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ORIGINAL ARTICLE

Shifts in soil microorganisms in response to warming are consistent across a range of Antarctic environments

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Because of severe abiotic limitations, Antarctic soils represent simplified systems, where microorganisms are the principal drivers of nutrient cycling. This relative simplicity makes these ecosystems particularly vulnerable to perturbations, like global warming, and the Antarctic Peninsula is among the most rapidly warming regions on the planet. However, the consequences of the ongoing warming of Antarctica on microorganisms and the processes they mediate are unknown. Here, using 16S rRNA gene pyrosequencing and qPCR, we report highly consistent responses in microbial communities across disparate sub-Antarctic and Antarctic environments in response to 3 years of experimental field warming (+0.5 to 2 °C). Specifically, we found significant increases in the abundance of fungi and bacteria and in the *Alphaproteobacteria*-to-*Acidobacteria* ratio, which could result in an increase in soil respiration. Furthermore, shifts toward generalist bacterial communities following warming weakened the linkage between the bacterial taxonomic and functional richness. GeoChip microarray analyses also revealed significant warming effects on functional communities, specifically in the N-cycling microorganisms. Our results demonstrate that soil microorganisms across a range of sub-Antarctic and Antarctic environments can respond consistently and rapidly to increasing temperatures.

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Introduction

The Antarctic Peninsula is among the most rapidly warming regions on the planet (Steig *et al.*, 2009). However, Antarctica is still the coldest, windiest, driest and most isolated continent on Earth. Consequently, Antarctic terrestrial food webs are relatively simple, being characterized by the general absence of insect and mammalian herbivores. In the relative absence of grazing, most energy and biomass is channeled into a detritus trophic pathway (Davis, 1981), which is dominated by microbial activities. Thus, soil microorganisms have a disproportionate importance in nutrient cycling and other ecosystem processes in ice-free terrestrial ecosystems in the Antarctic. In spite of the importance of microorganisms in Antarctic soils and the rapid warming of the Antarctic Peninsula, very little is known about how these microbial communities respond to global warming.

Some insight has been gained by comparing microbial communities across an Antarctic latitudinal gradient, as a proxy for long-term climatic trends (Yergeau *et al.*, 2007a–c, 2009), or via short-term laboratory incubations (Yergeau and Kowalchuk, 2008). The few field studies reported to date have shown modest effects of warming on soil organic matter decomposition, as well as other soil processes and bacterial temperature sensitivity (Treonis *et al.*, 2002; Bokhorst *et al.*, 2007a; Rinnan *et al.*, 2009). Based on this limited evidence, it was hypothesized, that the responses of Antarctic soils will be overridden by nutrient or water limitations

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(Wynn-Williams, 1996; Treonis *et al.*, 2002; Wasley *et al.*, 2006), similar to what is observed in Arctic soils (Ruess *et al.*, 1999; Rinnan *et al.*, 2007). However, rapid responses of mosses, nematodes, soil algae and cyanobacteria have been observed in some Antarctic soils (Kennedy, 1996; Wynn-Williams, 1996; Convey and Wynn-Williams, 2002). Currently, there is a clear gap in knowledge related to the underlying responses of key microbial parameters, like diversity, community composition, abundance and C- and N-cycle functions, to global warming. Given the central roles of microorganisms in Antarctic soil nutrient cycling, this knowledge is crucial to predicting the trajectory of Antarctic terrestrial environments following global warming.

Our objective was, therefore, to examine the impact of short-term, environmentally relevant field warming on sub-Antarctic and Antarctic soil microbial community composition, diversity, abundance and functions. We conducted *in situ* warming experiments for 3 years using open-top chambers (OTCs) at one sub-Antarctic (Falkland Islands, 52°S) and two Antarctic locations (Signy and Anchorage Islands, 60°S and 67°S, respectively) (see Supplementary Figure S1 for a map). At each location, we compared OTC plots with adjacent control plots in both densely vegetated and bare fell-field environments for a total of 12 treatments. Pyrosequencing (454 GS FLX Titanium, Roche Diagnostics, Laval, QC, Canada) of 16S rRNA gene amplicons was used to follow bacterial diversity and community composition, and functional gene microarrays (GeoChip 2.0; He *et al.*, 2007) were used to assess changes in functional gene distribution. Although the sampled soil environments were highly disparate, experimental warming through OTC manipulation induced a number of consistent changes in microbial communities across all the sampling sites, including decreases in the relative abundance of *Acidobacteria*, increases in the relative abundance of *Alphaproteobacteria* and weakened correlation between taxonomical and functional richness.

Materials and methods

Site descriptions

One sub-Antarctic site and two Antarctic sites were chosen at the following locations: Falklands Islands (sub-Antarctic zone; 51°76′S 59°03′W), Signy Island (South Orkney Islands, maritime Antarctic; 60°43′S, 45°38′W) and Anchorage Island (near Rothera Research station, western Antarctic Peninsula; 67°34′S, 68°08′W). At each location, two types of environments were selected: (1) 'vegetated', where dense vegetation cover was present with retention of underlying soil and (2) 'fell-field', represented as rocky or gravel terrain with scarce vegetation or cryptogam coverage, for a total of six contrasting environments. Densely vegetated and bare environments represent two contrasting environments for Antarctic soil microorganisms, with large differences in C and N inputs to soils (Yergeau *et al.*, 2007a). The dominant species in these plots at the start of the experiment were as follows: Falkland vegetated: *Empetrum rubrum* (vascular plant, mean cover 68%); Falkland fell-field: *Poa annua* (vascular plant, 26%); Signy-vegetated: *Chorisodontium aciphyllum* (moss, 76%); Signy fell-field: *Usnea antarctica* (lichen, 53%); Anchorage vegetated: *Sanionia uncinata* (moss, 48%); Anchorage fell-field: *Buellia latemarginata* (lichen, 30%) (Bokhorst *et al.*, 2007b). No significant changes in the vegetation composition and diversity of all sites were observed after 2 years of warming (Bokhorst *et al.*, 2007b). Vegetation composition and diversity were not measured after 3 years of warming. Table 1 summarizes soil characteristics as measured in the experimental plots. Additional soil characteristics are described by Bokhorst *et al.* (2007a) and Yergeau *et al.* (2007a).

