RESEARCH ARTICLE

The impact of different soil parameters on the community structure of dominant bacteria from nine different soils located on Livingston Island, South Shetland Archipelago, Antarctica

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Abstract

Microorganisms inhabit very different soil habitats in the ice-free areas of Antarctica, playing a major role in nutrient cycling in cold environments. We studied the soil characteristics and the dominant bacterial composition from nine different soil profiles located on Livingston Island (maritime Antarctica). The total carbon (TC) and total nitrogen (TN) values were high for the vegetated soils, decreasing with depth, whereas the values for the mineral soils were generally low. Soil pH was more acidic for moss-covered soils and neutral to alkaline for mineral soils. Numbers of culturable heterotrophic bacteria were higher at vegetated sites, but significant numbers were also detectable in carbon-depleted soils. Patterns of denaturing gradient gel electrophoresis (DGGE) revealed a highly heterogeneous picture throughout the soil profiles. Subsequent sequencing of DGGE bands revealed in total 252 sequences that could be assigned to 114 operational taxonomic units, showing the dominance of members of the *Bacteroidetes* and *Acidobacteria*. The results of phospholipid fatty acid analysis showed a lack of unsaturated fatty acids for most of the samples. Samples with a prevalence of unsaturated over saturated fatty acids were restricted to several surface samples. Statistical analysis showed that the dominant soil bacterial community composition is most affected by TC and TN contents and soil physical factors such as grain size and moisture, but not pH.

Introduction

Ice-free areas represent only 0.32% of the Antarctic continent (Ugolini & Bockheim, 2008) and they are characterized by extreme climate conditions, with subzero temperatures almost throughout the entire year, low water availability and low nutrient concentrations. The tip of the Antarctic Peninsula is determined by a polar maritime climate, resulting in positive summer air temperatures, repeated freeze–thaw events and a distinctly higher precipitation than in continental areas. Liquid water can be found during the summer months when temperatures rise above 0 °C. These conditions allow the presence of scarce vegetation (lichens, mosses, grasses) that has a direct influence on the composition of soil microbial communities by providing degradable polymeric organic matter in the form of plant litter or root-based low-molecular substances. These root exudates can be used by the soil microbiota as a carbon source (MacRae & Castro, 1966; Rovira, 1969; van Veen *et al.*, 1989; Ström *et al.*, 2003). Thus, vegetation and microbial activity have a small-scale influence on the soil chemical parameters and therefore also on the structural soil microbial composition.

Data on microbial communities in Antarctic terrestrial habitats are still quite rare. Nevertheless, during the last two decades, it became evident that these extreme habitats harbor a unique community of microorganisms, and an increasing number of studies have focused on unraveling the structural network, the adaptation mechanisms and the physiological properties of these microbial communities, also in the face of possible biotechnological applications (Nichols *et al.*, 1999, 2004; Aislabie *et al.*, 2006, 2008; Smith

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et al., 2006; Novis *et al*., 2007; Yergeau *et al*., 2007, 2009; Niederberger *et al*., 2008; Pointing *et al*., 2009). Furthermore, the response of bacterial communities on climate change has become the focus of investigations in the last few years (Bokhorst *et al*., 2007; Rinnan *et al*., 2009).

The application of culture-dependent approaches describes only a limited number of taxa due to the selection of certain microorganisms when using artificial growth conditions (e.g. medium, substrate, temperature, oxygen concentrations). Therefore, culture-independent methods have become an important alternative in environmental microbiology to investigate complex microbial systems in various Antarctic habitats for example lake and marine sediments (Purdy *et al*., 2003; Li *et al*., 2006), dry mineral soils (Aislabie *et al*., 2006; Smith *et al*., 2006; Shravage *et al*., 2007), sea ice (Brown & Bowman, 2001; Brinkmeyer *et al*., 2003), freshwater and sea water (Pearce *et al*., 2003; Gentile *et al*., 2006) and microbial mats (Taton *et al*., 2006; Fernández-Valiente *et al*., 2007). A number of investigations used the method of denaturing gradient gel electrophoresis (DGGE) to assess the diversity and community structure of bacteria in different antarctic and subantarctic terrestrial environments (Chong *et al*., 2009a, b; Foong *et al*., 2010). DGGE can visualize only numerically dominant bacteria, which make up at least 1% or more of the *in situ* population of the overall bacterial community (Muyzer *et al*., 1993).

Microbiological investigations of different Antarctic mineral soils showed that these environments can harbor high bacterial numbers of up to 10^9 cells g^{-1} dry soil (Ramsay & Stannard, 1986; Roser *et al*., 1993; Bölter, 1995; Cowan *et al*., 2002; Aislabie *et al*., 2006, 2008; Xiao *et al*., 2007; Cannone *et al*., 2008), which are comparable to numbers from temperate forest (Ekelund *et al*., 2001), glacial forefield soils (Sigler *et al*., 2002) and Siberian arctic tundra lowlands (Schmidt, 1999; Kobabe *et al*., 2004).

In this study, we investigated and compared different maritime Antarctic soils sites located on Livingston Island to identify the dominant phylogenetic bacterial groups as well as compositional patterns. Sites with and without vegetation were chosen for analyzing different soil parameters to show their impact on the dominant soil bacterial community and how the bacterial community could possibly be altered when plant growth increases in the maritime Antarctic due to future climate change.

Materials and methods

Study area

Hurd Peninsula is located in the southern part of Livingston Island, one of the largest islands in the South Shetland Archipelago (Fig. 1). Ice-free areas can be found mainly along the coast, whereas the central and northwestern part of the peninsula is covered by glaciers. Climatic conditions can be defined as cold maritime, with mean summer temperatures of ~2 °C, whereas daily maxima can be up to 10 °C. During the winter, temperatures are always below 0 °C, with minima of −35 °C (reviewed in Toro *et al*., 2007). The mean annual precipitation is much higher than in most continental areas of Antarctica, with values between 500 and 1000 mm (Bañón, 2001; Vieira & Ramos, 2003).

The main study site was located in an ice-free area northeast of the Bulgarian Antarctic Base characterized by basaltic and andesitic bedrock at 64 m a.s.l. The soils and sediments were mainly characterized by coarse grain-sized (> 2 mm) material and soil horizons can only be differentiated in the course of initial soil formation (e.g. humus accumulation, brownification), which was observed at some sites. Beyond this, sites were characterized by loose sediments, till deposits covered by lapilli-sized volcanic ashes and permafrost.

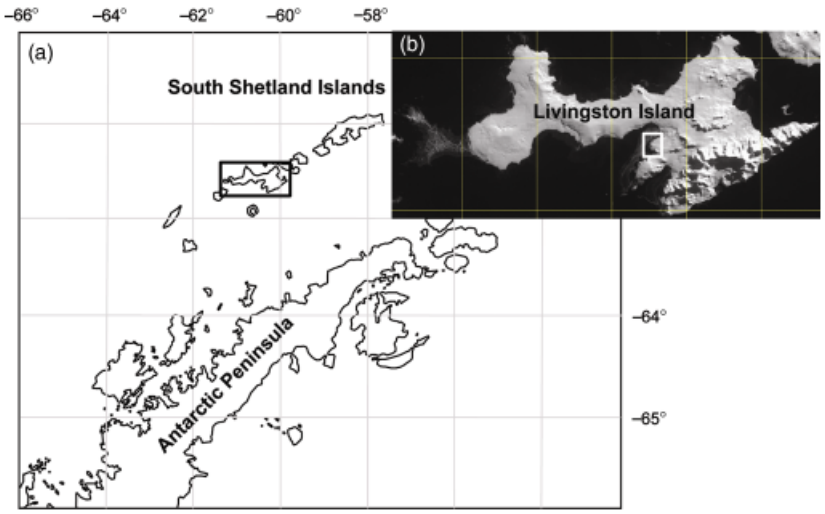

Fig. 1. (a) Location of Livingston Island within the South Shetland Archipelago (black rectangle). (b) The satellite image shows the Hurd Peninsula (white rectangle).

