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ORIGINAL ARTICLE

The effect of experimental warming on the root-associated fungal community of *Salix arctica*

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The effect of experimental warming on the root-associated fungal community of arctic willow (*Salix arctica*) was studied in three distinct habitats at a tundra site in the Canadian High Arctic. Plots were passively warmed for 5–7 years using open-top chambers and compared to control plots at ambient temperature. Fungal communities were assessed using terminal restriction fragment length polymorphisms. We found the following: (1) the root-associated fungal community in these high arctic tundra habitats is highly diverse; (2) site and soil characteristics are the most important drivers of community structure and (3) warming increased the density of different genotypes on individual root sections but has not (yet) affected the composition, richness or evenness of the community. The change in genotype density in the warmed plots was associated with an increase in PCR amplification efficiency, suggesting that increased C allocation belowground is increasing the overall biomass of the fungal community.

Introduction

By the end of the century, in the absence of significant curtailment in greenhouse gas emissions, models predict that the mean annual temperature north of 60°N will increase by 5–7 °C, with the greatest increase occurring during autumn and winter (ACIA, 2005).

Studies of the impacts of experimental warming on arctic tundra have shown that plants respond strongly and rapidly to experimental warming, with changes in plant phenology, reproductive effort and productivity (Henry and Molau, 1997; Arft *et al.*, 1999; Henry, 2002; Walker *et al.*, 2006), and increased carbon allocation to roots (Hill, 2006). At the plant community level, warming caused increased height and cover of deciduous shrubs and graminoids, decreased cover of moss and lichen (Cornelissen *et al.*, 2001), and decreased species diversity and evenness (Hollister *et al.*, 2005; Walker *et al.*, 2006), although the magnitude of these changes depends upon ecosystem type (Van Wijk

et al., 2004) and latitude (Walker *et al.*, 2006). The experimental results support conclusions that increased shrub abundance (Sturm *et al.*, 2001) and tundra greenness (Richter-Menge *et al.*, 2006) in arctic regions are the result of recent warming.

There have been few studies examining how warming affects root-associated microbial communities, even though major impacts of global warming may arise from the influences of increased soil temperature and CO_2 concentration on soil processes (Pendall *et al.*, 2004). Increased soil temperature can influence root growth, phenology and architecture, respiration and ion uptake, water and nutrient availability (Pregitzer *et al.*, 2000), but the impacts on soil microbial communities is varied and often unpredictable (Zak *et al.*, 2000). Responses of mycorrhizal fungi to simulated global change factors (CO_2, warming, shading and fertilization) are also variable, although several studies have found a not unexpected link between increased plant C allocation belowground and increased mycorrhizal colonization (Staddon *et al.*, 2002; Urcelay *et al.*, 2003; Fitter *et al.*, 2004; Heinemeyer and Fitter, 2004; Olsrud *et al.*, 2004; Clemmensen *et al.*, 2006). Some studies have found that experimental treatments alter mycorrhizal community composition (Rygiewicz *et al.*, 2000; Clemmensen and Michelsen, 2006; Parrent *et al.*, 2006), but these changes are rarely of

sufficient magnitude to significantly change the overall richness or diversity of the community.

Given the important role of root-associated fungi in nutrient acquisition, we set out to examine the interactions between site and soil characteristics and experimental warming on the composition and diversity of the root-associated fungal community of the ectomycorrhizal dwarf shrub, *Salix arctica*. Since roots were washed well before characterization, the root-associated fungal community should be dominated by mycorrhizal fungi, dark septate endophytes, hyaline septate endophytes, and possibly root-pathogens. Ectomycorrhizal and ericoid mycorrhizal fungi are important components of arctic systems (Gardes and Dahlberg, 1996), and dark septate endophytes and hyaline septate endophytes are ubiquitous in both alpine and arctic systems (Bledsoe *et al.*, 1990; Gardes and Dahlberg, 1996; Jumpponen, 1999; Cázares *et al.*, 2005). Although the contributions of mycorrhizas to nutrient uptake are well-known (Smith and Read, 1997), the function of dark septate endophytes and hyaline septate endophytes are less clear, as they appear to range from pathogenic to mutualistic (Jumpponen, 2001). Dark septate endophytes have been hypothesized to mimic the role of arbuscular mycorrhizal fungi, which are scarce in the high arctic (Bledsoe *et al.*, 1990). To investigate the potential impact of climate change on root-associated fungal communities, warming experiments were established in three sites: a lowland site on predominantly granitic parent materials, an upland site on granitic parent materials and an upland site on dolomitic parent materials.

