Bacterial diversity associated with ornithogenic soil of the Ross Sea region, Antarctica[1]

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Abstract: In the Ross Sea region of Antarctica, ornithogenic soils form on land under Adélie Penguin rookeries. Compared with mineral soils of the Ross Sea region, ornithogenic soils are generally high in microbial biomass, organic carbon, and total nitrogen and phosphorus, with high electrical conductivity and large variations in pH. The objective of this study was to assess the bacterial composition of ornithogenic soils from Cape Hallett and Cape Bird in the Ross Sea region using culture-independent methods. Soil clone libraries were constructed and those clones that occurred ≥3 times were sequenced. The bacterial diversity of the soils was dependent on the presence of penguins. *Firmicutes* most closely related to the endospore-formers (e.g., *Oceanobacillus profundus* and *Clostridium acidurici*) and (or) *Gammaproteobacteria* belonging to the genus *Psychrobacter* dominated soils currently occupied with penguins. In contrast, *Gammaproteobacteria*, closely related to cultured members of the genera *Rhodanobacter*, *Psychrobacter*, *Dokdonella*, and *Lysobacter*, dominated the soils previously colonized by penguins. Results of this study indicate that despite relatively high nutrient levels and microbial biomass, bacterial communities of ornithogenic soils were not more diverse than those of mineral soils of the Ross Sea region of Antarctica.

Key words: Antarctica, ornithogenic soil, bacterial diversity, colonized and previously colonized soil.

Résumé : Des sols ornithogènes se forment sur le sol, sous les colonies de manchots Adélie de la région de la Mer de Ross en Antarctique. Comparativement aux sols minéraux de la région de la Mer de Ross, les sols ornithogènes sont généralement riches en biomasse microbienne, en carbone organique ainsi qu'en azote et en phosphore totaux, et possèdent une forte conductivité électrique et de grandes variations de pH. L'objectif de cette étude était d'évaluer la composition bactérienne des sols ornithogènes du Cap Hallett et du Cap Bird dans la région de la Mer de Ross, par des méthodes indépendantes de la culture. Des banques de clones de bactéries des sols ont été construites et les clones apparaissant 3 fois ou plus étaient séquencés. La diversité bactérienne de ces sols était dépendante de la présence des manchots. Les espèces de *Firmicutes* les plus étroitement reliées aux organismes formant des endospores (*Oceanobacillus profundus* et *Clostridium aciduri*) et (ou) les espèces de *Gammaproteobacteria* appartenant au genre *Psychrobacter* étaient dominantes dans les sols actuellement occupés par les manchots. En revanche, les espèces de *Gammaproteobacteria* étroitement reliées aux membres cultivables des genres *Rhodonobacter*, *Psychrobacter*, *Dokdonella* et *Lysobacter* étaient dominantes dans les sols antérieurement colonisés par les manchots. Les résultats de cette étude indiquent que malgré les niveaux relativement élevés de nutriments et de biomasse, les communautés bactériennes des sols ornithogènes ne sont pas plus diversifiées que celles des sols minéraux de la région de la Mer de Ross, en Antarctique.

Mots-clés : Antarctique, sols ornithogènes, diversité bactérienne, sols colonisés et antérieurement colonisés.

[Traduit par la Rédaction]

Introduction

Antarctic soils form in ice-free areas with the largest expanse in the McMurdo Dry Valleys of the Ross Sea region. In the Adélie Penguin (*Pygoscelis adeliae* (Hombron & Jacquinot, 1841) rookeries of the Ross Sea region, the abundance of penguins causes significant inputs of organic matter from the marine environment to the soil. These soils, termed ornithogenic, are the only soils south of the Antarctic circle containing high concentrations of organic matter (Speir and Cowling 1984). Organic matter is brought to the rookery during summer when the penguins are ashore and is added to the soil as penguin guano, feathers, eggshells, and bird remains.

Microbial biomass, respiration, and nitrogen mineralization activity are typically higher in ornithogenic than in mineral soils, although inhibited by increasing phosphorus inputs associated with penguins (Tscherko et al. 2003; Barrett et al. 2006). In summer, midday soil respiration rates measured at

Received 27 October 2008. Accepted 28 October 2008. Published on the NRC Research Press Web site at cjm.nrc.ca on 29 January 2009.

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[1]This article is one of a selection of papers in the Special Issue on Polar and Alpine Microbiology.
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Seabee Hook, Cape Hallett, on an ornithogenic soil were an order of magnitude greater than that of mineral soils located in Northern and Southern Victoria Land (Barrett et al. 2006). Orchard and Corderoy (1983) showed that decomposition rates in ornithogenic soils of Cape Bird were dependent on soil temperature and moisture. Barrett et al. (2006), however, reported that microbial biomass carbon (C), but not soil organic C or moisture contents, significantly influence rates of in situ soil respiration. Enzyme activities in ornithogenic soils are also high, and include proteases, alkaline phosphatase, urease, and xylanase from soil microbes and possibly from penguin gut material and faecal organisms (Speir and Ross 1984).

Bacteria dominated the soil microbiota of ornithogenic soils under active penguin colonies; few yeasts or fungal hyphae were revealed by microscopic examination (Roser et al. 1993). Futhermore, *Archaea* were below detection limits in ornithogenic soils using microscopic or molecular tools[3] Total bacterial counts of approximately 1×10^{10} g^{-1} of dry soil occurred in surface soils (Ramsay and Stannard 1986; Roser et al. 1993), whereas the numbers of culturable bacteria varied from 1×10^5 to 1×10^7 (Ramsay and Stannard 1986; Pietr 1986). The highest numbers of heterotrophic culturable bacteria occur in surface organic layers and decrease with soil depth. Among the culturable heterotrophs, bacteria that degrade proteins, uric acid, and chitin were prevalent, as were endospore formers (Pietr 1986). In contrast to heterotrophs, the highest numbers of nitrifying bacteria have been detected in the soil layers deep in the soil profile (Pietr 1986).

There is very little published information on the bacterial composition of ornithogenic soils. Nonetheless, novel species of *Arthrobacter* and *Psychrobacter* have been isolated from Antarctic ornithogenic soils (Gupta et al. 2004; Bowman et al. 1996). In contrast, bacterial communities of mineral soils of the Ross Sea region have been well studied and shown to be dominated by the phyla *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Deinococcus-Thermus*, or *Gemmatimonadetes*, with the prevalence of each group varying between sites (Smith et al. 2006; Shravage et al. 2007; Aislabie et al. 2008*a*; Niederberger et al. 2008).

The objective of this study was to characterize the bacterial composition of ornithogenic soils from Cape Hallett and Cape Bird in the Ross Sea region using culture-independent methods. The high levels of soil C and N combined with relatively high levels of microbial biomass led us to postulate that the bacterial composition of ornithogenic soils will be more diverse than that reported for mineral soils. The soil bacterial composition is related to soil properties and compared with that of mineral soil from the Ross Sea region. Some of the data presented in this paper have been published previously (Aislabie et al. 2008*a*). However, we include new data and phylogenetic analysis of the prevalent clones retrieved from ornithogenic soil.

Materials and methods

Site descriptions, soil sampling, and chemical analysis

Soil samples were collected from Cape Hallett (CH), Northern Victoria Land (72°19′S, 170°13′E), and Cape Bird (CB) on the northern tip of Ross Island (77°13′S, 166°26′E) in Southern Victoria Land.