Experimental design

The experiment was established during the 2003–2004 austral summer. The design consisted of 12 paired 2×2 m plots per environment. Half the plots were covered with standard-type OTC, warming underlying plants and soils passively (see Marion *et al.*, 1997 for further details), while the other half served as control plots. The Falkland Islands fell-field habitat was not sufficiently extensive at the study site to allow for such a design, and only 6 plots (3 OTCs and 3 controls) were delineated in this environment. The remaining OTCs and controls were placed in the vegetated environment, resulting into 18 Falkland Islands vegetated plots (9 OTCs and 9 controls). Soil moisture and temperature were monitored hourly, along with wind speed and direction, photosynthetically active radiation and precipitation. OTCs at our sampling sites passively increased annual soil temperature by $\sim$0.8 °C at a soil depth of 5 cm (Bokhorst *et al.*, 2007a). This increase was, however, not constant throughout the year, and varied between the different locations sampled (Bokhorst *et al.*, 2007a). Warming in Antarctica is expected to not only increase average temperatures, but also reduce the frequency of freeze-thaw events, and our OTC treatments mimicked this scenario for the Antarctic environments studied (Table 1). Detailed trends in temperature and other environmental factors modified by the OTCs are provided by Bokhorst *et al.* (2007a). It must be noted that OTCs can affect numerous environmental variables besides temperature including wind speed, snow cover and relative humidity, although previous studies have estimated that these unwanted effects are generally small (Marion *et al.*, 1997; Hollister and Webber, 2000). Among the unwanted effects that could have affected our results more strongly are prolonged snow accumulation in spring, delay in soil thaw in spring and occurrence of extreme temperatures in summer (Bokhorst *et al.*, 2011).

Table 1 Mean summer soil temperature (at a 5-cm depth), soil freeze-thaw cycles (FTCs), soil moisture, extractable soil nitrogen content and soil respiration for control and OTC plots at the Falkland, Signy and Anchorage Islands on vegetated and fell-field sites

	Summer mean soil T (°C)	*FTC (year^{-1})*	*Soil moisture (%)*	*NH_4^+ (mg kg^{-1})*	*NO_3^- (mg kg^{-1})*	*% N*	*Resp. (mg CO_2 m^{-2} h^{-1})*
Falkland Islands							
Vegetated							
Control	10.7 (0.3)	0.3 (0.6)	0.27 (0.05)	24.5 (13.5)	0.8 (0.6)	0.90 (0.07)	216.8 (26.3)
OTC	10.7 (0.5)	1.7 (2.1)	0.20 (0.09)	21.6 (8.5)	1.0 (0.6)	0.96 (0.06)	288.5 (68.0)
Fell-field							
Control	11.0 (0.3)	12.3 (14.6)	NA	15.7 (9.9)	23.5 (6.8)	0.92 (0.19)	NA
OTC	11.7 (0.1)	13.7 (22.0)	NA	19.3 (12.6)	48.4 (49.0)	0.93 (0.31)	NA
Signy Island							
Vegetated							
Control	1.9 (0.3)	150.0 (12.3)	0.17 (0.04)	47.6 (18.0)	4.2 (0.6)	1.94 (0.47)	11.2 (5.3)
OTC	2.4 (0.2)	127.0 (17.6)	0.17 (0.03)	44.8 (13.0)	3.0 (0.6)	1.96 (0.38)	14.7 (4.8)
Fell-field							
Control	2.0 (0.2)	129.7 (18.8)	0.07 (0.03)	NA	NA	NA	NA
OTC	2.7 (0.2)	104.0 (21.3)	0.06 (0.03)	NA	NA	NA	NA
Anchorage Island							
Vegetated							
Control	0.8 (1.5)	67.3 (30.0)	0.25 (0.02)	146.9 (150.3)	7.9 (4.3)	3.30 (0.34)	5.0 (1.9)
OTC	2.3 (0.8)	51.7 (14.0)	0.26 (0.01)	89.2 (13.4)	35.6 (34.1)	3.25 (0.22)	5.5 (2.1)
Fell-field							
Control	2.8 (0.1)	76.0 (8.2)	NA	NA	NA	NA	NA
OTC	3.4 (0.1)	47.0 (9.2)	NA	NA	NA	NA	NA

Abbreviations: NA, not available; OTC, open-top chamber.
Values in parenthesis represent s.d.