Methods

Study site
Samples were collected at the Alexandra Fiord lowland on Ellesmere Island, Nunavut, Canada, 78° 53′N, 75° 55′W. The mesic Lowland Granitic (LG) site was near sea level (30 m) and enclosed by 500–700 m plateaus to the east and west, Twin Glacier to the south, and the waters of Alexandra Fiord to the north. The growing season climate at the lowland site is relatively warm due to frequently clear skies, relatively warm air masses from the west and south (Labine, 1994), and reflection of sunlight from the glaciers and sea ice into the lowland (Freedman *et al.*, 1994). The soils are mainly regosolic cryosols (Muc *et al.*, 1994a) composed of granitic parent materials (Sterenberg and Stone, 1994). The lowland site has been classified as a dwarf shrub–cushion plant community and is dominated by *Cassiope tetragona*, *Dryas integrifolia* and *S. arctica*, along with *Vaccinium uliginosum*, *Saxifraga oppositifolia* and *Luzula nivalis* (Muc *et al.*, 1994b). The upland sites were located on top of the western plateau at 500 m. These sites were xeric with sparse vegetation and exhibit polar desert

and semi-desert conditions. Vascular plant diversity was approximately 40% lower than the lowland site (Batten and Svoboda, 1994). The two upland sites were distinguished by soil type: the Upland Granitic (UG) site occurred on granitic parent materials similar to the lowland site, and the Upland Dolomitic (UD) site developed from dolomitic parent materials, which were characterized by high concentrations of calcium magnesium carbonate $(CaMg (CO_3)_2)$, high pH, and low organic matter deposition. The UG site was dominated by *S. arctica* and *S. oppositifolia*, with *C. tetragona* and *D. integrifolia* as minor components. The UD site was dominated by *D. integrifolia*, *S. arctica* and *S. oppositifolia*; *C. tetragona* was absent at this site (Batten and Svoboda, 1994).

Experimental warming
To experimentally increase temperature, open-top chambers (OTCs), which covered $0.8 \, m^2$ and were 0.3 m high, were used to passively warm plots at each of the sites. OTCs increase air temperatures by 1–4 °C (Marion *et al.*, 1997), although their effect on soil temperature is more variable, with increases and decreases being observed (Hollister *et al.*, 2006). OTCs were installed at the LG site in 1995 and at the two upland sites in 1993 (Stenström *et al.*, 1997).

Field collection
Two specimens of *S. arctica* were harvested from each of three OTC plots at each site in August 2000. Two plants that were found between 0.5 and 1.5 m from each OTC were harvested as ambient controls. Plants and surrounding soil were kept in Ziploc bags ($18 \times 20 \, cm$) in a permafrost cooler at approx. -1 °C until transported to the laboratory.

For soil analysis, two 300 g samples of soil were collected in August 2001 within 1 m of the harvested ambient plants. Soils were dried and sieved (2 mm) to remove rocks and course debris from the samples. One hundred grams from each of the two replicates was mixed and sent to the Ministry of Forests Analytical Laboratory (Victoria, British Columbia, Canada) for the following analyses: pH in water, total C and N using combustion elemental analysis, cation exchange capacity and exchangeable cations using 0.1 N barium chloride extraction, available NH_4-N and NO_3-N extracted with 2 N KCl, and available P using the Mehlich III protocol.

Sampling from roots
Plant roots were kept cold during transport and stored at 4 °C prior to sampling. Roots were immersed in water for up to 24 h at 4 °C prior to washing to loosen adhering materials. The root systems were then vigorously washed with running tap water and collected in a 0.5 mm sieve. Roots were cut into 2 cm sections then immersed in sterile

distilled water on a numbered grid. The root segment that traversed or was closest to a randomly selected grid point was selected. One root tip was excised from each segment, rinsed with sterile distilled water, placed in a 1.5 ml microcentrifuge tube and then frozen at $-40\,°C$ until DNA was extracted. Thirty root segments were randomly selected from each plant. Leaf tissue was also sampled to confirm that plant DNA would not amplify with the chosen primers.

DNA extraction and ITS-terminal restriction fragment length polymorphism analysis
DNA was extracted from root tips using a CTAB protocol (Gardes and Bruns, 1996) which was modified by excluding the freeze–thaw cycle and by including an additional phenol/chloroform-iso-amyl alcohol (1:1) purification step (Lee and Taylor, 1990). The pellet was re-suspended in 50 μl of TE-8 buffer. Plant tissues were extracted with the same protocol except the extra phenol/chloroform-isoa-myl alcohol purification step was not included, and pellets were re-suspended in 100 μl of TE-8. All extractions were stored at $-20\,°C$.