Cape Hallett is a peninsula formed from a basalt shield volcano. Near the north end of the peninsula is Seabee Hook, a low-lying gravel spit formed by the gradual buildup of beach ridges. On Seabee Hook ornithogenic soils occur on beach ridges either currently occupied or previously occupied by Adélie Penguins (for further details see Hofstee et al. (2006)). Soils of the spit were disturbed owing to the establishment of Hallett Station. Human disturbance of the soil resulted from building and roading activities and contamination with hydrocarbons. Ornithogenic soil samples were collected from 2 locations on the spit (CH9 and CH13) and 1 location (CH1) west of the spit and adjacent to Willet Cove in January 2004. CH1 was a previously colonized penguin nesting area and CH9 was from a currently occupied mound. CH13 was a previously colonized penguin nesting area on disturbed soil near a fuel storage tank, and was collected from within the zone of an oil spill that occurred in 1994 (Gilmore 2001). Total petroleum hydrocarbons levels were 5 760 and 10 300 $\mu g \cdot g^{-1}$, respectively, for samples 13/1 and 13/4 collected from CH13. Chromatograms of the residual hydrocarbon contamination were indicative of highly weathered material.[4]

At Cape Bird soil samples were collected in January 2003 from an occupied site within the northern Adélie Penguin rookery (for further details see Heine and Speir (1989)). Soils inhabited by penguins contain stones (3–10 cm in diameter) used for nest building combined with organic matter (i.e., guano, dead birds, feathers, and eggshells in various stages of decomposition) in the upper 30–50 cm, overlying gravelly sand (Heine and Speir 1989; Hofstee et al. 2006).

Soil pits were dug at each of the sites, and single composite samples (ca. 1 kg) were collected from the surface and from several lower layers for chemical and microbial analyses using an ethanol-swabbed trowel. Soil samples for analysis were taken from CH1 (0–5 and 30–60 cm depths), CH9 (0–10, 10–25, and 40–50 cm depths), and CH13 (0–3 and 30–40 cm depths). The depths chosen were based on pedological descriptions of the soil materials. Soil samples were placed in sterile Whirl-Pak bags (Nasco), frozen at –20 °C, and transported to New Zealand for processing. We previously published soil clone library data for CH9 samples 9/1 and 9/2 (Aislabie et al. 2008*a*).

Soil subsamples (<4 mm fraction) were analysed for water content, pH, electrical conductivity (EC), nitrate and ammonium-N, and total phosphorus (P) using standard methods (Blakemore et al. 1987). Total organic C and N were determined in a Leco FP-2000 analyser at 1050 °C. Soil chemical properties are rated from very low to very high following Blakemore et al. (1987).

Total microbial counts were determined using epifluorescence microscopy with diphenylamidino indophenol (DAPI) staining (Bottomley 1994). For enumeration of culturable microbes in soil from Cape Hallett, soil (10 g wet mass) was shaken for 1h at 4 °C in 90 mL of 0.1% (*m*/*v*) sodium pyrophosphate (pH 8) containing 30 g glass beads (3 mm) and then diluted in phosphate-buffered saline. Numbers of

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culturable heterotrophic bacteria were then determined by plating soil dilutions (1×10^{-3} to 1×10^{-7}) onto R2A (Difco) agar plates and incubated at 15 °C for 4 weeks.

Bacterial community analysis

To extract DNA, 0.5 g of soil was transferred to a sterile 2 mL screw cap microtube (Porex Bio Products Inc.) containing 0.5 g each of 0.1 and 3.00 mm silica–zirconium beads (BioSpec Products Inc., Bartesville Oklahoma). The tubes were amended with 270 μL of phosphate buffer (100 mmol/L Na_2HPO_4, pH 8.0) and 30 μL of lysozyme solution (10 mg/mL) and incubated for 1 h at room temperature. Equal volumes (300 μL) of lysis buffer (100 mmol/L NaCl, 500 mmol/L Tris, pH 8.0, 10% sodium dodecyl sulfate (SDS)) and chloroform – iosamyl alcohol (24:1) were added to the tubes and they were shaken twice vigorously (45 m/s) for 40 s in a Bio 101 FastPrep FP120 instrument (Savant Instruments Inc., Holbrook, New York). The tubes were then spun at 13 100*g* for 5 min to sediment debris and 650 μL of supernatant was recovered for subsequent DNA purification. Proteins and SDS were precipitated by adding 360 μL of 7 mol/L ammonium acetate and pelleted with a microfuge at full speed for 5 min, and the upper aqueous layer was recovered. DNA was precipitated by adding 0.54 volumes of isopropanol, incubating for 15 min at room temperature, and then centrifuging at 13 100*g* for 5 min. The supernatant was discarded and the pellet washed with 1 mL of 70% ethanol. Following removal of the supernatant, the pellet was allowed to air dry for 30 min, then it was dissolved in 50 μL of sterile distilled water.

Bacterial 16S rRNA genes were amplified from extracted soil DNA by PCR using the oligonucleotide primers PB36 (5′-AGRGTTTGATCMTGGCTCAG-3′) and PB38 (5′-GKTACCTTGTTACGACTT-3′), corresponding to *Escherichia coli* positions 8–27 and 1492–1509, respectively (numbering per Brosius et al. (1981)) (Foght et al. 2004). PCR was carried out in a final volume of 25 μL using 1 μL of template DNA, 3.0 mmol/L MgCl, 200 μmol/L each dNTP, 0.5 μL of BSA solution (10 mg/mL), 0.2 μmol/L of each primer and 0.5 U Platinum *Taq* polymerase (Invitrogen) with 1× concentration of the supplied buffer (Applied Biosystems, Foster City, California). PCR amplification was carried out using a program of 94 °C for 3 min followed by 25 cycles of 94 °C, 45 s; 55 °C, 45 s; and 72 °C, 90 s. Duplicate PCR reactions were performed on each soil sample and the resulting products pooled and then purified using a High Pure PCR Purification Kit (Roche) according to the manufacturer's instructions.

Amplicon libraries were constructed in a T-tailed pGEM vector. For each clone library, 90 randomly selected colonies were prepared for restriction fragment length polymorphism (RFLP) analysis. The inserts within the plasmid vectors were recovered by PCR from whole cell lysates. Lysates were prepared from overnight broth culture of clone libraries prepared in microtitre trays. A 50 μL sample of each culture was transferred to a new plate containing 100 μL of water. The suspensions were heated at 95 °C for 30 min and 2 μL of the suspension used as the template for PCR reaction. Vector-specific primers pGEM-F (5′-GGCGGTCGCGGGAATTCGATT-3′) and pGEM-R (5′-GCCGCGAATTCACTAGTGATT-3′) were used for PCR amplification under the same program and conditions as described for primers PB36 and PB38 except that no BSA was added when amplifying from the vector.

The PCR products from clone libraries were screened for similarity using RFLP analysis using *Hae*III. Fingerprint profiles were compared for similarity using the program GelCompar II (version 2.2 Applied Maths; Kortrijk, Belgium). Any ribotype containing three or more members was selected for DNA sequencing; a ribotype being defined as any unique pattern of DNA fragments found when the amplified 16S rRNA genes of the clones were digested with *Hae*III. Purification and sequencing of bacterial 16S rRNA gene PCR products representative of the RFLP patterns obtained was performed by Macrogen (www.macrogen.com). Sequences were screened for chimeras using CHIMERA_CHECK (version 2.7) of the Ribosomal Database Project (RDP). Partial 16S rRNA gene sequences were matched to good sequences (>1200 bp) in the RDP II – Release 9 (RDP) using the Sequence Match search tool. The taxonomic assignment of the sequences was determined using the RDP Classifier tool with a 95% confidence threshold. The sequences were deposited in GenBank.

Soil clone library diversity measures

We characterized the bacterial clone library composition by ribotype richness and dominance using the RFLP abundances. The total ribotype richness in the soil samples was determined using the S_{chao1} estimator (Kemp and Aller 2004) and dominance was estimated from the Berger and Parker (1970) index, which is the abundance of the most numerous ribotype expressed as a proportion of total ribotype abundance [note: evenness = 1 – Dominance]. Clone library coverage was determined using Good's coverage (Kemp and Aller 2004).