Volcanic ashes precipitated by eruption of the nearby Deception Volcano (Calvet *et al.*, 1993) were prevalent in the profiles, either as sole layers or mixed into the surface debris. Vegetation was found at different sites, with lichens preferring rocky ground, whereas mosses were restricted to small depressions that contain finer soil material.

The last major glacial retreat on Livingston Island started about 5–4 ka BP (Björck *et al.*, 1991, 1996; Björck & Zale, 1996). In the ice-free areas, continuous permafrost can be found in altitudes above 35 m a.s.l., whereas below this altitude, the distribution of permafrost is isolated (Ramos & Vieira, 2003; Schwamborn *et al.*, 2008).

Soil sampling

During the austral summer period of 2005, soil samples from ice-free areas of Hurd Peninsula (Livingston Island) were taken (Fig. 2). Sites were chosen by different habitat properties (e.g. with or without vegetation, surface characteristics) and exposition. Altogether, nine soil profiles were excavated and described and 38 samples were taken from defined layers for soil chemical, soil physical and soil microbiological analyses (Table 1). Five profiles (T1-1 to T1-5) were situated along a transect from the top of a hill to a small depression. The remaining profiles (SP-A to SP-D) were chosen by distinct site characteristics. Soil samples of each depth were taken from an area of 25×25 cm and homogenized directly in the field. Subsamples for soil chemical and soil physical analyses were transferred into sealable plastic bags and stored at 4 °C until further processing. Subsamples for microbiological analyses were placed in 250 or 500 mL sterile Nalgene® boxes, which were frozen immediately. Continuous cooling at −20 °C was ensured for microbial samples by the transfer with the research vessels *MS Las Palmas* and *RV Polarstern* to Germany.

Soil analysis

Grain size distribution was determined using freeze-dried, homogenized and sieved soil samples (< 2 mm) of about 15 g (Schlichting *et al.*, 1995). Water content was determined after freeze-drying the soil samples. The total carbon (TC) and total nitrogen (TN) contents were analyzed on a milled sample using an automatic element analyser (Elementar Vario EL Bundy III). Conductivity and pH were measured on a 1 : 2.5 soil : deionized water slurry that was shaken for 1 h in the dark using a MultiLab 540 (WTW, Germany).

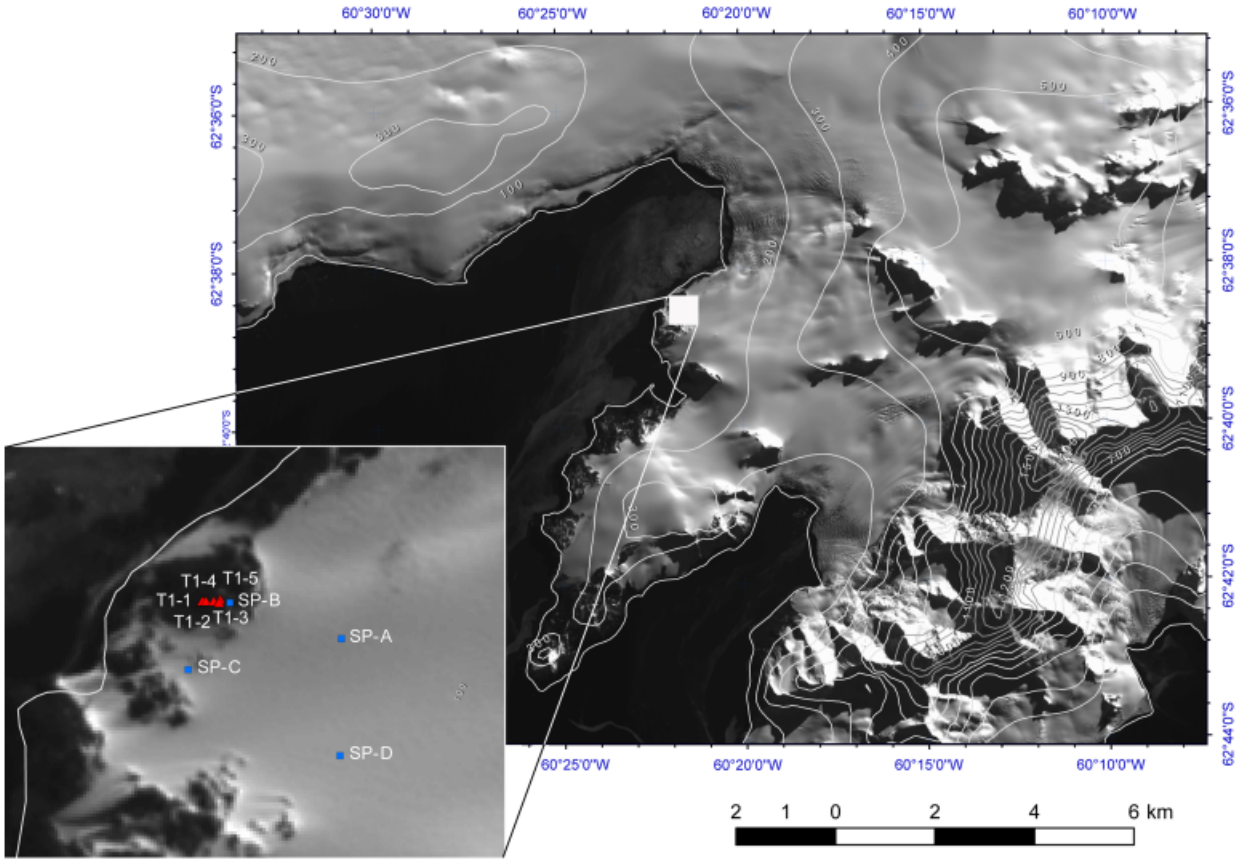

Fig. 2. Detailed picture of the Hurd Peninsula with the position of the sampling sites.