The primer pair ITS1 and ITS4 (White *et al.*, 1990) was used to amplify the nrDNA internal transcribed spacer region from root DNA extracts. The ITS1 forward primer was labeled with Cy5 dye and the ITS4 reverse primer was labeled with Cy5.5 dye to allow detection by an OpenGene fragment analyzer (Bayer Healthcare, Tarrytown, NY, USA). PCR cock-tails consisted of 0.2 mM dNTPs, 0.5 μM primers, 2.5 mM $MgCl_2$, 1/10th volume of $10\times$ PCR buffer and 0.72 U of Platinum Taq DNA polymerase (Invitrogen, Burlington, Canada) in a final volume of 19 μl to which was added 1 μl of a 1:10 dilution of the DNA extract. Samples which did not amplify successfully at a 1:10 dilution were redone using 1 μl of undiluted DNA extract. The following program was used for PCR on a PTC-100 thermo-cycler (MJ Research): $94\,°C$ (4 min); $48\,°C$ (1 min); $72\,°C$ (2 min); ($94\,°C$ (30 s); $48\,°C$ (30 s); $72\,°C$ (1 min 30 s) $\times$ 34 cycles); $72\,°C$ (6 min 30 s). Samples were run on a 0.7% agarose gel to confirm amplification.

Digestions using *Alu*I or *Hin*fI (Invitrogen) and 6 μl of the PCR product were done in 10 μl reactions following the manufacturer's recommendations and incubated at $37\,°C$ for at least 3 h. One microliter of the digested samples was mixed with 1.65 μl of loading dye mixture that contained formamide and two sets of internal markers at 101, 200 and 351 bp; one set labeled with Cy 5.0, the other with Cy 5.5. The samples containing the loading dye mixture were denatured at $80\,°C$ for 2 min then quenched on ice. Two microliters were then loaded onto a 6% polyacrylamide gel with the laser power set at 50%, temperature at $53\,°C$, and current at 1250 V and run for 60 min on an OpenGene System Long Tower Sequencer (Bayer International). Terminal fragments that exceeded a threshold value based upon the size

of the marker peaks were determined using Gen-eObjects 3.1 fragment analysis software (Bayer Healthcare, Tarrytown, NY, USA) for each primer–enzyme combination (that is, ITS1-*Alu*I, ITS1-*Hin*fI, ITS4-*Alu*I and ITS4-*Hin*fI).

Data analysis
Peaks were binned to identify genotypes, and then genotype frequency was tabulated for each enzyme–primer combination. Genotype density per root tip, richness and evenness were calculated separately for each of the four enzyme–primer combinations then averaged to provide an estimate of each parameter. Genotype frequency tables from each of the four enzyme–primer combinations were com-bined for use in perMANOVA, ordination and two-way cluster analyses.

Genotype density was standardized by dividing by the total number of root tips sampled per plot because the number of root tips from each plant specimen varied. Standardization assumed that genotypes were absent from samples that were unsuccessful after at least two separate PCR ampli-fications at different dilutions. A two-way analysis of variance (ANOVA) was run using Statistica, vers. 6.1 (StatSoft, Tulsa, OK, USA) to investigate the effects of site and treatment on genotype density. To determine if density was correlated with increased biomass, a two-way ANOVA was run using a measure of amplification efficiency (number of root tips amplified/number of root tips attempted).

Genotype richness was measured as the number of unique genotypes per plot. Hurlbert's probability of interspecific encounter index, as implemented in EcoSim 7.72 (Gotelli and Entsminger, 2006), was used to assess evenness. Because genotype density varied significantly between sites and treatments, genotype richness and evenness were standardized using individual-based rarefaction as implemented in the program EcoSim v. 7.72 (Gotelli and En-tsminger, 2006). Rarefaction ensured that differ-ences in genotype richness or evenness were not artifacts caused by differences in sampling intensity. Two-way ANOVA was performed for both richness and evenness.

Nonmetric multidimensional scaling using PC-ORD, version 5.03 (McCune and Mefford, 1999) was used to examine the distribution of plots in genotype space. Canonical Correspondence Analy-sis (CCA) (McCune and Mefford, 1999) was used to examine whether soil properties (that is, pH, CEC, available NH_4, available NO_3, available P, C/N ratio and exchangeable cations) explained differences in genotype frequency and composition among sites. Soil properties were transformed by log_{10} except for pH and C/N ratio. A Monte Carlo test was used to test linear relationships between the genotype frequency and soil properties. A two-way, nested ANOVA was used to test if warming affected soil properties (Table 1): (p.p.m. for P and available N),