Discrimination of bacterial communities was examined by semi-strong-hybrid (SSH) multidimensional scaling ordination (Faith et al. 1987; Belbin 1991), with the Gower (1971) metric as the measure of community distance. The analyses included samples from mineral soils of the Ross Sea region described by Aislabie et al. (2008*a*) to provide for a robust characterization of bacterial community structure across the mineral to ornithogenic gradient. Data input into the ordination was restricted to community-dominant ribotypes (CD-ribotypes, those unique clones with absolute frequencies ≥3 members within individual sample clone libraries), combined with total abundance of ribotypes within bacterial phyla. The ordination was implemented in the PATN software package (Belbin 1995). Principal axis correlation (PCC) (Belbin 1991) analysis was then performed in PATN to examine directionality and correlation, in the ordination space, of gradients in the measured soil parameters.

Phylogenetic methods

Because of their prevalence in ornithogenic soil *Firmicutes* and *Gammaproteobacteria* were chosen for phylogenetic analysis. In addition to the clones recovered in this study, sequences were included from GenBank on the basis of RDP results and sequence quality. For phylogenetic analyses of the *Gammaproteobacteria*, we also included the bacterial cultures CH14i and CH23i, isolated from Cape Hallett

groundwater (Aislabie et al. accepted), and CH51i, CH83i, and CH71i from Cape Hallett soil.[4]

Multiple sequence alignment and phylogenetic analysis of 16S rRNA gene sequences were performed using CLUSTAL_X (Thompson et al. 1997) and PHYLIP version 3.65 or 3.66 (Felsenstein 1989). Distance matrices and unrooted phylogenetic trees were constructed using the Kimura 2-parameter (Kimura 1980) and neighbor-joining (Saitou and Nei 1987) methods, respectively. Phylogenetic trees were bootstrapped using 1000 replicates and branch lengths, as determined by the method of Fitch and Margoliash (1967), and were superimposed on the consensus branching orders.

Results

Soil properties

Properties of the ornithogenic soil from Cape Hallett varied depending on whether the soil was currently occupied by penguins (Table 1). Notably, surface soil from the occupied site (CH9) had higher organic C and total N contents, higher salinity (measured as electrical conductivity, EC), and higher water content than those of the previously colonized sites (CH1 and CH13). The soil properties also varied with depth. Compared with the surface soils, those from the subsurface had lower levels of organic C, total N and P, EC, and water content.

The pH of the soil from sites CH9 and CH13 was strongly alkaline (pH > 7.6) and that from CH1 was moderately acidic (pH 5.6–5.9). Total N, much of which occurs as NH_4^+-N, was high (>0.6 mg/kg) in the surface soil and low (<0.3 mg/kg) in the subsurface. Total P was very high (>0.12%) at all sites. The EC was typically very high (>0.7 mS/cm) except for subsurface soil from sites CH1 and CH13. The organic C in the soils was rated as high (10%–20%) in CH9/1, medium (4%–10%) in CH1/2 and CH9/2, and low or very low (<4%) for the remaining samples. The C/N ratio was very low (<10). The C/N ratio of the soil from the previously colonized penguin nest sites was higher than that of the occupied site, indicating decreased inputs of N combined with a loss of N over time owing to leaching or metabolism by soil bacteria.

The soil from Cape Bird (CB1 and CB2) was most similar to that of the upper layers from the occupied mound (CH9/1 and CH9/2) at Cape Hallett (Table 1). The soil had high levels of organic C in the surface soil, and both soil depths had high levels of total N and P, NH_4^+-N, EC, and a low C/N ratio. In contrast to Cape Hallett soil from the occupied site, Cape Bird soil had lower soil moisture and acid pH.

Numbers of bacteria were higher in surface soils than subsurface soils for all sites, except for CH13 where higher numbers of bacteria in the subsurface may reflect hydrocarbon contamination (Table 1). Total direct counts revealed >1 × 10^8 cells/g dry mass. No fungal hyphae or yeast were observed. Numbers of culturable aerobic heterotrophs were <21% of the total direct counts in the Cape Hallett soil.

Structure of soil bacterial communities

16S rRNA gene clone libraries were prepared from the total community DNA extracted from soil from 3 locations at Cape Hallett and 1 at Cape Bird. Between 70 and 90 clones per sample were screened by RFLP (Table 2). The clones

Table 1. Chemical properties of ornithogenic soil samples collected from Cape Hallett and Cape Bird, Ross Sea region, Antarctica.

Sample Id	Soil depth (cm)	Water content (% dry mass)	pH	Organic C (%)	Total N (%)	C/N ratio	Nitrate-N (mg/kg)	NH_4^+-N (mg/kg)	Total P (%)	EC (mS/cm)	Total counts (g^{-1} dry mass)[a]	Aerobic heterotrophs (g^{-1} dry mass)[a]
Cape Hallett (previously colonized soil)												
CH1/2	Surface (0–5)	20.2	5.6	4.3	0.9	5	70.1	18.6	3.6	1.2	1.6×10^9 (±0.1)	1.1×10^8 (±0.2)
CH1/5	Subsurface (30–60)	7.9	5.9	0.1	0.04	3	4.3	70.5	0.5	0.1	4.3×10^7 (±0.7)	1.4×10^5 (±0.4)
Cape Hallett (occupied soil)												
CH9/1	Surface (0–10)	30.5	7.7	11.7	4.7	2	385	8748	3.6	15.4	6.8×10^9 (±0.6)	1.1×10^8 (±0.1)
CH9/2	Subsurface (10–25)	27.6	8.0	9.4	4.2	2	378	9296	5.1	13.5	1.8×10^9 (±0.2)	6.1×10^6 (±0.6)
CH9/4	Subsurface (40–50)	3.2	8.3	0.2	0.2	1	41.9	1350	0.5	1.5	2.6×10^8(±0.2)	2.4×10^3 (±0.6)
Cape Hallett (previously colonized disturbed soil)												
CH13/1	Surface (0–3)	9.3	8.0	3.7	0.7	6	42.8	584	1.4	0.7	1.5×10^9(±0.1)	1.9×10^9 (±0.3)
CH13/4	Subsurface (30–40)	5.5	7.9	1.5	0.3	6	37.2	624	0.9	0.5	1.3×10^9 (±0.1)	2.7×10^8 (±0.2)
Cape Bird (occupied soil)												
CB1	Surface (0–5)	7.5	6.5	22.6	17.0	1.3	734	46 610	2.6	25.3	3.0×10^9 (±0.4)	ND
CB2	Subsurface (10–20)	6.3	6.9	2.1	1.6	3.3	19.5	16 244	4.0	10.3	5×10^8 (±0.3)	ND

Note: ND, not determined; EC, electrical conductivity.
[a]Mean count (±1 SD).

Table 2. Number of clones in the soil clone libraries, the number of unique ribotypes and those that occurred ≥3 times (community-dominant (CD) ribotypes), percentage of clones assigned to CD ribotypes, and dominance, richness estimates, and sampling coverage for each sample.

Sample location	Sample depth (cm)	No. of clones	No. of unique ribotypes	No. of CD ribotypes	Clones assigned to CD ribotypes (%)	Ribotype richness (S_{chao1})	Ribotype dominance	Good's coverage
Cape Hallett (previously colonized soil)								
CH1/2	Surface (0–5)	82	31	6	63	63	0.29	0.76
CH1/5	Subsurface (30–60)	70	34	5	53	95	0.27	0.64
Cape Hallett (occupied mound soil)								
CH9/1	Surface (0–10)	87	27	7	76	115	0.22	0.78
CH9/2	Subsurface (10–25)	86	27	5	75	135	0.55	0.76
CH9/4	Subsurface (40–50)	82	36	4	60	256	0.39	0.62
Cape Hallett (previously colonized disturbed soil)								
CH13/1	Surface (0–3)	83	57	4	29	78	0.10	0.45
CH13/4	Subsurface (30–40)	90	35	5	62	101	0.39	0.71
Cape Bird								
CB1	Surface (0–5)	88	4	3	99	5	0.83	0.99
CB2	Subsurface (10–20)	80	19	4	78	36	0.53	0.85
Average across all ornithogenic soils[a]		83±10	30±14	5±1	66±19	98±71	0.40±0.22	0.73±0.15
Average across mineral soils[a,b]		106±29	50±16	7±3	53±14	87±44	0.14±0.07	0.63±0.13

[a]Mean (±1 SD).
[b]From Aislabie et al. (2008*b*).

were grouped within 4–57 ribotype patterns per clone library. Although many of the ribotypes were rare, from 3 to 7 occurred ≥3 times in each library (CD-ribotypes), and between 29% and 99% of the clones in the libraries were assigned to CD-ribotypes. The S_{chao1} estimates of total ribotype richness varied from 5 to 256, and were higher in samples taken from the subsurface. The dominance index varied from 0.1 to 0.83. There was no association between patterns of richness and dominance. Coverage, the estimated proportion of ribotypes in the environment represented in our clone libraries, ranged from 0.45 to 0.99.