Table 1. Sampling site description

Samples	Coordinates	Site description
T1-1	S62°38′24.2″, W60°21′41.7″	Silty sand in a bedrock depression; covered with a moss layer
T1-2	S62°38′24.2″, W60°21′41.2″	Silty sand with gravel and stones; lichens adherent on rocks
T1-3	S62°38′24.2″, W60°21′40.2″	Silty sand with gravel and stones; bands of gravel between 5–9 cm and 20–23 cm depth
T1-4	S62°38′24.3″, W60°21′39.5″	Silty sand with the watertable at 15 cm depth; moss cover on top
T1-5	S62°38′24.1″, W60°21′39.4″	Sandy volcanic ashes with gravel; till starting at a depth of 40 cm
SP-A	S62°38′28.3″, W60°21′12.1″	Sandy volcanic ashes with gravel; snowbed nearby, permafrost starting at 20 cm depth
SP-B	S62°38′24.5″, W60°21′36.4″	From 0 to 5 cm a volcanic ash layer with gravel and stones; from 5 to 35 cm sandy silt, permafrost occurred at 35 cm depth
SP-C	S62°38′31.2″, W60°21′45.8″	From 0 to 10 cm silty sand with gravel and stones, followed by sandy silt with gravel; lichens on top
SP-D	S62°38′40.1″, W60°22′12.8″	From 0 to 10 cm sandy volcanic ashes with gravel and stones; from 10 to 15 cm depth fine, silty-clayey material with gravel; from 15 to 20 cm sandy silt with gravel; lichens on top

The numbers of cultivable heterotrophic bacteria were determined by plating serial soil solutions (until 10^{-6}) on modified BR agar plates (Ganzert *et al.*, 2011) with incubation at 10 °C for 7 days.

Analysis of phospholipid fatty acid (PLFA) and phospholipid ether lipids (PLEL)

Lipids were extracted from 10 to 20 g sample material, according to the Bligh–Dyer method as described elsewhere (Knief *et al.*, 2003). The resulting lipid material was fractionated into neutral lipids, glycolipids and phospholipids on a silica-bonded phase column (SPE-SI; Bond Elute, Analytical Chem International, CA) by elution with chloroform, acetone and methanol, respectively. An aliquot of the phospholipid fraction was taken for PLFA analysis. After mild alkaline hydrolysis, the lipid extract was supplemented with nonadecanoic acid methyl ester as an internal standard. The analyses of the fatty acid methyl ester extracts were performed using GC–MS as described previously (Lipski & Altendorf, 1997).

An aliquot of the phospholipid fraction was used for PLEL analysis according to Gattinger *et al.* (2003). After the formation of ether core lipids, ether-linked isoprenoids were released following the cleavage of ether bonds with HI and reductive dehalogenation with Zn in glacial acetic acid. The extracts were supplemented with nonadecanoic acid methyl ester as an internal standard and subjected to GC/MS analysis under operating conditions described by Gattinger *et al.* (2003).

DNA extraction and PCR amplification of 16S rRNA genes from soil samples

DNA was extracted in triplicate from 0.5 to 1.0 g of well-homogenized soil material using the UltraClean™ Soil DNA Isolation Kit (MoBio Laboratories Inc.), following the manufacturer's instructions. DNA triplicates were pooled and 16S rRNA gene fragments were generated using PCR with the general primer pair GC_341F-907R for Bacteria (Muyzer *et al.*, 1993, 1995). A 50 μL reaction mixture contained PCR buffer, 0.25 mM of each dNTP, 2 mM $MgCl_2$, 0.4 μM of each primer, 1.25 U Taq DNA Polymerase (Eppendorf) and ~20 ng of DNA template. PCR was performed using an iCycler Thermal Cycler (Bio-Rad). The amplification conditions consisted of an initial denaturation step at 94 °C for 4 min, followed by 36 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1:30 min, with a final elongation step of 20 min at 72 °C. PCR products were checked on 2% agarose gels stained with SYBR Gold (Molecular Probes, Invitrogen).

DGGE and sequencing of environmental DNA bands

All samples were separated on 8% polyacrylamide gels in 1 × TAE buffer using a D-Code System (Bio-Rad). The denaturing gradient ranged from 20% to 60% (100% denaturant consisted of 7 M urea and 40% (v/v) deionized formamide). The gels were run at 60 °C at a constant voltage of 100 V for 14 h. After electrophoresis, the gels were stained for 35 min with SYBR Gold (1 : 10 000 diluted) and visualized under UV light using a GeneFlash system (Syngene). DGGE runs were repeated to ensure an identical banding pattern for further processing. For sequencing of DGGE bands, selected DGGE bands were cut out with a sterile scalpel and were transferred to sterile 0.5 mL Eppendorf tubes. DNA was eluted overnight in 30 μL of sterile water at 4 °C. The DNA fragments were then amplified for sequencing using the same conditions as described above, except using the forward primer without a GC clamp. After

purification, using a QIAquick PCR Purification Kit (Qiagen), DNA bands were commercially sequenced by Eurofins MWG GmbH (Martinsried, Germany) and GATC Biotech (Konstanz, Germany) using the forward primer without a GC clamp.

Phylogenetic analysis

Phylogenetic analysis of partial 16S rRNA gene sequences ($\geq$150 nucleotides) was performed using the ARB software package (Ludwig *et al.*, 2004; http://www.arb-home.de). The automatic alignment tool of the SILVA database (Pruesse *et al.*, 2007; http://www.arb-silva.de) was used for automatic sequence alignment and alignments were then corrected manually. Using the neighbor-joining method (Saitou & Nei, 1987) of the ARB program, distance matrices were created to group the sequences into operational taxonomic units (OTUs) using the program DOTUR v. 1.53 (Schloss & Handelsman, 2005). One OTU is described as a cluster of DNA sequences that share $\geq$97% sequence similarity (Roselló-Mora & Amann, 2001). To assess the affiliation of the dominant soil bacteria, the RDP classifier with a confidence threshold of 95% was used (Wang *et al.*, 2007).

Statistical analysis of data

For data processing, OTU numbers and environmental properties of the different depths of each sampling position were grouped to make the different sites comparable. The OTU numbers were normalized by log transformation, whereas the environmental parameters were normalized using a standard normal distribution (*zero mean and unit variance*) according to the following equation:

$$z' = \frac{x - \bar{x}}{S}, \quad (1)$$

where z' is the normalized value, x is the parameter that was measured, $\bar{x}$ is the mean and S is the SD (Leyer & Wesche, 2007). A matrix was compiled with the transformed data for both OTUs and environmental properties. The matrices were then processed to perform a canonical correspondence analysis (CCA) using the program CANOCO v. 4.5 (ter Braak, 1986). This analysis can provide us with information regarding the way in which the community is influenced and shaped by certain environmental parameters.

For the pairwise comparison of the dominant community structure of the different soils, the Sørensen index (S) was calculated according to the following equation:

$$S = \frac{2(ab)}{(a+b)}, \quad (2)$$

where a and b are the number of OTUs obtained from the DGGE profiles of samples a and b, and ab is characterized by the number of OTUs shared by both samples.

Results

Soil characteristics

The soil characteristics of nine soil profiles (Table 1) of Livingston Island (maritime Antarctica) were analyzed with regard to moisture, grain size fractions, TC and TN content, pH, conductivity (E_C) and the number of culturable heterotrophs (see Table 2). Most of the investigated mineral soils showed a sandy texture interspersed with gravel. Soil profiles SP-B, SP-C and SP-D were dominated by silt from a depth of $\geq$10 cm. Clay content was clearly under-represented in most of the soil profiles. Only profiles T1-2, T1-3, T1-5 (40–50 cm) and SP-D (10–20 cm) showed elevated clay fraction values (10–20%). Profiles T1-1 and T1-4 were covered by mosses and showed an incipient humus accumulation in the uppermost part. The TC and TN contents were low within most of the soil profiles, with values ranging from $<$ 0.10% to 1.36% (TC) and $<$ 0.10% to 0.13% (TN), respectively, except for the two uppermost layers of the profiles T1-1 and T1-4 due to the moss layer. The values for pH ranged from neutral to alkaline, except for profile T1-1 and the top layer of soil profile T1-4, where pH values ranged from pH 4.8 to 6.1. Conductivity was low in all the soil samples. The moisture content of the investigated mineral soils ranged from 2.6% to 15.6%, with differences depending on their exposure and site characteristics. Profiles SP-A and SP-B were characterized by underlain permafrost, whereas for the other profiles, no frozen ground was observed.