Soil sampling and analysis
Soil sampling for molecular analyses was carried out in October 2006 at the Falkland Islands, in January 2007 at Signy Island and in February 2007 at Anchorage Island. So, at the time of sampling experimental warming was in place for 3 years. At all sites, five 1-cm-diameter (from 2 to 3 cm to up to 15 cm deep, depending on soil depth) cores were sampled from each plot. One OTC in Signy Island fell-field environment was destroyed by the wind during the winter of 2006 and this OTC–control pair was therefore not sampled. Soil samples were frozen at −20 °C within 24 h and kept at this temperature until used in molecular analyses. Soil sampling for soil analyses was carried out in October 2005 at the Falkland Islands, in January 2006 at Signy Island and in February 2006 at Anchorage Island. To this end, 10-cm-diameter cores were sampled from each plot and kept at -20 °C until use. Soil physical and chemical analyses were carried out using standard methods (Carter, 1993). Soil respiration was measured directly in the field in October 2005 at the Falkland Islands, in January 2006 at Signy Island and in February 2006 at Anchorage Island, as previously described (Bokhorst *et al.*, 2007a).

Nucleic acid extractions, real-time PCR and PCR-DGGE
Nucleic acids were extracted from 500 mg soil subsamples following bead-beating disruption in a CTAB buffer and subsequent phenol-chloroform purification (Yergeau *et al.*, 2007a). After extraction, nucleic acids extracted from the five within-plot replicates were pooled to provide a single representative DNA sample. Real-time PCR was performed using ABsolute QPCR SYBR green mixes (AbGene, Epsom, UK) on a Rotor-Gene 3000 (Corbett Research, Sydney, Australia) with the primers and amplification conditions detailed in Supplementary Table S1. The general PCR protocol consisted of a single denaturation step (15 min at 95 °C), followed by 40 cycles of denaturation (60 s at 95 °C), annealing (60 s at annealing temperature) and synthesis (60 s at 72 °C). Fluorescence was read at the end of the synthesis step or after an additional 15 s reading step (to remove the contribution of primer dimers to fluorescence). For fungal PCR-denaturing gradient gel electophoresis (DGGE), DNA was amplified by PCR using fungal 18S rRNA gene-specific primers FR1-gc/FF390 (Vainio and Hantula, 2000) and subjected to DGGE in a 40–55% denaturant gradient on a D-Code Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA), as detailed previously (Yergeau *et al.*, 2007a).

16S rRNA gene pyrosequencing
Soil DNA samples were PCR amplified using the adapter–multiplex identifier–primer combinations detailed in Supplementary Table S2. Each primer combination was used on four different soil DNA extracts. The sequence-specific 16S primers were UnivBactF 9 and BSR534/18, amplifying positions 9–534 (V1–V4) of the 16S rRNA gene of *E. coli*. PCR conditions consisted of one denaturation cycle (5 min at 95 °C), 30 cycles of denaturation (30 s at

95 °C), annealing (30 s at 55 °C) and elongation (45 s at 72 °C) and one elongation cycle (10 min at 72 °C). The amplicons were gel purified using the QIAquick Gel Extraction kit (Qiagen, Mississauga, ON, Canada), quantified using the Quant-iT PicoGreen dsDNA assay kit (Invitrogen, Burlington, ON, Canada), diluted and pooled. Pooled amplicons were sequenced using the 454 GS FLX Titanium platform (Roche Diagnostics) at the Center for Applied Genomics, The Hospital for Sick Children, Toronto, ON, Canada. In each of the four regions of the sequencing plate, 18 barcoded amplicon samples were loaded (except one region with 16 samples). Due to technical issues at the sequencing center, sequencing yielded a relatively low yield of sequences (163 542 raw reads for one sequencing full plate). Sequence data were analyzed using mostly the RDP pyrosequencing pipeline (http://pyro.cme.msu.edu/). Sequences from each of the four regions were separated based on the adapter (A or B) using a custom Perl script. The sequences were then deconvoluted and binned according to their multiplex identifier (only accepting perfect matches), and the multiplex identifier and the forward primer were trimmed using the 'Pipeline Initial Process' tool. This resulted in 69 distinct data sets (one sample failed to sequence). Using the 'Pipeline Initial Process' tool, we also removed all sequences that contained undetermined bases (*N*), had an average expected quality score lower than 20 or were shorter than 150 bp. The data sets were pooled together and submitted to the RDP Classifier tool, where sequences matching plant plastids (at a bootstrap cutoff of 50%) were identified. The number of plastid sequences per individual data set ranged from 0 to 347, and these sequences were removed before subsequent analyses. The resulting data sets had between 347 and 3775 sequences, with an average of 1497 sequences (standard deviation: 641) having an average length of 385 bp. Part of the variation in sequence numbers was due to the removal of the sequences determined to be of plastid origin. The sequences of each data set were individually classified using the RDP Classifier tool with an 80% bootstrap cutoff. Sequences were also mapped against the GreenGenes core data set using Blast and used in FastUnifrac to calculate UniFrac distances between each pair of samples (Hamady *et al.*, 2009). The AmpliconNoise package (v 1.21) was used for diversity calculations (Quince *et al.*, 2011). In the process, data sets were normalized to 765 sequences (the number of sequences in the smallest data set, with the exception of four samples that had a very low number of sequences and were discarded for this analysis).

GeoChip microarray hybridization

Fifty nanograms of extracted soil DNA was amplified using whole-genome amplification as described previously (Wu *et al.*, 2006). Several samples (24 out of 72) failed to amplify and were not processed any further. The amplified DNA was fluorescently labeled with Cy5 dye (GE Healthcare, Piscataway, NJ, USA) by using random primers and the large (Klenow) fragment of DNA polymerase I (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The microarray hybridizations were carried out for 4 h at 50 °C in the presence of 50% formamide, as previously described (Yergeau *et al.*, 2007b). The hybridized microarray slides were scanned using a ScanArray 500 (Perkin-Elmer, Boston, MA, USA). Scanned images were processed in ImaGene, version 5.0 (Biodiscovery, El Segundo, CA, USA). Raw data from ImaGene were preprocessed as previously described (He *et al.*, 2010), and GeoChip normalized intensity was summed for each of the gene categories present on the array (138 categories), of which 12 were used in further analyses (C- and N-cycle genes).