Numerically dominant ribotypes in soil clone libraries

16S rRNA gene sequencing was limited to those clones numerically dominating the respective samples (i.e., CD-clones with relative frequencies ≥3). Between 29% and 99% of the clones in the libraries were therefore treated as the CD-ribotypes. Of the 34 CD-ribotypes sequenced, all had ≥0.600 similarity to 16Sr RNA gene sequences in RDP (June 2008), and 7 were ≥0.950 similar (Tables 3 and 4). Twelve CD-ribotypes grouped within the *Proteobacteria* (with 11 assigned to the *Gammaproteobacteria* and 1 to the *Alphaproteobacteria*), 11 within the *Firmicutes*, 7 in the *Actinobacteria*, 3 in the *Bacteroidetes*, and 1 within *Deinococcus-Thermus* (Tables 3 and 4). The closest matches of the CD-ribotypes were from various sources including soil and Antarctic or marine ecosystems. The CD-ribotypes were taxonomically affiliated to heterotrophic bacteria that grow under aerobic (e.g., *Rhodanobacter* and *Dokdonella*) or anaerobic conditions (e.g., unclassified *Clostridiales*).

Bacterial composition of the ornithogenic soils and phylogenetic analysis

The distribution of the CD-clones within the samples analysed, the closest relatives of the clones sequenced, and their assignment to bacterial phyla, family, and (or) genus by the RDP Classifier are given in Tables 3 and 4 for soils from Cape Hallett and Cape Bird, respectively. Between 1 and 8 clones were sequenced per ribotype depending on the distribution of the ribotypes between the samples and the number of clones belonging to the ribotype. *Gammaproteobacteria* (Fig. 1) and *Firmicutes* (Fig. 2) were selected for phylogenetic analysis as they were well represented in ornithogenic soil. In general, there was good agreement between the naïve Bayesian classification method of RDP Classifier (Tables 3 and 4) and the neighbor-joining method (Figs. 1 and 2) employed for the analysis of the 16S rRNA gene sequences obtained during this study.

Gammaproteobacteria were prevalent in soil from Cape Hallett but not detected in soil from Cape Bird (Tables 3 and 4). All but one of the gammaproteobacterial ribotypes was assigned to the families *Xanthomonadaceae*, *Moraxellaceae*, or *Pseudomonadaceae*. Six of the 11 gammaproteobacterial ribotypes belonged to the family *Xanthomondaceae* and 4 ribotypes clustered with the genera *Rhodanobacter* (C, D, and E) and *Lysobacter* (I). Three ribotypes belonging to *Moraxellaceae* clustered with *Psychrobacter* (G and H) or *Alkanindiges* (A). A single ribotype belonged to the *Pseudomonadaceae* (J) and clustered with unclassified bacterium CHNDP38 recovered from swine effluent. One ribotype (K) was only loosely related to *Ectothiorhodospira shapsohnikovii* and clustered with uncultured soil bacterium M54_Pitesti (Fig. 1).

Five of the gammabacterial CD-ribotypes (B, C, D, E, and F) were exclusive to the previously colonized site CH1 and were most closely related to uncultured bacterial clones, some of which were detected in lake sediment receiving penguin droppings. Ribotype I was affiliated with *Lysobacter* and detected exclusively in surface soil from the previously colonized disturbed site (CH13). Clones belonging to ribo-

Table 3. Taxonomic affiliation and abundance and distribution of bacterial groups (ribotypes) in soil from Cape Hallett (CH), Antarctica, as defined by restriction fragment length polymorphism (RFLP) analysis of amplified 16S rRNA gene sequences and sequencing.

Ribotype	Distribution of ribotypes in soil							Clones sequenced	Accession No.	Closest match (GenBank Accession No., seqmatch score; habitat, assignment by RDP classifier)
	1/2	1/5	9/1	9/2	9/4	13/1	13/4			
Gammaproteobacteria										
A							35	13/4_9A, 13/4_4G	FJ380171, FJ380170	Uncultured bacterium EV818BHEB5102702SAS67 (DQ256350, 0.844 and 0.843; subsurface water, *Alkanindiges*)
B	21							1/2_1D, 1/2_4D	FJ380129, FJ380132	Cimanggu media isolate 88 (AF229452, 0.795 and 0.799; hot springs, unclassified *Xanthomonadaceae*)
C	4	19						1/2_6C	FJ380133	Uncultured bacterium AKAU4141 (DQ125888, 0.911; soil, *Rhodanobacter*)
								1/5_6F	FJ380139	Uncultured bacterium KD7-52 (AY218713, 0.930; Antarctic lake sediment,* *Rhodanobacter*)
								1/5_1C	FJ380138	Uncultured bacterium KD8-16 (AY218686, 0.937; Antarctic lake sediment,* *Rhodanobacter*)
D	3	2						1/5_10F	FJ380141	Uncultured bacterium AKAU3857 (DQ125745; 0.925; soil, *Rhodanobacter*)
E		3						1/5_7D	FJ380140	Uncultured bacterium KD7-52 (AY218713, 0.922; Antarctic lake sediment,* unclassified *Xanthomonadaceae*)
F	7							1/2_1G, 1/2_2D	FJ380130, FJ380131	Uncultured bacterium KD8-80 (AY218694, 0.881 and 0.914; Antarctic lake sediment,* *Dokdonella*)
G			12			5		9/1_5E	EU009756	*Psychrobacter vallis* CMS 56 (AJ584832, 0.949; Antarctic cyanobacterial mat, *Psychrobacter*)
								13/1_7C	FJ380164	*Psychrobacter aquimaris* SW-210 (AY722804, 0.938; seawater, *Psychrobacter*)
								13/1_9G	FJ380166	*Psychrobacter maritimus* (T) Pi2-20 (AJ609272, 0.962; sea ice, *Psychrobacter*)
H			6					9/1_6E	EU009757	*Psychrobacter* sp. M176 (EF061890, 0.961; unknown, *Psychrobacter*)
I						8		13/1_2E, 13/1_7E	FJ380162, FJ380165	*Lysobacter* sp. 3070 (AM111012, 0.950 and 0.962; deep sea sediment, *Lysobacter*)
J							6	13/4_10H	FJ380169	Swine effluent bacterium CHNDP38 (DQ337540, 0.950; swine effluent holding pit, unclassified *Pseudomonadaceae*)
K						7		13/1_5G	FJ380163	Uncultured bacterium M54_Pitesti (DQ378269, 0.974; soil, *Gammaproteobacteria*)
Alphaproteobacteria										
L						4		13/1_8F	FJ380167	Uncultured bacterium C58 (EU234233, 0.882; river water, unclassified *Rhizobiales*)
Firmicutes										
M		1	10	47	32		9	9/1_3D, 9/1_3E, 9/2_10B	FJ380150, FJ380151, FJ380154	*Oceanobacillus profundus* CL-MP28 (DQ386635, 0.677–0.686; deep sea sediment, unclassified *Bacillaceae*)

Table 3 (*continued*).