In about half of the investigated mineral soil profiles (T1-1, T1-4, SP-A, SP-B, SP-D), the number of culturable heterotrophic bacteria decreased with increasing depth, whereas in T1-2, T1-3, T1-5 and SP-C, the numbers of culturable heterotrophs were relatively consistent throughout the entire soil profiles (Table 2). The highest numbers of culturable heterotrophic bacteria were observed in the surface layer of profiles T1-1 and T1-4 (1.4×10^8 CFU g^{-1} dry soil and 3.0×10^6 CFU g^{-1} dry soil, respectively).

Lipid biomarker analyses

The overall PLFA content decreased with increasing depth in almost all the profiles studied (Table 3). Only profiles SP-C and SP-D showed high PLFA concentrations for the bottom samples. The highest values were obtained in profiles T1-1, T1-4 and T1-5, respectively. While most top layers showed a predominance of unsaturated fatty acids, all the other samples were dominated by straight-chain saturated fatty acids (Table 3). For most profiles, the even-numbered compounds tetradecanoic, hexadecanoic and octadecanoic acid (14:0, 16:0 and 18:0) were constantly present throughout the profile (Supporting Information, Table S1). Branched-chain fatty acids could be detected throughout the entire profiles T1-1, SP-C and SP-D and within the

Table 2. Soil geochemical and soil geophysical descriptions of the sampling sites

Site	Depth (cm)	Moisture (%)	Sand (%)*	Silt (%)*	Clay (%)*	Total C (%)	Total N (%)	pH	EC ($\mu S\,cm^{-1}$)	Number of culturable heterotrophs (g^{-1} dry soil)
T 1-1	0–4	7.1	ND	ND	ND	26.50	0.84	4.81	ND	1.2×10^8
	4–7	4.7	73.9	20.4	5.7	2.22	0.20	6.04	88	1.8×10^5
	7–14	5.5	56.1	39.3	4.6	0.46	< 0.10	6.10	40	7.8×10^4
T 1-2	0–5	3.3	44.1	36.4	19.5	0.11	< 0.10	7.22	38	7.2×10^4
	5–12	4.1	59.6	25.7	14.7	< 0.10	< 0.10	7.80	34	5.5×10^4
	12–24	3.9	49.1	31.4	19.5	< 0.10	< 0.10	7.82	37	4.7×10^4
T 1-3	0–9	3.6	60.7	28.4	10.9	0.13	< 0.10	7.58	49	2.3×10^4
	9–16	6.5	49.6	40.0	10.4	0.11	< 0.10	7.16	34	4.1×10^4
	16–23	3.6	73.8	18.0	8.2	< 0.10	< 0.10	7.92	31	1.7×10^4
	23–30	8.3	50.5	38.0	11.5	< 0.10	< 0.10	7.23	31	1.3×10^4
T 1-4	0–5	10.0	ND	ND	ND	9.34	0.32	5.97	ND	1.7×10^6
	5–8	9.2	75.4	15.7	8.9	2.31	0.19	6.77	112	5.4×10^3
	8–13	5.4	79.3	16.4	4.3	0.28	< 0.10	7.53	48	5.2×10^4
	13–18	5.9	83.9	12.5	3.6	0.14	< 0.10	7.83	39	2.5×10^4
T 1-5	0–10	3.3	88.4	10.3	1.3	< 0.10	< 0.10	7.81	30	5.0×10^4
	10–20	3.8	86.3	12.1	1.6	< 0.10	< 0.10	8.01	28	1.9×10^4
	20–30	4.5	81.8	16.7	1.5	< 0.10	< 0.10	8.16	30	8.7×10^3
	30–40	5.0	84.5	14.3	1.2	< 0.10	< 0.10	7.94	30	2.3×10^3
	40–50	3.1	45.8	36.3	17.9	0.23	< 0.10	8.45	100	4.3×10^3
SP-A	0–5	6.6	80.1	15.8	4.0	< 0.10	< 0.10	7.71	36	1.0×10^4
	5–10	7.0	81.6	15.0	3.4	< 0.10	< 0.10	8.04	31	2.0×10^4
	10–15	7.4	89.7	8.3	2.0	< 0.10	< 0.10	8.24	30	1.5×10^4
	15–20	9.9	92.4	6.4	1.2	< 0.10	< 0.10	8.61	39	1.9×10^4
	20–25	15.6	91.6	7.0	1.4	< 0.10	< 0.10	8.49	57	1.8×10^4
SP-B	0–5	2.6	93.0	5.6	1.4	0.15	< 0.10	7.48	42	1.6×10^3
	5–10	9.9	57.5	39.5	3.0	0.19	< 0.10	7.69	34	7.4×10^2
	10–15	7.8	46.7	50.2	3.1	0.11	< 0.10	7.83	34	3.1×10^2
	15–20	10.3	41.3	56.4	2.4	< 0.10	< 0.10	7.78	33	1.9×10^3
	20–25	6.4	31.4	64.1	4.6	0.10	< 0.10	7.84	37	3.9×10^3
	25–30	11.1	51.0	45.1	3.9	0.14	< 0.10	8.02	41	ND
	30–35	10.3	36.9	59.6	3.6	< 0.10	< 0.10	7.91	37	1.5×10^1
SP-C	0–10	2.7	69.1	24.2	6.7	0.21	< 0.10	7.07	53	2.2×10^4
	10–20	5.6	41.3	50.8	7.9	0.38	< 0.10	7.48	44	2.0×10^4
	20–30	5.3	45.0	49.0	6.0	0.17	< 0.10	7.49	35	1.1×10^4
SP-D	0–5	4.5	75.6	18.9	5.5	0.45	< 0.10	7.26	65	8.4×10^4
	5–10	5.1	71.9	20.1	8.1	0.48	< 0.10	7.41	74	5.0×10^4
	10–15	6.2	12.0	71.2	16.8	1.21	0.12	7.37	91	5.3×10^4
	15–20	5.4	42.0	47.3	10.7	1.36	0.13	7.24	101	4.5×10^3

*Part of the grain size fraction < 2 mm.
ND, no data.

uppermost layer of the profiles SP-A and SP-B. For deeper soil layers of profiles SP-A and SP-B as well as for profiles T1-2 and T1-5, no or only low concentrations of branched-chain fatty acids were detectable. PLEL characteristic for *Archaea* could not be detected in any of the samples.