Table 1 Mean and standard error of soil analysis variables

Site Treatment	Lowland Granitic		Upland Dolomitic		Upland Granitic	
	Ambient	OTC	Ambient	OTC	Ambient	OTC
CEC (Ba) (cmol+kg^{-1})	6.767±0.973	5.623±0.936	11.097±1.268	8.953±0.768	7.747±0.644	8.260±0.626
Exch Ca (cmol+kg^{-1})	4.115±0.651	3.419±0.643	7.587±0.841	5.841±0.523	4.052±0.378	4.170±0.207
Exch Fe (cmol+kg^{-1})	0.006±0.005	0.002±0.001	<0.001	<0.001	0.002±0.001	0.002±0.001
Exch Mg (cmol+kg^{-1})	2.298±0.288	1.926±0.274	3.408±0.440	3.008±0.290	3.441±0.367	3.892±0.426
pH (H$_2$O)	5.553±0.199	5.480±0.046	8.037±0.099	8.173±0.007	5.867±0.428	6.157±0.055
Total C%	0.947±0.309	0.817±0.162	5.627±1.007	5.883±0.963	0.870±0.475	0.543±0.082
Total N %	0.064±0.019	0.054±0.011	0.058±0.006	0.047±0.003	0.074±0.041	0.044±0.006
NO$_3^-$ N (p.p.m.)	52.443±7.806	40.550±5.034	10.203±1.760	10.363±0.214	33.557±17.146	28.637±2.844
NH$_4^+$ N (p.p.m.)	1.483±0.690	1.343±0.216	0.710±0.064	0.467±0.033	6.210±2.394	2.997±0.066
Avail P (p.p.m.)	3.767±0.442	3.757±0.436	0.483±0.242	0.003±0.003	1.977±0.502	1.723±0.243

concentration (exchangeable cations and CEC), or ratio (C/N, pH), which could explain some of the variation due to site or treatment.

PerMANOVA performs distance-based multivariate ANOVA. The implementation of perMANOVA in PC-ORD, version 5.03 (McCune and Mefford, 1999) using the Relative Sorensen Index was used to explore whether site or treatment (warming) was a statistically significant factor explaining differences in genotype frequency and composition. The Relative Sorensen Index standardizes the data by sample units, thereby shifting the emphasis from absolute frequency of genotypes to relative proportions within sample units (McCune and Mefford, 1999).

Two-way cluster analysis, as implemented in PC-ORD, version 5.03 (McCune and Mefford, 1999) using the Relative Sorensen Index and the flexible β ($\beta = -0.25$) method for linking groups, permitted examination of the distribution of the major genotypes between plots.

Genotype diversity was also investigated by examining genotype rank abundance curves, which were generated by taking the natural log of genotype abundance (measured as number of occurrences of a genotype relative to the total number of individual genotypes observed) and plotting it against the genotype abundance rank. Niche apportionment analyses were conducted using PowerNiche (Drozd and Novotny, 1999), an Excel-based macro which helps determine if rank abundance curves fit niche apportionment models based on the works of Tokeshi, Sugihara, and MacArthur (see Magurran, 2004). The selection and division exponents were varied for each test of the power model with 250 replications. Different models were tested by changing the selection ($=k$) and division exponent ($=m$), which is indicative of which niche apportionment model is chosen. The following models were tested: the broken stick model ($k=1$, $m=1$), the random fraction model ($k=0$, $m=1$), the power fraction model ($0<k<1$, $m=1$), and Sugihara's sequential breakage model ($k=0$, $m=0:25:0:75$) (Drozd and Novotny, 1999).

Results

Our experimental design utilized three sites with three randomly placed replicates of OTC and ambient control treatments within each site. For each site/treatment, we sampled approximately 30 root tips (Mean 30.69 ± 5.61 s.d.) from each of two plants. To alleviate concerns that root tips from individual plant replicates were not independent estimates of genotype density or richness (that is, root tips from the same plant were more similar), we ran preliminary two-way, nested ANOVA with plant specimen nested within replicates of the treatment (warmed plots or control). These preliminary analyses confirmed that plant replicate was not a significant factor ($P = 0.551–0.876$). We conclude that root tip samples from a single plant are no more similar than the pooled sample from both plants, which is consistent with a model of colonization where most colonization of flushing roots is via soil-borne inoculum rather than from existing inoculum on the roots. On this basis, we felt justified in eliminating plant specimen replicates from the final analyses, and therefore we did not violate the assumption of independence because only the two independent variables, site and treatment, were used in our final ANOVA model.

The main effect of 5–7 years of warming was to increase genotype density (number of different genotypes per root tip) on roots. Density increased significantly in warmed plots at all sites ($F(1, 12) = 15.003$, $P = 0.00221$). Density also varied significantly among sites, being significantly lower for the UD site compared to the other two sites ($F(2, 12) = 29.396$, $P = 0.00002$) (Figure 1). There was no significant interaction between site and treatment ($F(2, 12) = 0.73268$, $P = 0.50093$). Amplification efficiency did not vary significantly by site ($F(2, 30) = 0.13547$, $P = 0.87384$) or by treatment ($F(1, 30) = 2.7909$, $P = 0.10520$) at the 95% confidence level, but this will be addressed further in the Discussion section.