Ribotype	Distribution of ribotypes in soil 1/2	1/5	9/1	9/2	9/4	13/1	13/4	Clones sequenced	Accession No.	Closest match (GenBank Accession No., seqmatch score; habitat, assignment by RDP classifier)
								9/2_3A, 9/2_11C, 9/4_2D, 9/4_5B, 13/4_8E	EU009752, FJ380155, FJ380159, FJ380157, FJ380168	Uncultured bacterium AKIW500 (DQ129377, 0.675–0.685; urban aerosol, unclassified *Bacillaceae*)
N				4				9/2_3F	EU009753	*Oceanobacillus profundus* CL MP28 (DQ386635, 0.725; deep sea sediment, unclassified *Bacillaceae*)
O				4				9/2_11H	EU009754	*Oceanobacillus profundus* CL MP28 (DQ386635, 0.710; deep sea sediment, unclassified *Bacillaceae*)
P			5					9/1_11C	EU009751	Uncultured bacterium AKIW500 (DQ129377, 0.600; urban aerosol, unclassified Bacilli)
Q		1	19	6	7			9/1_2A, 9/1_8C, 9/2_2E, 9/1_11H, 9/4_8E	EU009750, FJ380148, FJ380156, FJ380149, FJ380158	*Clostridium acidurici* (M59084, 0.648–0.657; soil, unclassified *Clostridiales*)
R			9					9/1_4A, 9/1_7A	EU009748, FJ380147	*Sporosarcina aquimarina* SAFN-008 (AY167819, 0.911 and 0.888; space craft facility, *Sporosarcina*)
S			5					9/1_2F	EU009749	Swine effluent bacterium BBDP62 (DQ337518, 0.805; swine effluent, *Trichococcus*)
T				3				9/2_4H	EU009755	*Erysipelothrix rhusiopathiae* Pecs 56 (AB055907, 0.690; unknown, *Erysipelothrix*)
Actinobacteria										
U		7						1/5_6D	FJ380144	*Tetrasphaera* sp. Ellin176 (AF409018, 0.842; soil, unclassified *Intrasporangiaceae*)
V					6			9/4_8A	FJ380160	Uncultured actinobacterium AKYG619 (AY921831, 0.776; farm soil, unclassified *Actinobacteria*)
W	5							1/2_2A, 1/2_12A	FJ380134, FJ380135	*Patulibacter minatonensis* (T) (AB193261, 0.804 and 0.809; soil, *Patulibacter*)
X	4							1/2_1C	FJ380136	Uncultured bacterium AKAU3561 (DQ125568, 0.954; soil, unclassified *Microbacteriaceae*)
Y		3				1		1/5_6A, 1/5_6E	FJ380142, FJ380143	*Mycobacterium kumamotonense* CST7274 (AB239925, 0.880 and 0.890; clinical specimen, *Mycobacterium*)
Z							3	13/4_1B	FJ380172	*Arthrobacter rhombi* (Y15884, 0.932; Greenland halibut, *Arthrobacter*)
A1							3	13/4_8A	FJ380173	*Arthrobacter* SPC 26 (AM931709, 0.965; Antarctic sediment, *Arthrobacter*)
Bacteroidetes										

Table 3 (*concluded*).

Ribotype	Distribution of ribotypes in soil						Clones sequenced	Accession No.	Closest match (GenBank Accession No., seqmatch score; habitat, assignment by RDP classifier)
	1/2	1/5	9/1	9/2	9/4	13/1	13/4			
A2	8	5						1/2_3B	FJ380137	Uncultured bacterium KD4-13 (AY218628, 0.896; Antarctic lake sediment,* unclassified *Sphingobacteriales*)
								1/5_4A	FJ380145	uncultured bacterium; KD8-73 (AY218691, 0.891; Antarctic lake sediment,* unclassified *Sphingobacteriales*)
A3				4				9/4_1C	FJ380161	Uncultured bacterium ELB25-087 (DQ015772, 0.741; Antarctic lake, unclassified *Saprospiraceae*)

*Contaminated with penguin droppings.

type A and affiliated with *Alkanindiges* were most abundant and found exclusively in subsurface soil from CH13 that was located in an oil spill zone (Table 3). Ribotypes affiliated with *Psychrobacter* (G and H) were prevalent in surface soil from the occupied and disturbed sites (Table 3). The sole CD-ribotype assigned to the *Alphaproteobacteria* occurred exclusively in soil from the disturbed site and were not closely related to known genera (Table 3).

Most of the CD-clones retrieved from soil occupied by penguins from Cape Hallett (CH9) and Cape Bird (CB) belonged to the *Firmicutes* and were most closely related, albeit distantly, to heterotrophic endospore-forming bacteria including *Oceanobacillus profundus*, *Clostridium acidurici*, and *Sporosarcina aquimarina* (Tables 3 and 4). Eight of the 11 *Firmicutes* ribotypes, however, could not be assigned to recognized genera using RDP Classifier, indicating that they likely belong to novel species. Five *Firmicutes* CD-ribotypes (M, N, O, A4, and A5) belonging to the Bacillaceae, formed 2 clusters, 1 of which clustered together with uncultured bacterium AKIW500 and uncultured bacterium TP80. Their closest affiliations with known organisms were a group of moderately halotolerant bacilli including the genera *Paucisalibacillus*, *Salinibacillus*, *Oceanobacillus*, *Lentibacillus*, and *Virgibacillus*, and a cluster containing *Sporosarcina* species (Fig. 2). Two ribotypes were common to both sites. Ribotypes M and N from Cape Hallett soil were identical to ribotypes A4 and A5, respectively, from Cape Bird soil.

The second most common *Firmicutes* CD-ribotype (Q) in the Cape Hallett soil belonged to the order *Clostridiales* and was distantly related to *C. acidurici* and *Clostridium purinilyticum* and *Sporosarcina* species (Fig. 2). Ribotype R clustered among the *Sporosarcina*, a member of the *Planococcaceae*. Ribotypes S and A6 belong to the *Carnobacteriaceae* and clustered with *Trichococcus* and *Atopostipes suicloacalis*, respectively. Ribotype T clustered with *Erysipelothrix* species within the family *Erysipelotrichaceae* (Fig. 2).

A few ribotypes were also assigned to *Actinobacteria*, *Bacteroidetes*, and *Deinococcus-Thermus* (Tables 3 and 4). Some of the actinobacterial ribotypes were affiliated with the genera *Patulibacter*, *Mycobacterium*, and *Arthrobacter*, whereas others could not be assigned to known genera using RDP classifier. The closest relatives of many of the clones assigned to *Bacteroidetes* or *Deinococcus-Thermus* are from Antarctic sources.

Discrimination analysis

Only the first 2 axes of ordination are presented as these accounted for 86% of the variance in bacterial community composition (stress values: axis 1 = 0.277; axis 2 = 0.137; axis 3 = 0.068). The SSH ordination, depicting dispersion of sites shown in Fig. 3A, clearly indicates separation of mineral and ornithogenic soils on the basis of bacterial community structure. Further, within the ornithogenic soils, the penguin-occupied sites are differentiated. Figure 3B illustrates the gradients in the measured soil parameters, and the centroids for the bacterial phyla, in the ordination space. All gradients significantly ($p < 0.01$) correlated with the ordination space. The fit of bacterial phyla to the ordination space is robust ($p < 0.01$), except for *Alphaproteobacteria*. These analyses indicate strong association of the CD-ribotypes be-

Table 4. Taxonomic affiliation and abundance and distribution of bacterial groups (ribotypes) in soil from Cape Bird, Antarctica, as defined by restriction fragment length polymorphism (RFLP) analysis of amplified 16S rRNA gene sequences and sequencing.