Statistical analyses

All statistical analyses were carried out in R (v.2.9.0, The R Foundation for Statistical Computing, Vienna, Austria). Our experimental design was paired, consisting of OTC plots with immediately adjacent control plots. We, therefore, carried out a non-parametric paired statistical test (Wilcoxon's sign-rank test for paired values) that compared each OTC plot with the directly adjacent control plot as a global test of the difference between OTC and control plots. Significance of the test statistic indicates that for a majority of the paired plots, the difference between the OTC and the control is in the same direction. This type of experimental design and statistical testing takes into account the high heterogeneity of the sampling sites by only comparing adjacent plots. Most of the data are presented as average with the associated s.d., but based on this type of test, large s.d. does not necessarily mean that differences are not significant. Within-plot heterogeneity was previously shown to be relatively minor (Yergeau *et al.*, 2007a). Correlations between different data were calculated using Pearson's correlation coefficient (*r*). Ordinations were carried out using principal coordinate analysis based on Unifrac distances (bacterial 16S rRNA gene), Jaccard similarity (fungal PCR-DGGE) or Bray-curtis distances (GeoChip microarray). PerMANOVA (Anderson, 2001) was carried out on Unifrac distances (bacterial 16S rRNA gene), Jaccard similarity (fungal PCR-DGGE) or Bray-curtis distances (GeoChip microarray and bacterial phylum class and genus relative abundance) and a full factorial design (location, OTC and vegetation crossed). When large numbers of similar variables were tested for significance, levels of significance were corrected for multiple comparisons using the method of Dunn-Sidak (Sokal and Rohlf, 1995). The corrected significance levels (to achieve a family-wise error rate of 0.05) were 0.00730 (soil factors), 0.0102 (bacterial phyla),

0.000245 (bacterial genera) and 0.00427 (GeoChip gene categories).

Data deposition
DNA sequences produced in the current study were deposited in GenBank under accession numbers HM641909–HM744649. Microarray data discussed here have been deposited in NCBI GEO and are accessible through GEO series accession number GSE22825 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE22825).

Results

Although the study sites examined varied both in their environmental conditions and their responses to *in situ* warming, we were most interested in examining whether general responses to warming could be identified. To this end, we examined microbial community responses to warming via a range of approaches and sought to discern site-specific vs general community responses to warming.

Environmental parameters
The experimental sites were spread across >1900 km and had very distinct vegetation covers. This resulted in large differences in environmental characteristics across the study range (Table 1). Average summertime soil temperature was significantly higher in OTC plots (+0.70 °C on average; $P<0.001$), while the number of annual soil freeze-thaw cycles was not significantly different at the corrected significance level ($P=0.024$) (Table 1). Average soil moisture was not significantly different between OTC and control plots (Table 1). Most of the environmental variables co-varied, with annual number of soil freeze-thaw cycle being significantly and negatively correlated with average summertime soil temperature ($r=-0.777$, $P<0.001$) and to average soil moisture ($r=-0.589$, $P=0.0039$). Soil extractable ammonium and nitrate and total nitrogen contents were not significantly different between OTC and control plots, even though there was a general increase in total soil nitrogen and nitrate (Table 1). Similarly, soil total carbon, C:N ratio, potassium, phosphate and pH were not significantly different. Soil respiration was significantly higher in OTC plots (+35.3 mg CO_2 m^{-2} h^{-1} or +32.6 % of control on average, $P=0.0024$; Table 1).

Soil microbial community structure, composition and abundance
No consistent differences between OTC and control plots were observed in the general soil community structure as the ordination of bacterial and fungal community structure data revealed that the main structuring factors were sampling site and vegetation type (Figures 1a and b). This visual interpretation was confirmed by PermANOVA tests that only showed significance for location, vegetation and their interaction (Table 2).

In contrast, even though the effect of vegetation and location was stronger, PerMANOVA using phylum/class level information for all phyla (and *Proteobacteria* classes) revealed significant differences between OTC and control plots (Table 2). We then tested individually the five most abundant phyla/classes (*Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria*, *Ktedonobacteria* and *Verrucomicrobia*) for significant differences between OTC and control plots across all the different environments. The relative abundance of *Acidobacteria* was significantly lower in OTC plots as compared with control plots ($P<0.001$; Figure 2a; Supplementary Table S3). In contrast, the relative abundance of *Alphaproteobacteria* was higher in OTC plots as compared with control plots ($P=0.027$; Figure 2a; Supplementary Table S3), even though this was not significant at the Dunn-Sidak corrected significance levels of 0.0102 (correction for multiple comparisons, see Materials and methods).

Bacterial abundance was significantly higher in OTC plots as compared with control plots, but only in plots with dense vegetation cover ($P=0.046$; Figure 2b). Fungal abundance was significantly higher in OTC plots as compared with control plots, across all environments ($P=0.010$; Figure 2c). Archaea only formed a minor part of the soil community as measured by qPCR (0.0001–0.01% of bacterial abundance and 0.01–1% of fungal abundance) and were not significantly different between OTC and control plots.

Bacterial taxonomic richness (number of operational taxonomic units at 97% similarity level), diversity and evenness based on 454 sequencing were not significantly different between OTC and control plots (not shown).