Genotype richness also varied significantly among sites ($F(2, 12) = 4.3367$, $P = 0.03825$), with the lowest
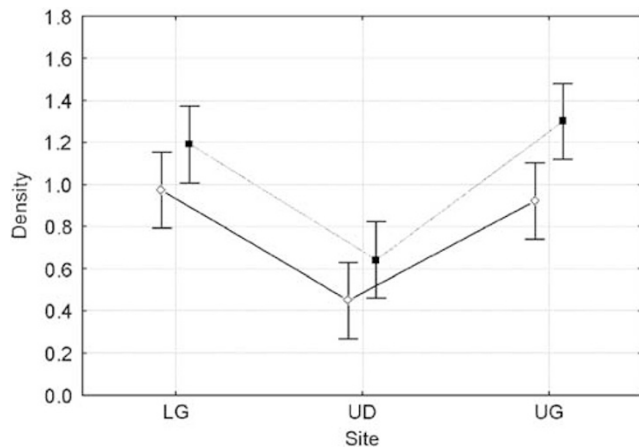
Figure 1 The effect of site and treatment on genotype density (number of genotypes per root tip). Error bars indicate 95% confidence intervals. Open diamonds indicate ambient control plots, filled squares indicate OTC warmed plots. OTC, open-top chamber.
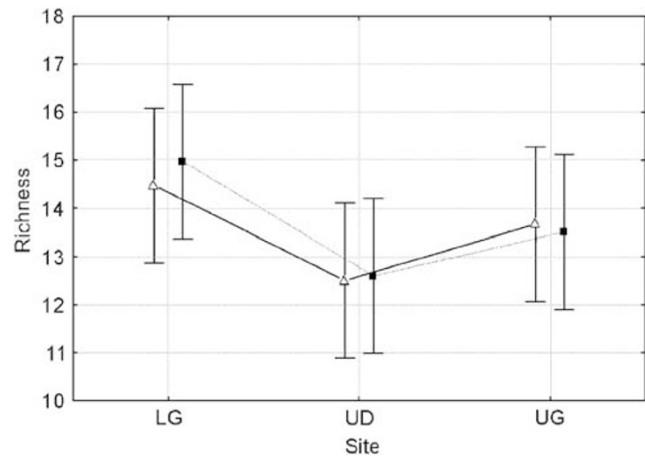
Figure 2 The effect of site and treatment on genotype richness (number of genotypes per plot). Richness was standardized by individual-based rarefaction to compensate for differences in density between samples. Error bars indicate 95% confidence intervals. Open triangles indicate ambient control plots, filled squares indicate OTC warmed plots. OTC, open-top chamber.

richness observed on the UD site and the highest on the LG site (Figure 2), but there was no effect of treatment on richness ($F_{(1, 12)} = 0.05493$, $P = 0.81865$). There was no significant interaction between site and treatment ($F_{(2, 12)} = 0.09930$, $P = 0.90621$). Evenness did not vary significantly by site or treatment.

Preliminary analysis using nonmetric multi-dimensional scaling (data not shown) of plots in genotype space showed that communities were strongly structured by site but not by treatment. Site and soil characteristics (Table 1) were the main factors differentiating communities according to the results from CCA (Figure 3). The dimensions for the CCA analysis were 18 plots and 360 genotypes for the main matrix and 18 plots and 8 variables for the environmental factors matrix. For the final CCA, Ca was excluded because of its high correlation with CEC ($r = 0.94$). Three axes were interpreted; the first axis ($r^2 = -0.694$) explained 11.3% of the variance, the second ($r^2 = -0.705$) 9.9% and the third ($r^2 = 0.121$) 7.3%. Results from the Monte Carlo test indicated that eigenvalues ($P = 0.002$) and species–environment correlations ($P = 0.01$) were significantly higher than expected by chance. The root-associated fungal communities from the LG and UG sites were more similar to each other than to the UD site, reflecting the similarity in soil properties. Both granitic sites were more strongly associated with available P, but the LG site was more strongly associated with Fe and nitrate N availability while the UG site was more strongly associated with ammonium N. The upland sites were more strongly associated with Mg availability. The UD site was strongly associated with pH, C/N ratio and CEC, as would be expected on a site with dolomite as parent material (Figure 3). Results from the two-way nested ANOVA on the different soil properties confirmed that soil properties differed significantly among sites, but did not change due to warming.

PerMANOVA analysis of community composition based upon the Relative Sorensen index supported the CCA results. Genotype composition differed significantly among sites ($F_{(2, 12)} = 5.0301$, $P = 0.0002$), but not treatments ($F_{(1, 12)} = 0.94759$, $P = 0.4644$).

Two-way cluster analysis (Figure 4) produced much the same groupings as the CCA analysis, with sites clustering together but treatments not consistently grouped within site clusters. With regard to the distribution of genotypes among the sites, cluster 268h55 to 286h5 contained genotypes that were widely distributed among all sites; cluster 341h55 to 173a55 genotypes were also widely distributed, but had a greater frequency in the upland sites; cluster 265h55 to 115a5 represents genotypes that are largely restricted to LG and UG sites but with higher frequency on the UG site; cluster 292h5 to 82h5 represents genotypes that predominate in the LG site, although they are found to a lesser extent in the UG site; cluster 274h5 to 331h5 contains genotypes that are more strongly represented in the UG samples, but also occur sporadically in other samples. Members of cluster 215h55 to 171a5 are sporadically distributed among plots without a clear pattern. When comparing the genotype distribution between control and warmed plots, most of the dominant genotypes within each site were present in both treatments. Some genotypes may be responding to warming, but it was difficult to detect consistent genotype responses, which is consistent with the results of the statistical analyses.