Ribotype	Ribotype distribution CB1	CB2	Ribotype sequenced	Accession No.	Closest match (GenBank Accession No., seqmatch score; habitat, genus)
Firmicutes					
A4	73	42	J1, J2, J3, J4	FJ380177, FJ380178, FJ380179, FJ380180	*Oceanobacillus profundus* CL-MP28 (DQ386635, 0.665–0.727; deep sea sediment, unclassified *Bacillaceae*)
A5	8	2	J8, J9	FJ380183, FJ380184	Uncultured bacterium TP80 (EF205590, 0.755–0.759; geothermal spring mat, unclassified *Bacillaceae*)
			J10	FJ380185	*Oceanobacillus profundus* CL-MP28 (DQ386635, 0.701; deep sea sediment, unclassified *Bacillaceae*)
A6	6	10	J5, J7	FJ380181, FJ380182	*Atopostipes suicloacalis* PPC79 (T) (AF445248, 0.703–0.723; swine manure storage pit, *Atopostipes*)
Deinococcus/Thermus					
A7		6	AL	FJ380187	*Deinococcus saxicola* sp. AA1444 (T) (AJ585984, 0.938; Antarctic rock, *Deinococcus*)
Bacteroidetes					
A8		4	AB	FJ380186	Uncultured soil forest bacterium DUNssu136 (AY913343, 0.772; soil, unclassified *Flexibacteraceae*)

longing to *Firmicutes* with the high-nutrient, high-EC conditions of the penguin-occupied sites (CH9 and CB). Whereas *Gammaprotobacteria* were widely represented in our samples, these bacteria are indicated to be most prevalent in previously colonized ornithogenic soils. In contrast, CD-ribotypes in the phyla *Actinobacteria*, *Betaproteobacteria*, *Bacteriodetes*, *Acidobacteria*, and *Deinococcus-Thermus* were most prevalent in the mineral soils, with the latter 3 groups most strongly associated with alkaline pH and high C:N conditions.

Discussion

To determine the genetic diversity of bacterial communities associated with ornithogenic soil from Cape Hallett and Cape Bird, we used culture-independent methods involving amplification of soil 16S rRNA genes and RFLP. The identity of ribotypes that dominated the soil bacterial communities was identified by sequencing 16S rRNA genes and matching them to those in the RDP. As we have used this approach previously for Antarctic mineral soils (Aislabie et al. 2008*a*) we were able to compare the structure of the soil bacterial communities of the ornithogenic soils with those of mineral soils. We speculate on the adaptations of the bacteria in the soil samples on the basis of what is known of their putative close relatives and soil conditions.

Bacterial diversity in ornithogenic soil compared with mineral soil

Relatively low richness, combined with varied degrees of dominance has led us to conclude that the bacterial communities of soils from the Ross Sea region are less diverse than those of northern latitudes (Aislabie et al. 2008*a*). When comparing clone library statistics for ornithogenic soils with mineral soils from the Ross Sea region it appears that despite high levels of organic C, N, and microbial biomass, ornithogenic soils are not more diverse than mineral soils (Table 2). To help comparison of the bacterial diversity of ornithogenic soil with that of mineral soil from the Ross Sea region we calculated the S_{chao1} estimator of ribotype richness (Kemp and Aller 2004) and the Berger–Parker dominance index (Berger and Parker 1970). The S_{chao1} value of the ornithogenic soils averaged 98 ± 71 and is similar to that for mineral soils (87 ± 44). In contrast, the dominance index for ornithogenic soils averaged 0.40 ± 0.22 compared with 0.14 ± 0.07 for the mineral soils. Thus, whereas bacterial communities in Antarctic ornithogenic soils can be ribotype rich relative to communities in Antarctic mineral soils, their structure is also one where a few ribotypes are dominant in prevalence.

Commonly, surface soils exhibit high richness and low dominance (Tringe et al. 2005). Bacterial dominance has been reported, however, for soils from saturated subsurface environments (Zhou et al. 2002). Zhou et al. (2002) proposed that the dominance structure was a result of competitive interactions enabled by excess water that allows for a high level of connectivity (low spatial isolation) and ample opportunity for the dispersal of microbes and nutrients. Under these conditions, the authors suggested that most bacterial species are eliminated by competitive exclusion, whereas a few species, better adapted to local conditions, dominate. According to the Zhou et al. (2002) model, we expect low dominance in the unsaturated soils of the Ross Sea region owing to high spatial isolation. However, this prediction is only partially consistent with our results. We suggest that dominance in Antarctic soils is likely brought about by the interplay of available nutrients (both quality and quantity are important) and adaptation to the harsh environmental conditions.

Bacterial composition of ornithogenic soil influence of nutrients

The bacterial groups dominating ornithogenic soils depended on the influence of penguins. For those sites currently occupied by penguins, *Firmicutes* and the gammaproteobacte-

Fig. 1. Unrooted neighbour-joining tree of *Gammaproteobacteria* clones retrieved from ornithogenic soils of the Ross Sea region, Antarctica (in bold face), and reference sequences retrieved from GenBank. Bootstrap percentage values >70% are indicated above the nodes. 16S rRNA gene sequences from Cape Hallet isolates CH14i, CH23i, CH51i, CH71i, and CH83i (Aislabie et al. 2009) are included for comparison. The scale bar shown represents a 10% difference in nucleotide composition.