Soil microbial functions
Bacterial taxonomic richness (number of operational taxonomic units at 97% similarity level) was significantly correlated ($r=0.4886$, $P=0.0180$) with functional richness (number of genes detected on the GeoChip) in control plots, but not in OTC plots ($r=0.3214$, $P=0.1554$; Figure 3). The number of functional genes detected on the GeoChip was significantly lower in the OTC plots as compared with the controls (average for control: 213.3 and for OTC: 68.4, $P=0.0039$). In fact, for a range of genes families having key functions in the N and C cycles (*amoA*, cellulase, chitinase, laccase, *nifH*, *nirK*, *nirS*, *nosZ*, *pmoA* and urease) the number of variants detected on the GeoChip was generally lower in OTC plots as compared with control plots (Supplementary Table S4). This large difference between OTC and control plots was also reflected in the ordination of the general functional community structure, where OTC plots grouped mostly together

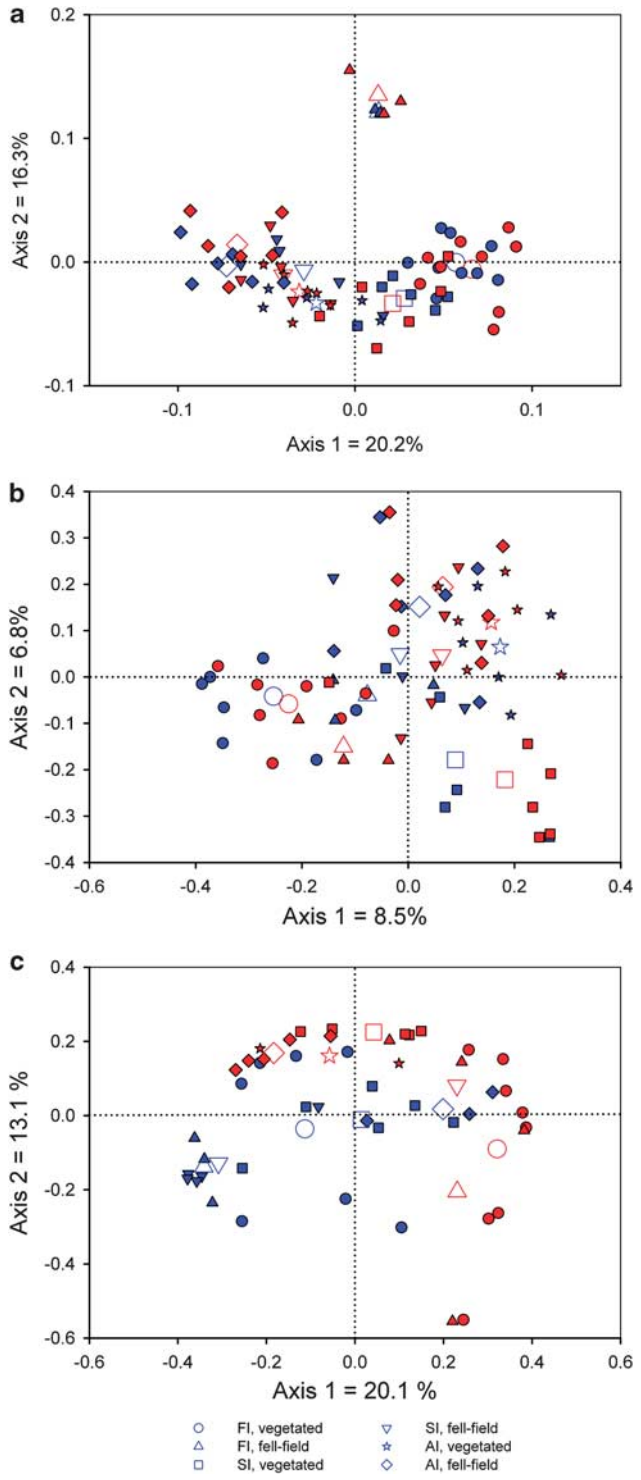

Figure 1 Principal coordinate analysis based on (**a**) Unifrac distance calculated from bacterial 16S rRNA gene sequences, (**b**) Jaccard similarity calculated from DGGE patterns obtained from PCR-amplified fungal 18S rRNA genes or (**c**) Bray-Curtis distance calculated from GeoChip microarray hybridization patterns for soils from Falkland, Signy and Anchorage Islands. The positions of the larger empty symbols are the mean position for the replicates. Red symbols: OTC plots; blue symbols: control plots.

(Figure 1c) and in the associated PerMANOVA tests that showed a significant effect of the OTC treatment (Table 2).

Similarly, PerMANOVA revealed a significant effect of OTC on GeoChip data at the gene category level (relative abundance of all variants of a gene summed together; Table 2). The relative abundance of urease genes detected on the GeoChip was significantly lower in OTC plots ($P=0.0034$). In contrast, the relative abundance of nitrogenase genes (*nifH*) measured on the GeoChip was higher in OTC plots ($P=0.027$) even though this was not significant at the Dunn-Sidak corrected significance level of 0.00427 (correction for multiple comparisons, see Materials and methods). The abundance of the bacterial ammonia monooxygenase (*amoA*) genes as measured by qPCR and the GeoChip microarray was not significantly different between control and OTC plots. No significant trend was observed for the archaeal version of the *amoA* gene in qPCR assays (Supplementary Table S5). Furthermore, no significant differences between OTC and control plots were observed for the abundance of bacterial chitinase (*chiA*), nitrite reductases (*nirK* and *nirS*), particulate methane monooxygenase (*pmoA*) and archaeal methyl-coenzyme A reductase (*mcrA*) in qPCR assays (Supplementary Table S5).