Niche apportionment analyses indicated that the root-associated fungal communities we studied exhibited a log normal distribution. Analyses with
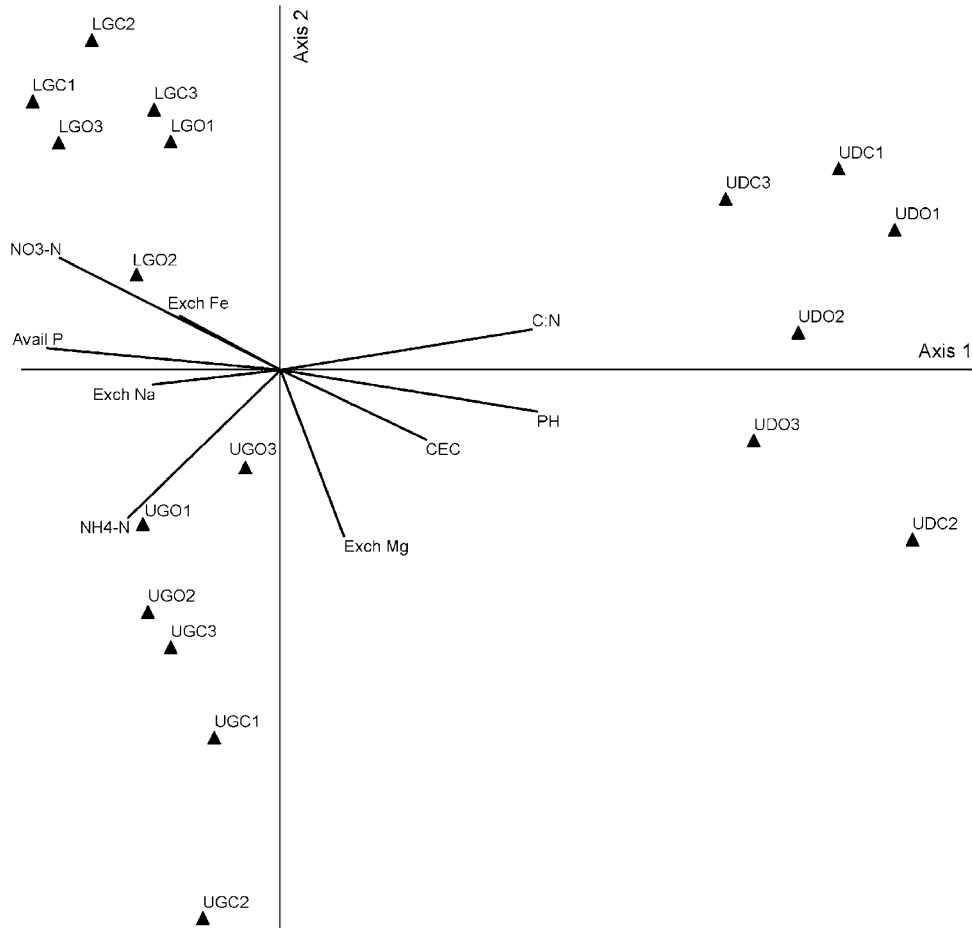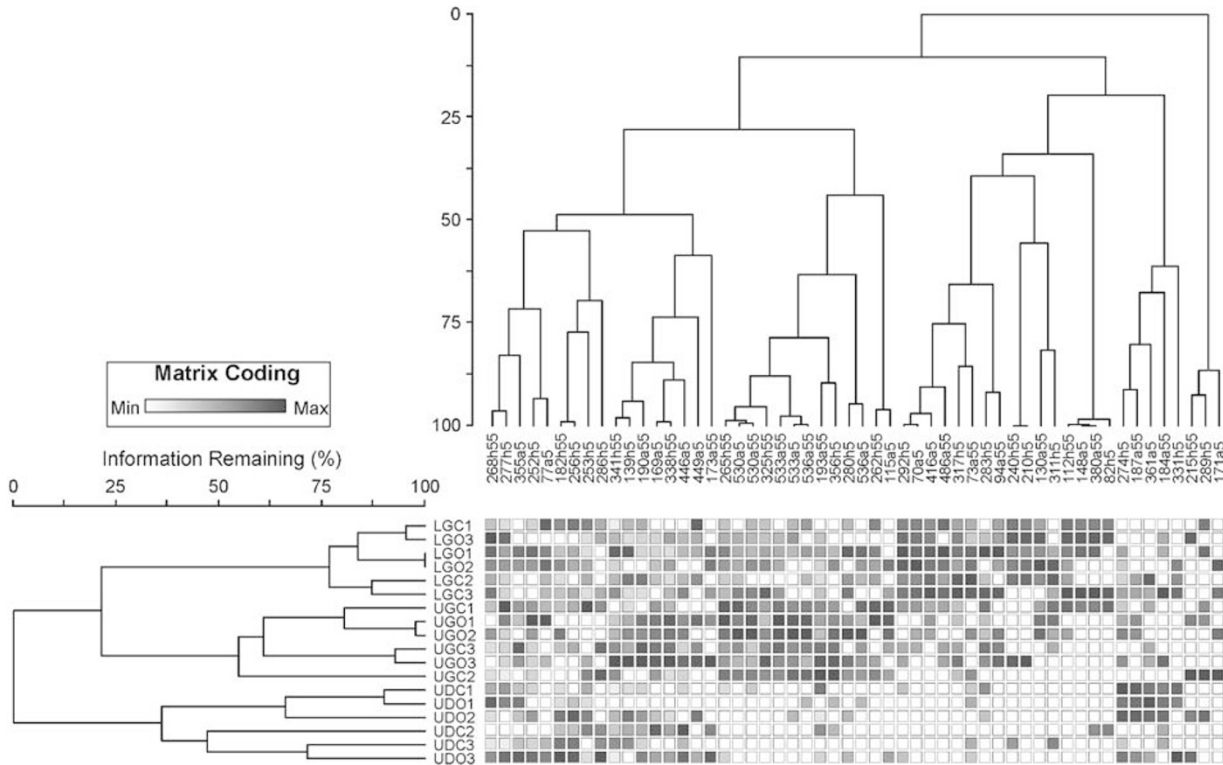
Figure 3 CCA biplot of warming treatments and site with soil properties. Coding is as follows: the first two characters represent site, LG, UD, UG; the third character signifies the treatment: C (ambient control) or O (OTC warmed); and the fourth character indicates the replicate number. Soil properties are plotted as vectors radiating from the center, and were used to assess the relationship between genotype and soil properties. CCA, Canonical Correspondence Analysis; LG, Lowland Granitic; OTC, open-top chamber; UD, Upland Dolomitic; UG, Upland Granitic.