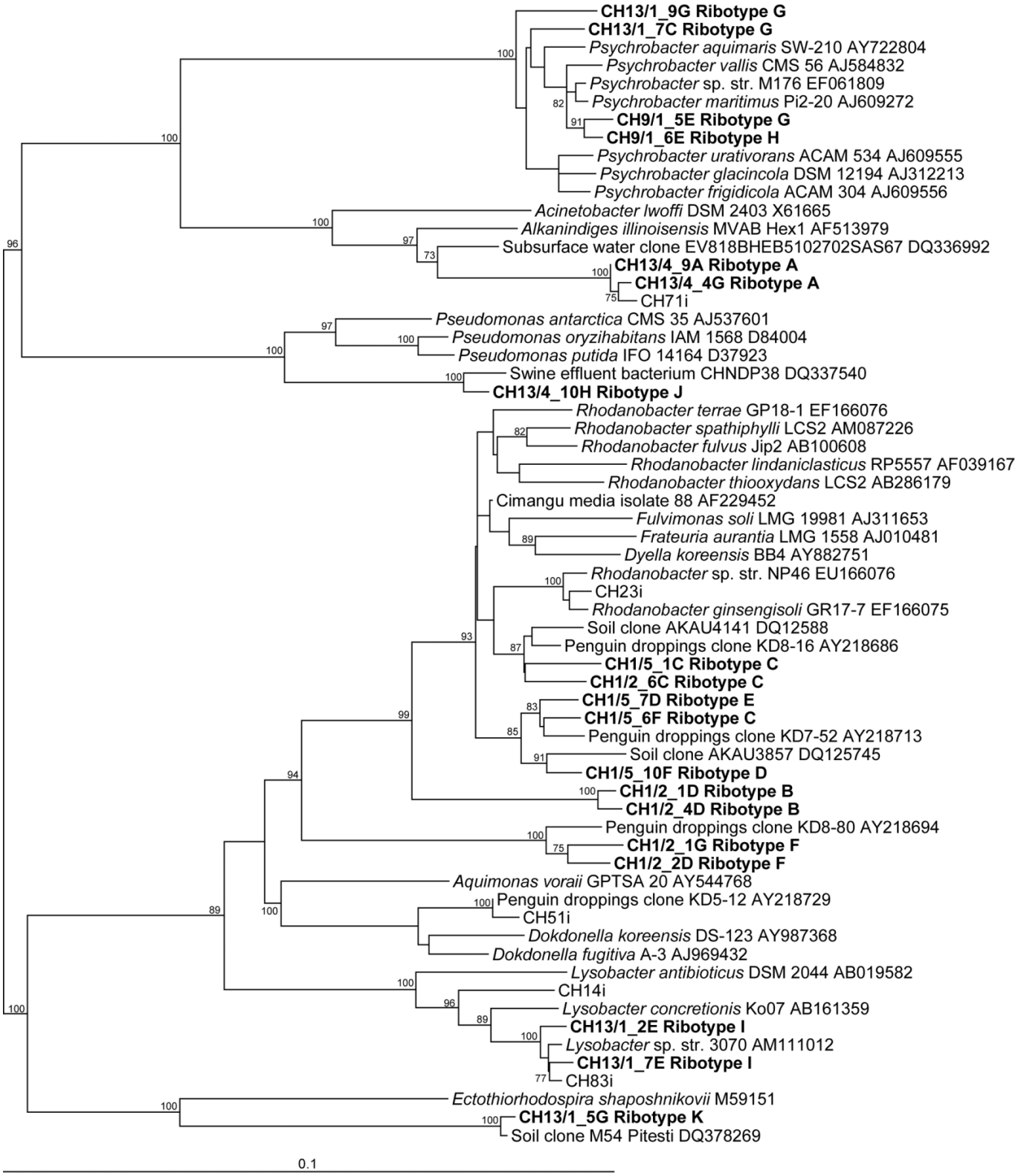
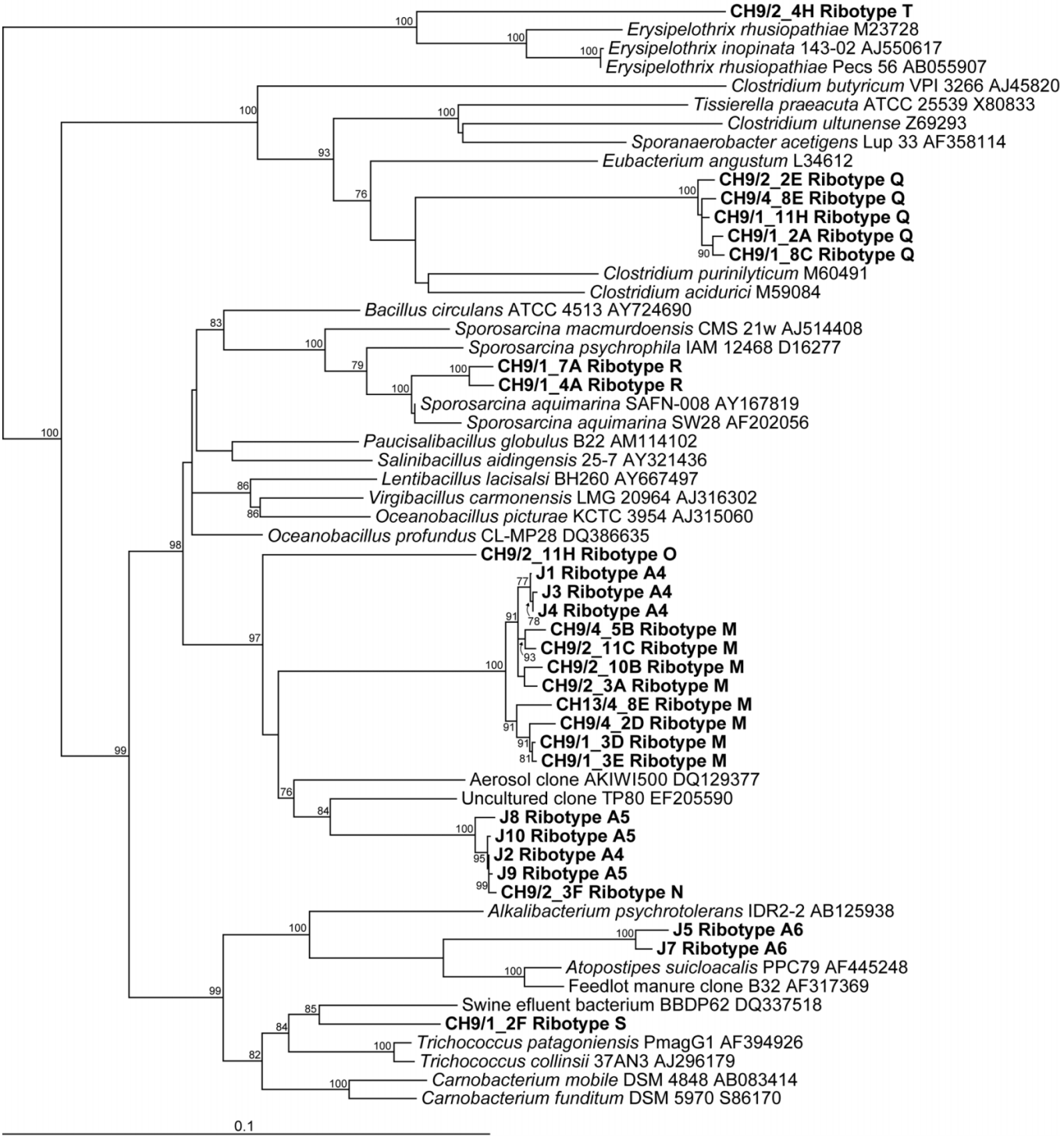


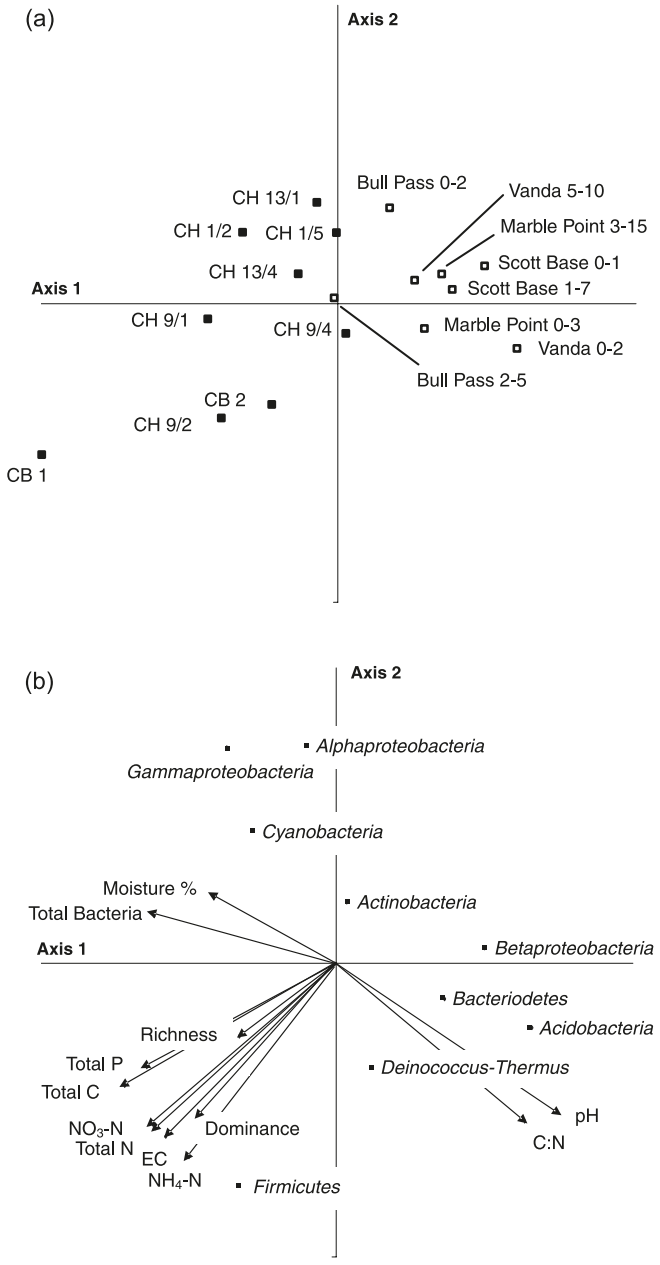
Fig. 2. Unrooted neighbour-joining tree of *Firmicutes* clones retrieved from ornithogenic soils of the Ross Sea region, Antarctica (in boldface), and reference sequences retrieved from GenBank. Bootstrap percentage values >70% are indicated above the nodes. The scale bar shown represents a 10% difference in nucleotide composition.



rium *Psychrobacter* were prevalent, whereas *Gammaproteobacteria* belonging to the family *Xanthomonadaceae* and *Actinobacteria* dominated previously colonized soil. This implies that *Firmicutes* and *Psychrobacter* are best adapted to conditions in the occupied soil that are typically high in nutrients and saline (Table 1). In contrast to the occupied soils, accumulated organic matter has declined in soils from previously colonized nest sites because of decomposition (Zdanowski et al. 2005). C, N, P, and salts may also have been leached or washed from the previously colonized soil with the snowmelt (Hofstee et al. 2006).

