N-fixation rates were significantly higher in surface soil samples than those from −10 to −20 cm. Studies of N-poor acidic forest soils have indicated a distinct difference in *nif*H RFLP patterns between litter layers and soil samples (Widmer *et al.*, 1999), and a decrease in pattern complexity from shallow to deep soil samples (Shaffer *et al.*, 2000). Denitrification is generally thought to be more strongly associated with anaerobic conditions. However, Braker *et al.* (2001) used an alternative denitrification marker (*nir*S) to investigate changes in community structure in ocean sediment cores based upon the transition to an anaerobic environment and as depth increased only a slight decrease in denitrifier diversity was detected in deeper samples even though a strong redox gradient was present. Enzyme analysis and gene probing of forest soils have also shown that denitrifier abundance decreases with depth despite lower levels of oxygen in deep samples (−25 cm) that should favor anaerobes (Mergel *et al.*, 2001; Rösch *et al.*, 2002). Our findings are not inconsistent with these studies, although we did not measure absolute abundance of *nos*Z or *nif*H.

The CH and RW sites were not well differentiated in any of the analyses, reflecting their general similarity in plant community composition and environmental factors. CH and RW shared many *nos*Z genotypes in common, and grouped together when gene frequency and abundance were examined by NMS. The communities were somewhat more divergent when comparing *nif*H relative abundance with NMS but still grouped closely together in the ordination, indicating their similarity (see Supplementary Information).

High *nos*Z richness was found at both CH and RW; likely due to comparable soil moisture regimens, a relatively rich plant community and similarly low soil pH. Brodie *et al.* (2002) and Stres *et al.* (2004), based upon bacterial communities in disturbed grassland and upon denitrifiers in forest soils, respectively, claim that plant uniformity and higher pH seem to be more consistent factors associated with high microbe diversity than site vegetation. This was not supported at CH or RW. Instead, *nos*Z denitrifier richness was comparatively high despite a relatively rich mixed plant community and acidic soils. Despite their similarities, there were differences in how CH and RW responded to warming and how communities were structured by depth. Warm-

ing had no significant effects on either microbial community at CH, but PERMANOVA showed a significant effect on *nif*H communities at RW, likely due to the significant reduction in *nif*H genotype richness at that site. Depth was not a major factor in structuring the *nos*Z communities at CH or RW, but *nif*H exhibited both an altered structure with depth at CH, and significantly fewer genotypes in deeper samples at RW. The lack of consistent responses to warming and depth at these very similar sites suggests that we required a higher sampling intensity to resolve the heterogeneity at these sites.

The upland sites were remarkably different from those on the lowland, likely due to the xeric nature of their soils and relatively sparse vegetation. They shared several plant species in common, although there were strong differences in relative abundance with more plant cover by *Salix arctica* in the UG site and absence of *Cassiope tetragona* at the UD site. However, they differed greatly in soil pH; the granitic site averaging pH 5.2 and the dolomitic site much more alkaline at pH 7.9. The communities differed considerably in genotype richness, with UG having lower richness than UD for both genes, which is likely due to the much lower pH of the UG site. These communities also showed less consistent responses to warming and depth than the SM site.

The UD site is a harsh xeric site with a high pH and low nutrient and organic carbon availability. The UD *nos*Z community showed altered community structure in response to warming, and a substantial, bordering on significant decrease in genotype richness. The UD *nos*Z community also differed in structure and richness by depth. The *nif*H community on this site was less impacted by warming, although community structure, but not richness, was affected by depth. Warming appears to have destabilized the *nos*Z community at this site, while having little impact upon the nitrogen fixers. In contrast, the UG *nos*Z community exhibited little impact from warming and was not structured by depth. However, the *nif*H community was much more affected by warming at the acidic pH, showing a significant change in community structure and a substantial, bordering on significant decline in genotype richness. Depth was also a factor structuring the *nif*H community at this site, although there was no corresponding decrease in richness with depth.

Soil parent materials and pH were likely major drivers of community structure at these sites, and it is interesting that *nos*Z and *nif*H communities had different responses to these constraints. This indicates that predicting the effects of warming on microbial communities will be difficult, given the complex interactions among environmental factors.

It is not uncommon for warming studies lasting more than a decade to detect persistent microbial community shifts (Rinnan *et al*., 2006). However, changes in physiological function have been noted without corresponding changes in community diversity (Gomez *et al*., 2004; Nicolaisen *et al*., 2004), and community shifts that do not affect microbial activity have been documented (Avrahami and Conrad, 2003; Deslippe *et al*., 2005). Callaghan *et al*. (2004) argue that extreme arctic environmental conditions restrict the metabolic potential of diverse arctic microbial communities, which contributes to the difficulty in establishing links between rates of microbially mediated processes and changes in microbial community structure (measured in terms of genetic diversity). We found that warming had complex effects on community structure or richness of microbial communities at this high arctic site. The two harshest and most xeric sites on the upland plateau, UG and UD, provided an interesting contrast in how the two communities responded: warming was a significant factor for the *nos*Z community at the UD site, while just a few meters away at the UG site, the *nif*H community showed the greatest response. The only site where there were unequivocal effects of OTC warming was SM, the most hydric site. Given that wet sedge meadows are among the most productive terrestrial ecosystems in the Arctic, this result should be investigated further to examine whether alterations in N-cycling rates accompany changes in community structure and richness.

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