DGGE analysis of nine mineral soil profiles

Nine Antarctic mineral soil profiles from Livingston Island were investigated using DGGE fingerprints to classify the prominent bacterial representatives existing in these habitats. The DGGE banding pattern showed a heterogeneous and diverse picture within the vertical profiles and between the different sites (Fig. 3). Distinct DNA bands were detectable (up to 15), even in soil layers with very low contents of carbon and nitrogen. Within one profile (SP-D), the pattern of DNA bands showed almost no change with depth. Within the other profiles, a clear vertical difference in the number and position of DNA bands could be observed. Even for neighboring soil layers with similar soil chemical and soil physical properties (see Table 2), for example profiles T1-5 (from 0 to 40 cm), SP-A and SP-B, different DNA banding patterns in terms of number and position of the DNA bands could be observed. In profile SP-C, the number of DNA bands decreased with increasing depth.

Table 3. Fractions of straight-chain saturated/unsaturated and iso-/anteiso-branched-chain fatty acids (FA) from the PLFA profiles of selected Antarctic mineral soil samples (per gram wet soil)

Site location	Depth (cm)	Straight-chain saturated FAs (ng FA g^{-1})*	Straight-chain monounsaturated FAs (ng FA g^{-1})†	Iso-branched-chain FAs (ng FA g^{-1})‡	Anteiso-branched-chain FAs (ng FA g^{-1})§
T1-1	0–4	1490	45	536	203
	4–7	205	6	132	55
	7–14	128	2	52	38
T1-2	0–5	61	-	8	1
	5–12	33	-	-	-
	12–24	34	-	-	-
T1-3	0–9	ND	ND	ND	ND
	9–16	ND	ND	ND	ND
	16–23	ND	ND	ND	ND
	23–30	ND	ND	ND	ND
T1-4	0–5	460	892	143	69
	5–8	152	118	55	30
	8–13	35	11	15	6
	13–18	31	-	-	-
T1-5	0–10	879	230	-	-
	10–20	62	-	-	-
	20–30	68	-	1	-
	30–40	48	-	-	-
	40–50	42	-	-	-
SP-A	0–5	244	274	104	55
	5–10	82	-	-	-
	10–15	75	-	-	-
	15–20	53	-	-	-
	20–25	57	-	-	-
SP-B	0–5	157	231	54	42
	5–10	39	6	1	2
	10–15	32	1	1	-
	15–20	28	1	-	-
	20–25	34	-	-	-
	25–30	40	3	1	1
	30–35	24	3	1	-
SP-C	0–10	141	469	74	46
	10–20	55	25	6	4
	20–30	274	78	20	39
SP-D	0–5	178	242	78	66
	5–10	95	73	30	27
	10–15	82	19	16	13
	15–20	110	17	19	16

*10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0.

†16:1cis7, 16:1cis8, 16:1cis9, 16:1cis11, 17:1cis9, 17:1cis11, 18:1cis7, 18:1cis9, 18:1cis11.

‡13:0 iso, 14:0 iso, 15:0 iso, 16:0 iso, 17:0 iso.

§13:0 anteiso, 15:0 anteiso, 17:0 anteiso.

ND, no data.

Phylogenetic analyses of dominant soil bacteria

To investigate the microbial life in mineral soils of Livingston Island, altogether 292 DNA bands of different intensities were cut out from the DGGE gels and were reamplified for sequencing. In total, 252 sequences of ≥ 150 bp could be obtained from the nine soil profiles. Sequences from mineral soils were affiliated to *Alpha*-, *Beta*- and *Gammaproteobacteria*, *Bacteroidetes*, *Planctomycetes*, *Gemmatimonadetes*, *Acidobacteria*, *Nitrospirae*, *Firmicutes*, *Actinobacteria*, *Chloroflexi* and unclassified bacteria (Table 4). Sequences affiliated with *Bacteroidetes* were detectable in almost all soil profiles (except profile T1-2), displaying the major group in profiles T1-1 and T1-4 (Fig. 4). Sequences related to the phylum *Acidobacteria* were also present in all investigated soil profiles and were most prominent in profiles T1-2, SP-C

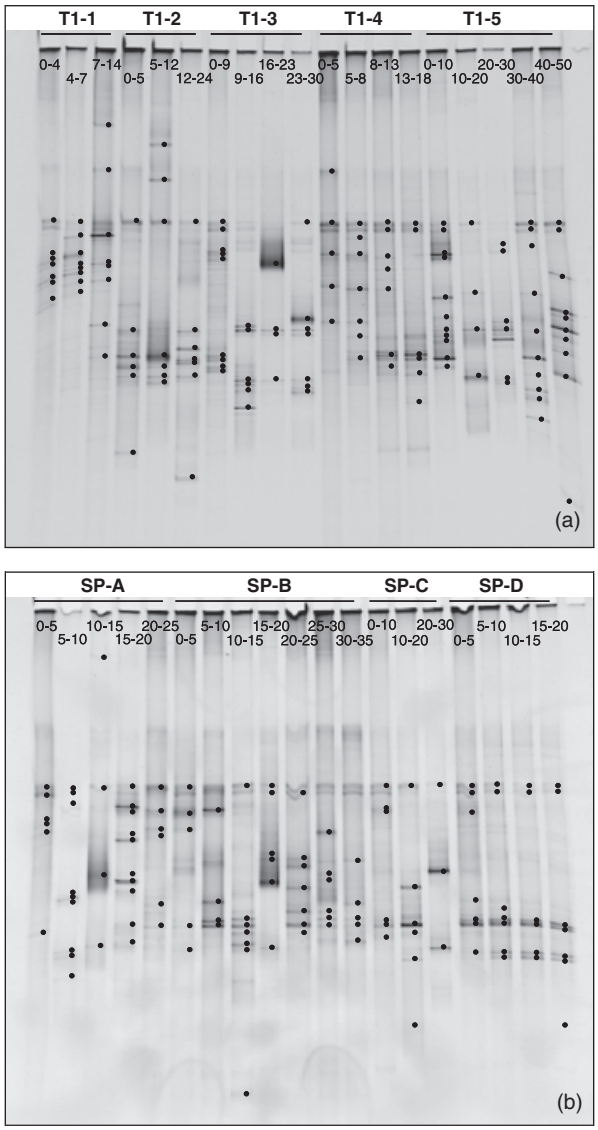

Fig. 3. DGGE fingerprints of nine different maritime Antarctic soil profiles. Circles indicate DNA bands that were cut for sequencing and that were subsequently used for phylogenetic analyses. Numbers in the DGGE picture indicate the depth in cm. (a) Soil profiles T1-1 to T1-5; (b) soil profiles SP-A to SP-D.

and SP-D. A much more diverse affiliation of sequences belonging to different bacterial phyla could be observed for the other soil profiles (T1-3, T1-5, SP-A, SP-B). Interestingly, sequences related to methanotrophic bacteria were found in different soil profiles, although no methane (CH_4) emission could be detected at the sampling sites (data not shown) and no archaeal PLEL markers were found. A sequence related to the genus *Methylocystis* was found in only one mineral soil profile (T1-5), whereas *Methylobacter psychrophilus* sequences could be found in four different profiles (T1-2, T1-3, T1-5, SP-A). Altogether, 114 OTUs could be defined. Most OTUs obtained from the DGGE patterns belong to the *Bacteroidetes* (36 OTUs), *Acidobacteria* (29 OTUs) and unclassified bacteria (20 OTUs), followed by *Firmicutes* (10 OTUs), *Actinobacteria* (5 OTUs) and *Betaproteobacteria* (5 OTUs). The remaining groups were represented to a much smaller extent, with two OTUs for the *Gammaproteobacteria*, *Gemmatimonadetes* and *Chloroflexi*, and with one representative for the *Alphaproteobacteria*, *Planctomycetes* and *Nitrospirae*, respectively. Members of the *Bacteroidetes*, which represented the largest group of OTUs, were mostly assigned to the class *Sphingobacteria*, with most sequences clustering in the family *Chitinophagaceae*. OTUs of the *Acidobacteria* could be grouped into five different subdivisions (Gp4, 6, 7, 13, 17), in which 66% of the *Acidobacteria* were affiliated to subdivision Gp4. For the unclassified bacteria, OTUs can be assigned distantly to *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, group OD1 and *Proteobacteria*. The *Firmicutes* sequences obtained were related to the families *Lactobacillaceae*, *Staphylococcaceae*, *Streptococcaceae* and *Clostridiaceae*.