PowerNiche based upon Tokeshi's niche apportionment models indicated that relative abundance curves of all four primer–enzyme combinations fit the power fraction model for the UG site, three of the four primer–enzyme combinations for the LG site also fit the power fraction model (the ITS4-*Alu*I abundance curve fit the broken stick model), and all four primer–enzyme combinations fit the broken stick model for the UD site.

Discussion

Two hypotheses could explain the observed increase in genotype density: (1) there was a general increase in fungal biomass in roots resulting in higher amplification efficiency and hence increased likelihood of detecting genotypes or (2) that we are observing the initial stages of a replacement process and are detecting both indigenous and colonizing fungi on roots. Since we did not directly measure biomass, we analyzed amplification efficiency with the expectation that a general increase in fungal

biomass on root tips would result in higher efficiency of amplification, whereas replacement would not. Although differences in amplification efficiency between ambient and warmed plots were not significant at the standardly accepted 95% confidence level, there was an increase in amplification efficiency with warming at all sites and the probability ($P = 0.10520$) was sufficiently high that hypothesis 1 remains the most parsimonious explanation for the increase in density; this hypothesis will be tested in future studies. This hypothesis is consistent with the lack of significant change in genotype richness or evenness, and examination of genotype distributions, which suggested that the increased density of genotypes on individual roots in warmed plots was due to colonization by existing genotypes rather than an influx of novel genotypes. Chapin *et al.* (1995) found significant increases in plant biomass after 9 years of experimental warming in the low arctic, and recent evidence indicates that large increases in belowground biomass are accompanying these changes (Hill, 2006). Previous studies have shown mycorrhizal biomass to increase

Figure 4 Two-way cluster analysis of fungal genotypes. Labels in the horizontal dendrogram are coded as follows: the first two characters represent site, LG, UD, UG; the third character signifies the treatment: C (ambient control) or O (OTC warmed); and the fourth character indicates the replicate number. Labels in the vertical dendrogram indicate genotype fragment size (first 2–3 digits), restriction endonuclease (h = *Hin*fI; a = *Alu*I), and Cy dye (Cy5 = forward primer; Cy55 = reverse primer). LG, lowland granitic; OTC, open-top chamber; UD, Upland Dolomitic; UG, Upland Granitic.

in response to warming (Clemmensen *et al.*, 2006); the increase in genotype density observed in our study is consistent with these observations.