Discussion

Consistent and rapid microbial shifts across sites

The most striking feature of our study is the consistency of the responses of several microbial parameters across the disparate experimental sites. These sites were spread across >1900 km, spanned two climatic regions and had very distinct vegetation covers. This resulted in large differences in biotic and abiotic characteristics across the study range. For instance, Anchorage Island had a simpler trophic structure as compared with the other locations, with almost no fungivorous or omnivorous nematodes (S Bokhorst, unpublished results). The Anchorage Island soils also had very high levels of inorganic nitrogen in the form of ammonia and nitrate due to the proximity of a bird colony. Even with these large differences, key soil microbiological parameters showed consistent responses to warming, which were also apparent at the functional level. Furthermore, our results demonstrate that sub-Antarctic and Antarctic soil microorganisms can respond rapidly (<3 years) to warming. This is not surprising, since similar swift responses of mosses, nematodes, soil algae and cyanobacteria were previously observed in Antarctic soil warming experiments (Kennedy, 1996; Wynn-Williams, 1996; Convey and Wynn-Williams, 2002). In contrast, the responses of some dry mineral Antarctic soils to warming were reported to be overridden by nutrient or water limitations (Wynn-Williams, 1996; Treonis

Table 2 PerMANOVA results for bacterial community structure (Unifrac distances and genus and phylum/class level relative abundance), fungal community structure (DGGE patterns) and functional community structure (GeoChip hybridization patterns at the gene category and probe levels)

	Bacteria						*Fungi*		*GeoChip*			
	Unifrac		*Genus*		*Phylum*		*DGGE*		*Gene cat.*		*Probes*	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
L	**26.84**	**0.001**	**21.85**	**0.001**	**16.93**	**0.001**	**4.27**	**0.001**	**2.19**	**0.012**	**2.47**	**0.001**
V	**35.36**	**0.001**	**23.57**	**0.001**	**7.58**	**0.002**	**3.30**	**0.001**	**4.24**	**0.001**	**2.92**	**0.002**
O	1.16	0.305	2.02	0.074	**2.83**	**0.035**	1.17	0.237	**5.40**	**0.001**	**4.37**	**0.001**
L × V	**10.48**	**0.001**	**13.82**	**0.001**	**2.27**	**0.028**	**2.58**	**0.001**	1.91	0.056	1.28	0.14
L × O	1.82	0.086	0.91	0.500	0.82	0.535	0.85	0.833	**4.68**	**0.001**	**3.68**	**0.001**
V × O	0.17	0.989	0.93	0.445	1.06	0.373	0.69	0.922	**3.02**	**0.013**	**2.04**	**0.014**
L × V × O	0.47	0.902	0.69	0.722	0.39	0.888	1.12	0.219	**3.83**	**0.006**	1.40	0.116

Abbreviations: L, location; O, OTC (open-top chamber); V, vegetation.
For the GeoChip, the gene category level was calculated as the sum of the relative abundance of all probes aiming at this gene.
Bold face values are significant at $P<0.05$.
P-values based on 999 permutations (lowest *P*-value possible is 0.001).

et al., 2002; Wasley *et al*., 2006). Similarly, in the Arctic, growth is generally limited by nitrogen or phosphorus deficiency (Jonasson *et al*., 1999), and experiments with nutrient additions typically induce much larger changes in microbial communities than warming (Ruess *et al*., 1999; Rinnan *et al*., 2007). It should be noted that the long-term biological changes in response to global warming might be very different from the short-term shifts observed in global warming experiments (Arft *et al*., 1999). Integrating experimental and gradient studies might help to distinguish between the short-term and transient effects of climate change vs long-term and lasting effects (Kennedy, 1996; Dunne *et al*., 2004). It should, however, be recognized that the use of such a gradient along the Antarctic Peninsula region is not straightforward due to parallel variations in the severity of the thermal and hydric environments, differences in precipitation balance and disparate geological histories across the study range (Kennedy, 1993).

Weakened linkage between taxonomic and functional richness

Bacterial taxonomical richness was significantly correlated with functional richness in control plots, but not in OTC plots. These results indicate that following warming, sub-Antarctic and Antarctic soil organisms are functionally more homogenous even though they remain as taxonomically diverse as before warming. One possible explanation for this result is a shift from specialist species (harboring specialized functional genes) to generalist species (harboring functional genes shared by many species). Indeed, an increase in the taxonomic diversity of generalist species will not result in an increase in functional diversity, since most species harbor more or less the same genes. In contrast, an increase in the taxonomic diversity of specialist species will result in an increase in functional diversity, since each species harbors a specific set of functional genes. This explanation is supported by the observed increases in the *Alphaproteobacteria* and decreases in the *Acidobacteria*. Consequences of such shifts may include a decrease in the range of functions the soils can perform and a reduced redundancy in some specialized soil functions. In fact, for a range of genes having key functions in the N and C cycles, the number of variants detected on the GeoChip was generally lower in OTC plots as compared with control plots. This result could also be related to the observed higher fungal abundance in OTC plots, as fungal genes are less well represented among the functional characteristics assayed by the GeoChip. Another alternative explanation is that the shifts in bacterial community composition caused by the OTCs resulted in a community which functional genes were less well represented on the GeoChip, and were thus not detected.