Statistical analyses

Overall, the Sørensen similarity index was low for all investigated sites, with values of $S \leq 0.4$, even for sites where the environmental parameters were comparable (Table 5).

The CCA plot shows clusters of the different sites as well as the weights and orientation of the environmental factors (Fig. 5). The first two axes accounted for 18.3% (PC1) and 15.4% (PC2) of the variation. TC, TN and pH were correlated with PC1, whereas the other factors [moisture, electric conductivity (E_C), sand, silt, clay] were correlated with PC2. Soil profile T1-1 is strongly correlated with TC and TN and the soil profiles T1-2, T1-5, SP-C and SP-D were correlated with clay. The other soil profiles (T1-3, T1-4, SP-A and SP-B) are mainly correlated with sand and moisture.

Discussion

Although the Antarctic continent is characterized by extreme climate conditions, recent studies in Antarctic soil environments revealed a large bacterial diversity, with a high proportion of sequences that were only distantly related to known sequences, indicating the existence of novel species or genera (Shivaji *et al.*, 2004; Saul *et al.*, 2005; Aislabie *et al.*, 2006, 2008; Smith *et al.*, 2006; Shravage *et al.*, 2007; Yergeau *et al.*, 2007; Niederberger *et al.*, 2008). Within this study, we identified the dominant bacterial groups in a broad variety of different soils from Livingston Island (maritime Antarctica) and identified the soil parameters affecting the community structure of dominant bacteria existing in these habitats.

Antarctic mineral terrestrial habitats were mostly poorly developed and initial processes in soil formation such as

Table 4. Closest phylogenetic affiliation of sequences obtained from DGGE bands of each single soil profile

Closest phylogenetic affiliation	T1-1	T1-2	T1-3	T1-4	T1-5	SP-A	SP-B	SP-C	SP-D
Alphaproteobacteria					1 (1)	1 (1)			
Rhizobiales						1			
Methylocystis					1				
Betaproteobacteria					2 (2)		3 (3)	3 (2)	
Polaromonas							1		
Comamonadaceae							1		
Burkholderia								2	
Burkholderiales								1	
Oxalobacteraceae					1				
Unclassified *Betaproteobacteria*					1		1		
Gammaproteobacteria		1 (1)	6 (2)		1 (1)	4 (1)			
Methylobacter		1	6		1	4			
Bacteroidetes	21 (9)		4 (3)	14 (12)	8 (3)	13 (7)	8 (4)	2 (2)	1 (1)
Chitinophagaceae	21		3	4	7	8	4	2	1
Chitinophaga					1				
Sphingobacteriales			1	1					
Unclassified *Bacteroidetes*				6			4		
Ferruginibacter				2		3			
Terrimonas				1					
Tannerella				1		2			
Planctomycetes						2 (1)			
Planctomycetaceae						2			
Gemmatimonadetes		2 (2)			2 (1)				1 (1)
Gemmatimonas		2			2				1
Acidobacteria	1 (1)	17 (8)	9 (4)	7 (3)	13 (5)	2 (1)	9 (7)	8 (3)	24 (7)
Group 4	1	9	5	7	12	2	6	7	24
Group 6		4	4				2		
Group 7		3			1		1		
Group 13								1	
Group 17		1							
Nitrospirae				1 (1)			1 (1)		
Nitrospiraceae				1			1		
Firmicutes			1 (1)		9 (4)	4 (2)	7 (2)		
Lactobacillus					4				
Bacillales					3				
Staphylococcus			1				5		
Staphylococcaceae							1		
Clostridiaceae					1				
Unclassified *Firmicutes*					1		1		
Streptococcus						4			
Actinobacteria			6 (3)			5 (3)	1 (1)	1 (1)	
Propionibacterium			5			3	1	1	
Corynebacterium			1						
Acidimicrobiales						1			
Unclassified *Actinobacteria*						1			
Chloroflexi				1 (1)	1 (1)		1 (1)		
Anaerolineaceae				1	1		1		
Unclassified bacteria	1 (1)		1 (1)	2 (2)	4 (4)	3 (3)	10 (7)	1 (1)	1 (1)
Total number of sequences (OTUs)	23 (11)	20 (11)	27 (14)	26 (19)	41 (22)	34 (19)	40 (26)	15 (9)	27 (10)

Numbers in parentheses indicate the number of OTUs.

humus accumulation, acidification and brownification could be observed only at a few sites. The different soil profiles revealed a highly heterogeneous picture with regard to bacterial composition and site-specific properties (e.g. water and carbon content, grain size distribution). Water content was generally much higher than in mineral soils from continental Antarctica, for instance the Larsemann Hills, Prydz Bay (unpublished data), Victoria Land (Aislabie

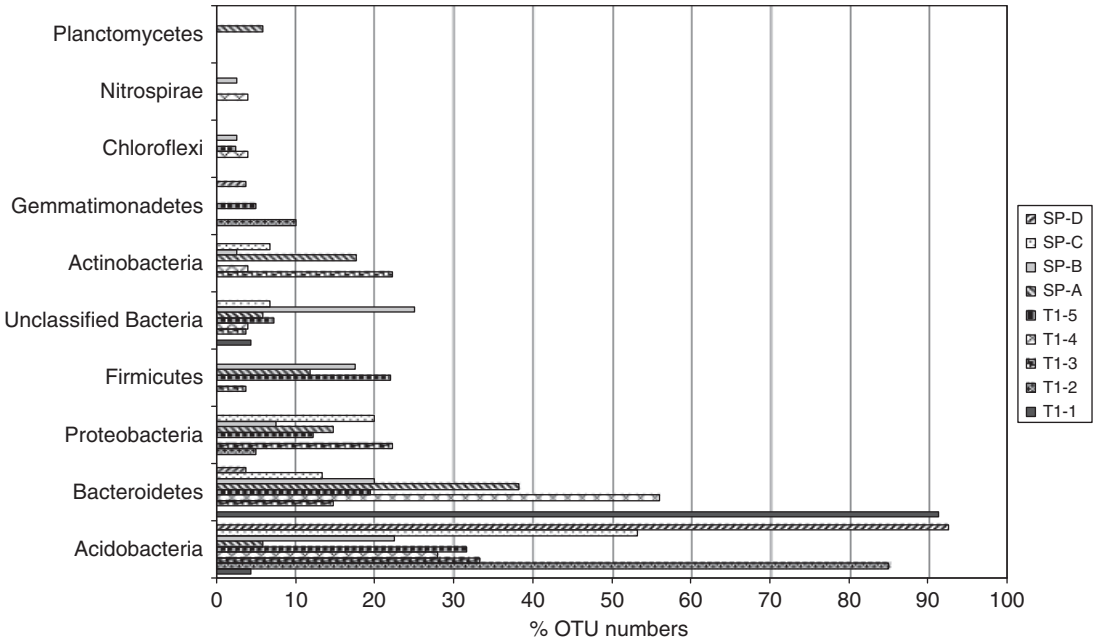

Fig. 4. Percentage abundance of the dominant community 16S rRNA gene sequences belonging to each taxonomic group for each individual soil profile, revealed by DGGE analysis.