The lack of a change in richness or evenness suggests that the entire root-associated fungal community benefited from the warmer temperatures and increased carbon allocation belowground. This finding is contrary to a previous study examining the effects of warming on ectomycorrhizal fungal communities of Douglas-fir seedlings. Rygiewicz *et al.* (2000) found an increase in the number of different mycorrhizal fungi on seedlings grown in controlled environment chambers with elevated temperatures compared to those grown in chambers with ambient temperatures. Differences in results between our study could be due to the following: (1) differences in how the hosts react to warming; (2) the age of the plant, as seedlings were used for their study while we sampled mature shrubs and (3) greater availability of fungal inoculum, which may be more widely dispersed at lower latitudes, providing greater opportunity for migration of new genotypes. We also did not find an initial decrease in diversity due to warming as some studies have found for plant diversity (Chapin *et al.*, 1995; Hollister *et al.*, 2005; Walker *et al.*, 2006), although Jónsdóttir *et al.* (2005) noted that there was no change in species richness of plant communities in

Iceland and suggest that initial conditions may dictate responses to warming.

Site and soil characteristics are important determinants of bacterial community structure at small scales (for example, Ritz *et al.*, 2004), but we expected root-associated fungal communities to be more strongly buffered by host interactions. In this study, we found that genotypes on a single host plant species in this restricted geographical area were strongly associated with specific sites along altitudinal and soil physicochemical gradients. Reasons for the low richness and density at the dolomitic site include a high C/N ratio, which is not conducive to the breakdown of organic matter, and low levels of NH_4, NO_3 and P, suggesting that both nitrogen and phosphorus may be limiting factors at the UD site. Although not statistically significant, P levels dropped to negligible levels in the warmed plots on the dolomitic site. The lack of available P could explain the paucity of plants and mycorrhizal fungi present at this site. However, some ectomycorrhizal fungi can solubilize calcium phosphate (Martin *et al.*, 2000); fungi that can fulfill this role may have a competitive advantage, which could be a factor in dictating the community composition of this site. The UG and LG sites were more similar in community composition, although the more xeric and harsh UG site had lower genotype richness.

Relative abundance distributions of species in Arctic plant communities have been reported to conform to a geometric series model (McKane et al., 2002), which is commonly associated with species-poor, harsh environments or species found in early succession (Magurran, 2004); therefore, we hypothesized that we would find similar distribution patterns indicating a species-poor, root-associated fungal community. This was not the case, as shown by genotype richness and niche apportionment models. Our arctic system had 25–35 genotypes for the two granitic sites (LG and UG), which is comparable to other studies using terminal restriction fragment length polymorphism analysis in temperate forests, where 19–35 genotypes were found in an oak shrub community (Klamer et al., 2002) and 26 genotypes in a 60-year-old pine plantation (Dickie et al., 2002). Our root-associated fungal communities exhibited a log-normal distribution, which is usually associated with more diverse ecosystems such as temperate and tropical forests (McKane et al., 2002), and typical of ectomycorrhizal fungal communities on temperate trees with more extensive root systems (Horton and Bruns, 2001). Analyses with PowerNiche based upon Tokeshi's niche apportionment models indicated that relative abundance curves for the LG and UG sites more closely fit the power fraction model while the broken stick model fit better for the UD site. In the power fraction model, abundant species are more likely to successfully invade or inhabit niche space (Tokeshi, 1999) or in this case colonize a segment of root. Once a root tip is occupied, other fungi may be challenged to outcompete the existing fungus. The genotypes that increased in density due to warming were generally dominant genotypes, which is consistent with the power fraction model. In the broken stick model, resources are evenly divided among species (Magurran, 2004). This model may have been better applied to the UD site because both the plant and fungal diversity is limited and dominated by fungi that can tolerate the low nutrient status and environmental extremes of this site. Those fungi that tolerate these constraints appear to allocate the limited resources more equitably.

Our findings suggest that the root-associated fungal community at these tundra sites were quite resilient to warming, as evidenced by the lack of change in genotype richness or evenness (Kårén and Nylund, 1997; Rygiewicz et al., 2000). Over longer time periods, it would be interesting to know if sites with higher richness, such as the LG and UG sites, are more resilient than the UD site. Also, it would be valuable to extend these results using DNA sequencing or genomic approaches, as genotype richness may have been partially masked by the lower taxonomic resolution of the terminal restriction fragment length polymorphism technique. How will global warming affect the root-associated fungal community on S. arctica? Commensurate with an increase in carbon allocation belowground, we predict an increase in colonization by root-associated fungi. Significant changes in community composition are likely to be slower, and to be highly localized due to the strong effect of site and environment upon community structure. However, we only examined a single host. If the hypothesis that plant diversity will increase with long-term warming is correct (Chapin et al., 1995; Arft et al., 1999; Walker et al., 2006), then this may alter the root-associated fungal community by providing new hosts and increase the potential for facilitation of multi-host fungi.

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114

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