Shifts in Acidobacteria *and* Alphaproteobacteria *and increased fungal abundance*

We observed shifts in the relative abundance of *Alphaproteobacteria* and *Acidobacteria* and increases in fungal biomass in response to the warming treatment. *Alphaproteobacteria* and *Acidobacteria* are major players in soil microbial communities around the globe, and previous studies have suggested that a higher *Alphaproteobacteria*-to-*Acidobacteria* ratio is indicative of higher soil nutrient availability (Smit *et al*., 2001; Thomson *et al*., 2010). The majority of *Acidobacteria* are thought to grow slowly and prefer soils with low nutrient availability, thereby showing a negative correlation with soil respiration (Fierer *et al*., 2007). *Alphaproteobacteria,* on the other hand, are mostly fast growing, preferring nutrient-rich environments and were shown to be positively correlated with

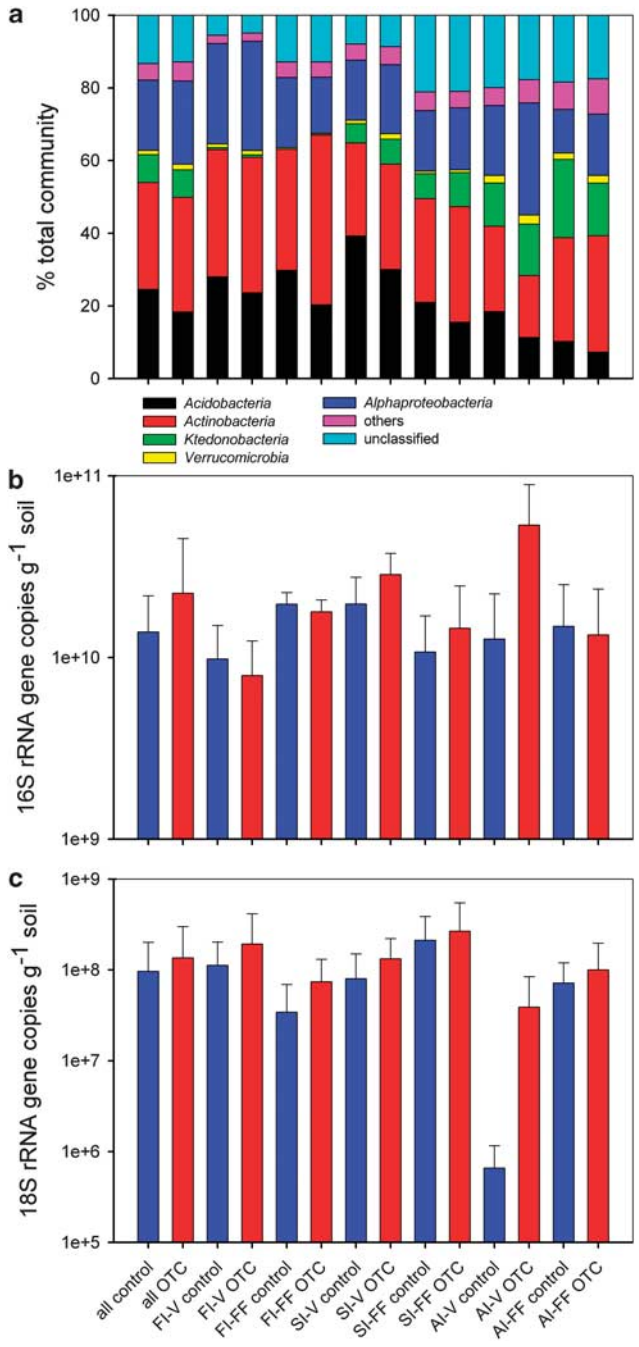

Figure 2 Bacterial community composition and microbial abundance in control and OTC plots at the Falkland, Signy and Anchorage Islands. (**a**) Mean bacterial phylum/class relative abundance in 16S rRNA gene libraries, (**b**) mean bacterial abundance measured by 16S rRNA gene qPCR and (**c**) mean fungal abundance measured by 18S rRNA gene qPCR. In (**b**) and (**c**), red bars: OTC plots, blue bars: control plots, error bar: s.d.