Table 5. Comparison of the dominant soil community OTUs (with a sequence similarity ≥97%) using the Sørensen index *S*

	T1-1	T1-2	T1-3	T1-4	T1-5	SP-A	SP-B	SP-C	SP-D
T1-1	1								
T1-2	0.00 (0.13)	1							
T1-3	0.08 (0.21)	0.16 (0.19)	1						
T1-4	0.00 (0.26)	0.07 (0.08)	0.13 (0.14)	1					
T1-5	0.06 (0.15)	0.18 (0.28)	0.17 (0.19)	0.15 (0.22)	1				
SP-A	0.07 (0.16)	0.13 (0.15)	0.30 (0.27)	0.11 (0.18)	0.20 (0.21)	1			
SP-B	0.11 (0.13)	0.16 (0.19)	0.25 (0.29)	0.18 (0.26)	0.13 (0.14)	0.13 (0.20)	1		
SP-C	0.20 (0.27)	0.10 (0.12)	0.35 (0.40)	0.15 (0.17)	0.13 (0.14)	0.21 (0.23)	0.23 (0.26)	1	
SP-D	0.00 (0.15)	0.29 (0.27)	0.17 (0.33)	0.07 (0.09)	0.19 (0.15)	0.07 (0.08)	0.06 (0.07)	0.21 (0.14)	1

The numbers in parentheses indicate the sequence similarity at the genus level (95%).

et al., 2006) or the Dry Valleys (Smith *et al.*, 2006). The higher moisture results from higher precipitation rates due to the frontal systems bringing warmer and moister air to the western part of the Antarctic Peninsula. Temperatures can change rapidly in soils or moss beds (up to 42 °C), forcing the microorganisms to design adaptation mechanisms for a survival over a wide temperature gradient (Lewis-Smith, 1986; Bölter *et al.*, 1989). The input, quantity and availability of organic compounds derived by plants, animals or autotrophic microorganisms are of importance for establishing a microbial food web. Thus, habitat conditions can vary on a micrometer scale, leading to distinct changes in bacterial community structures. However, higher carbon values were only found in two sites on Livingston Island, coinciding with a moss cover and high numbers of culturable heterotrophs. In general, cell numbers were comparable with the plate counts obtained from other Antarctic sites, ranging from 1.7×10^3 to 6.1×10^7 g^{-1} dry soil (Ramsay & Stannard, 1986; Saul *et al.*, 2005; Aislabie *et al.*, 2006, 2008).

In contrast to previous analyses of soil samples from other cold terrestrial habitats such as Siberian permafrost (Wagner *et al.*, 2005), no increase in the ratio of anteiso- to iso-fatty acids and a lack of unsaturated fatty acids for most of the samples were observed. Samples with a prevalence of unsaturated oversaturated fatty acids were restricted to several surface samples (T1-4 and SP-profiles). Adaptation mechanisms other than unsaturation that maintain the fluidity of the cell membrane can be changes in lipid polar head groups, changes in the protein content, changes in the carotenoid composition of the membranes and changes of

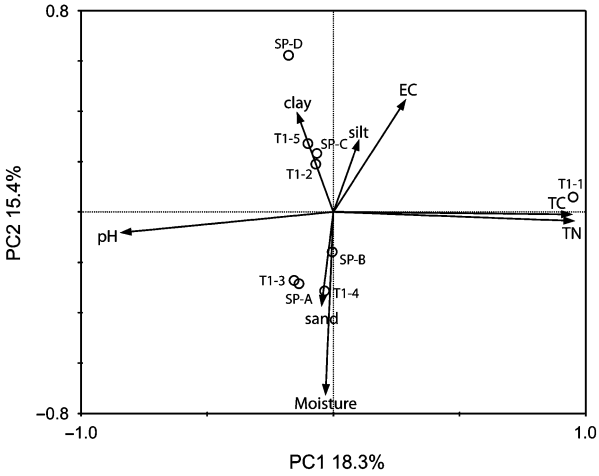

Fig. 5. CCA plot of the investigated soil profiles. Circles indicate the different sampling sites in relation to different soil physical and soil chemical parameters (T1-1 to T1-5; SP-A to SP-D). Arrows indicate the different weights and orientations of the investigated soil factors in the CCA plot.

the length of the fatty acids (Chintalapati *et al.*, 2004; Mangelsdorf *et al.*, 2009). The composition of the PLFA profiles suggested the dominance of a bacterial community; marker lipids for eukarya, such as linoleic acid (18 : 2 *cis*9,12), were detected in significant amounts only in the top layer of profiles T1-4, T1-5 and SP-B (Table S1), but were absent in deeper layers. Other eukaryotic polyunsaturated fatty acids such as linolenic acid were not found in any sample. Marker lipids for several bacterial taxa indicated the presence and spatial allocation of these groups. Tuberculostearic acid (18:0 10-methyl) is characteristic for several genera within the *Actinobacteria* and was found in the top layers of profiles T1-1 and T1-4.

DGGE patterns of the investigated soils showed a highly diverse bacterial community in most of the soil profiles. The sequences were related to *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Gemmatimonadetes*, *Planctomycetes* and *Proteobacteria*, revealing an establishment of a complex microbial community structure in different mineral soils under maritime Antarctic climate conditions. Most of these bacterial phyla, recently identified to be predominant in soils (Janssen, 2006), were detected with a high proportion of sequences that could not be affiliated to known genera. This is consistent with recent studies on other Antarctic soil environments (Shivaji *et al.*, 2004; Saul *et al.*, 2005; Aislabie *et al.*, 2006, 2008; Smith *et al.*, 2006; Shravage *et al.*, 2007). Yergeau *et al.* (2007) found all nine of the proclaimed dominant soil phyla in soil samples spanning a latitudinal transect from the Falkland Islands (South Atlantic) to the Ellsworth Mountains (West Antarctica). The majority of the sequences they detected were affiliated to *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Proteobacteria*. Niederberger *et al.* (2008) were also able to detect the nine 'soil' phyla, with a dominance of *Acidobacteria* (up to 50% of the clone library) and *Actinobacteria* in all samples.