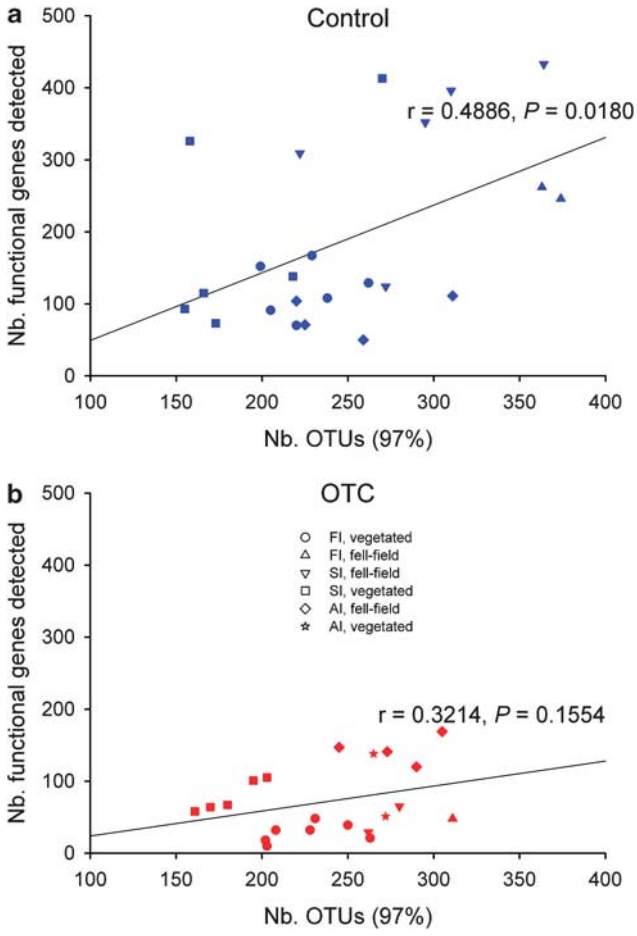

Figure 3 Taxonomic richness (number of operational taxonomic units, OTUs, at 97% similarity) in relation to functional richness (number of genes detected on the GeoChip) for control and OTC plots across all environments. Pearson's linear correlation and *P*-values are given next to the trend lines. Missing data points are due to absence of amplification during GeoChip microarray analyses (see Materials and methods for more details).

soil CO_2 production in the Canadian high Arctic (C Martineau, E Yergeau, LG Whyte and CW Greer, in preparation). Thus, the observed shifts are consistent with increased soil carbon turnover upon warming (Thomson *et al.*, 2010). Fungi were also consistently more abundant in OTC plots. Antarctic soil bacteria and fungi have optimal growing temperatures far above current field conditions (Zucconi *et al.*, 1996; Rinnan *et al.*, 2009), thus potentially enabling them to benefit rapidly from increased temperatures. Fungi have key roles in organic matter decomposition, thereby contributing significantly to soil respiration (Anderson and Domsch, 1973). However, in the longer term, increased fungal biomass might lead to lower respiration rates and increased carbon sequestration as fungi generally have higher carbon assimilation efficiencies than bacteria (Six *et al.*, 2006; Singh *et al.*, 2010). Bacterial abundance was also significantly higher in OTC plots, but only for vegetated environments. Several studies have demonstrated that soil respiration typically increases in global warming experiments (Aerts *et al.*, 2006), but little data are available with respect to the organisms and mechanisms involved. Although the microbial and respiration data were measured in different years of this study, the trends observed lead us to hypothesize that increases in Antarctic soil respiration

after warming are related to shifts in the relative abundance of *Alphaproteobacteria* and *Acidobacteria* and increases in fungal abundance.

Nitrogen cycle
Soil ammonium, nitrate and total nitrogen contents were not significantly higher in OTC plots. Previous studies have reported an increase in Arctic tundra N mineralization by ~70% following at least 3 years of warming (Aerts *et al.*, 2006), but this parameter was not measured in the present study. It is, therefore, still uncertain if the changes observed in soil functional gene groups translate in changes at the process level, and it would be very interesting for further studies to integrate measurements of N-cycle processes to clarify this point. Indeed, changes in N-cycle functions following global warming could have important consequences on Antarctic soil microorganisms and plants, since high latitude ecosystems are typically N limited (Jonasson *et al.*, 1999; Mataloni *et al.*, 2000). However, this might be of less importance at some Antarctic locations (for example, Anchorage Island) where nitrogen is abundant due to the proximity of bird colonies (Bokhorst *et al.*, 2007c).

General microbial community structure
No consistent shifts were observed in the general soil community structure at low taxonomic levels (genus/species levels) following warming. Ordination and PerMANOVA analysis of bacterial and fungal community structure data revealed that the main structuring factors were sampling site and vegetation cover. At low taxonomic levels, soil microbial communities were therefore mostly influenced by local soil characteristics, temperature regimes and vegetation cover. This makes good sense since the warming treatments imposed on the soils were rather subtle (~1 °C) in comparison with the large differences that exist between the sites (~10 °C). However, warming induced significant changes in bacterial community composition at higher taxonomic levels (phylum/class) and in fungal and bacterial abundance. These results highlight the ecological relevance and general coherence of some high-level taxonomic groups (Fierer *et al.*, 2007; Philippot *et al.*, 2010; Wessen *et al.*, 2010) and the appropriateness of using such groups to monitor large-scale responses of soils to perturbations (Singh *et al.*, 2010). For instance, our study shows that the effects of the ongoing global warming on maritime Antarctic soils might potentially be monitored by simple qPCR assays targeting *Alphaproteobacteria* and *Acidobacteria*. Interestingly, we also observed significant effects of warming on the functional community structure, indicating that shifts in microbial functions might be more closely related to shifts at high taxonomic levels rather than at the genus-species level. This relationship between high bacterial taxonomic levels and functions was already shown for CO_2 production, as mentioned above (Fierer *et al.*, 2007). Similarly, in the human gut, it has been shown that shifts in the *Firmicutes* and the *Bacteroidetes* can result in changes in the energy harvesting efficiency, which could play a role in obesity (Ley *et al.*, 2006; Turnbaugh *et al.*, 2006). One explanation for the relationships between high taxonomic levels and functions is that at high taxonomic ranks, most of the species level variation is averaged out, leaving a clearer ecological picture. It has also been suggested that there are barriers to horizontal gene transfer at high taxonomic levels, which create a cohesive force holding the members of these taxa together (Kurland *et al.*, 2003).

Concluding remarks
In conclusion, 3-year warming treatments resulted in consistent shifts in microbial community composition and abundance across a range of sub-Antarctic and Antarctic environments. We also found significant changes in functional genes and a potential link with soil respiration, indicating that these shifts in microbial community composition will probably have functional consequences. This study demonstrates that terrestrial ecosystem processes in the Antarctic region may be strongly affected by the ongoing global warming through changes in microbial communities.

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