Soil texture and mineral composition as well as nutrient content and nutrient quality can influence the microbial colonization of raw mineral soils. It is known that these soil characteristics can affect the metabolic activity of soil microorganisms and thus the microbial community (van Loosdrecht *et al.*, 1990; England *et al.*, 1993; Costerton *et al.*, 1995). Most of the sequences obtained from initial soils of Livingston Island belonged to the diverse phyla of the *Acidobacteria* and *Bacteroidetes*. Representatives of these groups were found in numerous terrestrial Antarctic habitats (Shivaji *et al.*, 1992; Bowman *et al.*, 1997; Brinkmeyer *et al.*, 2003; Abell & Bowman, 2005; Yergeau *et al.*, 2007; Niederberger *et al.*, 2008; Foong *et al.*, 2010). On Livingston Island, DGGE sequences related to *Acidobacteria* were restricted to soils with low carbon and nitrogen contents and neutral to alkaline pH values, whereas DGGE sequences related to *Bacteroidetes* could be found in almost all of the investigated profiles with a dominance in the moss-covered sites, where the pH is more acidic. Possibly, the input of organic compounds, provided by bryophytes (Bölter *et al.*, 2002), leads to the dominance of *Bacteroidetes* in the vegetated soils. This would be in accordance to Fierer *et al.* (2007), who found that additional carbon led to a positive correlation between the *Bacteroidetes* abundances and the carbon mineralization rates, while the abundance of *Acidobacteria* was negatively correlated with carbon mineralization rates. For the *Bacteroidetes*, this implies an important role in bryophyte-influenced Antarctic soils by degrading polymeric organic matter and providing low molecular substances for the microbial food web. Sequences belonging to *Bacteroidetes* were also found to make up an important part of the bacterial community in other cold habitats such as *Sphagnum*-covered permafrost-affected soils in Siberia (Pankratov *et al.*, 2006; Liebner *et al.*, 2008; Wagner *et al.*, 2009). However, a comparison of these data with those obtained by PLFA analyses shows that the characteristic iso-/anteiso fatty acids of the *Bacteroidetes* phylum did not dominate the lipid profiles of the samples. Therefore, this group may not represent the dominating bacterial population of the samples analyzed. This could be due to the different lysis efficiencies of bacterial cells during the DNA extraction, which could therefore lead to a preferential amplification of certain groups. Members of the phylum *Acidobacteria*, in which few isolates have been described so far (Kishimoto *et al.*, 1991; Eichorst *et al.*, 2007; Koch *et al.*, 2008; Kulichevskaya *et al.*, 2010), are widely distributed in a variety of soils (Barns *et al.*, 1999; Janssen, 2006) and other habitats, for example wastewater, sediment or hot springs (reviewed in Eichorst *et al.*, 2007). Sequences related to

Acidobacteria were also detected in Antarctic cold-desert soils (Aislabie *et al.*, 2006, 2008; Smith *et al.*, 2006; Niederberger *et al.*, 2008). They were exposed to similar geochemical conditions (low C and N values, neutral to alkaline pH) as in the bare maritime mineral soils of this study, where *Acidobacteria* represent a major portion in the overall community. Therefore, it is conceivable that members of the *Acidobacteria* play an important role in nutrient turnover in these cold, oligotrophic soils. Although somewhat speculative, it is possible that *Acidobacteria* are involved in a broad range of metabolic pathways, as they are phylogenetically highly diverse (Barns *et al.*, 2007), suggesting a large physiological potential. However, their relevance and function in the ecosystem is still unknown, even though the abundance can make up to 50% in soils (Dunbar *et al.*, 2002; Lipson & Schmidt, 2004; Niederberger *et al.*, 2008). Because only a few representatives of the *Acidobacteria* had been isolated so far, the lipid data available for this group are limited and therefore, interpretation of the PLFA profiles obtained with respect to the abundance of this group is not possible.

Additionally, a distinct number of OTUs, with up to 25% of the dominant bacterial phylotypes in the single soil profiles, could only be assigned to unclassified bacteria, leading to the suggestion that these cold soil habitats harbor a yet undescribed part of the microbial community with currently unknown physiological and ecological functions.

In four of the oligotrophic soil profiles (T1-2, T1-3, T1-5, SP-A), we were able to detect sequences that were affiliated to *Methylobacter psychrophilus* and *Methylocystis* sp., previously known from Arctic tundra wetlands (Omelchenko *et al.*, 1996; Liebner *et al.*, 2009). Accompanying CH_4 flux measurements at the same sites did not reveal any CH_4 emission, not even after intense rain, whereas Gregorich *et al.* (2006) reported a noticeable emission from lakeshore soils in an Antarctic dry valley. Lacking CH_4 emission from Livingston Island soil profiles could be firstly explained by complete oxidation of produced CH_4 through methanotrophic activity indicated by the presence of sequences related to CH_4-oxidizing bacteria or secondly methanogenic archaea might not be particularly abundant or even absent as a result of the aerobic conditions in most parts of the mineral soils, which are unfavorable for an anaerobic metabolism. This would be in accordance to our results of PLEL determination, where no archaeal biomarkers were detectable. The detection and description of methanotrophic bacteria that are able to use atmospheric CH_4 was shown in several studies (Holmes *et al.*, 1999; Bull *et al.*, 2000; Henckel *et al.*, 2000; Kolb *et al.*, 2005). Therein, sequences related to the genera *Methylobacter* and *Methylocystis*, as observed in our data, were found in very different soil types (tropical and temperate forest soils). An indication for the existence of such methanotrophs could be the negative average flux of CH_4 as it was observed for several nonornithogenic soils from maritime Antarctica (Sun *et al.*, 2002; Zhu & Sun, 2005). In contrast, ornithogenic soils, which are characterized by bird excrement (penguin guano), showed a positive CH_4 net flux comparable to values similar to mesic sites from northern high latitudes ($> 55^{\circ}$N; Bartlett *et al.*, 1992; Christensen *et al.*, 1995; Nakano *et al.*, 2000; Wille *et al.*, 2008). Further measurements as well as investigations regarding the activity and structure of methanotrophic communities could answer the question as to whether the southern ice-free polar regions, including the subpolar islands, can act as a sink of atmospheric CH_4.

The statistical analysis of the dominant soil phylotypes and the soil parameters determined showed a strong influence of the nutrient content on the community structure of the soil profile T1-1, with an obvious favoring of *Bacteroidetes* in moss-covered, acidic soils. In the other soil profiles, soil-physical parameters (clay, sand, moisture) became important for the community structure. Interestingly, pH is not positively correlated with the TC and TN content, although it is known to have an influence on the composition, conservation and availability of soil organic and inorganic components (Kemmitt *et al.*, 2006). This might be due to the low carbon and nitrogen values that favor a specific soil microbial community, which is not regulated by a large nutrient input derived from plants. In future scenarios, the predicted increase of the mean annual air temperatures in the maritime Antarctic could result in an increase in plant coverage together with a higher nutrient input and could therefore possibly lead to a change in the soil microbial community shifting from an oligotrophic, *Acidobacteria*-dominated or more diverse bacterial community to a less diverse microbial collective dominated by members of copiotrophic bacteria, for example representatives of the *Bacteroidetes* phyla.

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Statement

GenBank accession numbers: HQ153156–HQ153407

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Supporting Information

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

Table S1. PLFA profiles of Antarctic soil samples. Fatty acids are given in ng g^{-1} wet soil.

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