Guano deposition on the arid soils of Antarctica underlying penguin rookeries leads to accumulation of organic matter. Guano contains uric acid, the primary nitrogenous waste

Fig. 3. Semi-strong-hybrid (SSH) ordination and principal component correlation (PCC) of the bacterial community composition of ornithogenic soil (■) and mineral soil (□) (data for mineral soils taken from Aislabie et al. 2008*b*). (A) Site ordination. (B) Gradients of environmental variables and centroids for the prevalence of bacterial phyla fitted to the ordination.



product of birds, and undigested material such as chitin derived from krill the almost exclusive food source of Adélie Penguins (Staley and Herwig 1993). Although breaking down rapidly in moist environments (Lindeboom 1984), uric acid can remain in arid soils for a long time (Ramsay and Stannard 1986). Relatively high numbers of culturable uric acid degrading bacteria are present in ornithogenic soils, with 1×10^3 to 1×10^4 degraders/g dry soil at the surface declining to undetectable levels in lower levels (Pietr 1986). Under aerobic conditions, uric acid is degraded via allantoin to CO_2, NH_3, and water. Several bacteria are reported to degrade uric acid aerobically including members of the genera *Serratia*, *Pseudomonas*, *Alcaligenes*, and *Cytophaga* (reviewed by Staley and Herwig (1993)). In ornithogenic soils *Psychrobacter* have been implicated in uric acid degradation and demonstrated for some isolates (Bowman et al. 1996). *Psychrobacter* were prevalent in clone libraries from occupied soil from Cape Hallett but not Cape Bird (Tables 3 and 4). Furthermore, *Psychrobacter* species were prevalent in the culturable bacterial community of ornithogenic soil from Eastern Antarctica (Bowman et al. 1996) and have been isolated from penguin guano (Zdanowski et al. 2004).

Under anaerobic conditions uric acid is degraded to acetic acid, carbon dioxide, and ammonium. Among the bacteria that degrade uric acid anaerobically are members of the genera *Clostridium*, *Fusobacterium*, *Streptococcus*, and *Citrobacter* (reviewed by Staley and Herwig (1993)). The closest relatives of ribotype Q, are *C. acidurici* and *C. purinolyticum* (known as the purinolytic clostridia), which metabolize purines, uric acid derivatives, and pyrimidines for growth anaerobically using special pathways with their unique selenocysteine-containing enzymes (e.g., purine hydroxylase and xanthine dehydrogenase) (Self 2002). This implies that microbes belonging to ribotype Q may degrade uric acid under anaerobic conditions in penguin-colonized soils of Cape Hallett.

Xanthamondaceae and *Actinobacteria* dominating previously colonized soils may also be active in organic matter degradation. The closest relatives of the *Xanthamonadaceae* include bacteria belonging to the genera *Rhodanobacter*, *Dokdonella*, and *Lysobacter*. Members of these genera are commonly isolated from soil. Some *Rhodanobacter* species, for example, use amino acids rather than sugars for growth, produce proteases, and may grow under nitrate-reducing conditions (Lee et al. 2007). Similarly, *Lysobacter* are commonly isolated from decaying material in soil and are proteolytic (Bae et al. 2005). *Actinobacteria* isolated from soils are saprophytes, recognised for their ability to metabolize a wide range of substrates including amino acids and sugars (Buckley and Schmidt 2002). *Arthrobacter*, in particular, are ubiquitous in Antarctic soils and have been isolated from both mineral and ornithogenic soils (Gupta et al. 2004).

Carbon inputs to soil at CH13 include guano and a spill of hydrocarbons. When spilled on soils hydrocarbons may either serve as substrates for bacterial growth or prove toxic and inhibit bacterial growth and activity. The clone library statistics for CH13/1, including lower dominance and coverage (Table 2), suggest it is more diverse than the uncontaminated surface ornithogenic soils analysed (CH9/1 and CH1/2). This indicates that hydrocarbon spillage may have provided additional substrates for bacterial growth at this site, leading to a more diverse soil bacterial community. We have no evidence, however, that the dominant clones in soil CH13/1 are hydrocarbon degraders. Nonetheless, numbers of culturable hydrocarbon degraders at this site were higher than at uncontaminated sites, and we have isolated hydrocarbon degraders from the site including *Actinobacteria* belonging to the genera *Rhodococcus* and *Gordonia* (Aislabie et al. 2008*b*). In contrast to the surface soil (CH13/1), the clone library statistics for subsurface sample CH13/4, including higher dominance and coverage, indicate lower diversity. This contrasts with trends at the other sites where

bacterial diversity appears to increase with depth (Table 2). Hydrocarbon-contaminated CH13/4 is dominated by ribotype A (39% of library clones), which is taxonomically affiliated with the gammaproteobacterial genus *Alkanindiges*. Ribotype A clusters with the type strain *Alkanindiges illinoisensis* and an isolate CH71i from this site (Fig. 3).[4] CH71i, like the type strain, is a hydrocarbon degrader whose growth on R2A was enhanced in the presence of *n*-alkanes (Bogan et al. 2003).[4] Hydrocarbon contamination of Antarctic mineral surface soils can lead to enrichment of hydrocarbon degraders, specifically *Proteobacteria*, including members of the genera *Sphingomonas* and *Pseudomonas*, and *Actinobacteria* such as *Rhodococcus* (Saul et al. 2005). To our knowledge this is the first report of *Alkanindiges* in hydrocarbon-contaminated Antarctic soils.

Nitrogen in ornithogenic soils occurs as uric acid and NH_4^+-N with lesser quantities of nitrate (Speir and Cowling 1984; Table 1). Ammonium and nitrate readily serve as N sources for bacterial growth. Similarly uric acid, as noted above, is degraded under aerobic and anaerobic conditions and can serve as both a C and a N source. The detection of high nitrate levels in ornithogenic soil (Table 1) may be due to in situ nitrification, but known nitrifying bacteria were not among the closest matches of the dominant ribotypes detected in these soils.

Organic compounds or their degradation products or nutrients in surface guano layers mobilized in snowmelt would provide growth substrates for bacteria in lower layers. These substrates may include degradation products of chitin and uric acid (chitin and uric acid are poorly soluble in water), proteins, and amino acids.

Bacterial composition of ornithogenic soil: influence of osmotic stress

Ammonium is an excellent N source for bacteria, but it can be detrimental when present at molar concentrations. Laboratory experiments indicate that at these concentrations growth inhibition of *Corynebacterium glutamicum*, *Escherichia coli*, and *Bacillus subtilis* is due to enhanced osmolarity rather than an ammonium-specific effect (Müller et al. 2006). Although soil levels of ammonium were below these levels, it is possible that inhibitory concentrations may exist in soil water in summer when soils are dry between snowfalls. This may particularly apply to soils of Cape Bird where ammonium levels were extremely high (Table 1), probably reflecting lower levels of precipitation and available moisture at Cape Bird, leading to less leaching of ammonium from these soils compared with those of Cape Hallett (Barrett et al. 2006).

Recently, Lozupone and Knight (2007) reported that the major environmental determinant of bacterial community composition was salinity rather than extremes of temperature, pH, or other physical and chemical conditions present in the samples they analysed. Similarly, in this study osmotic stress was a major determinant for the dominance of *Firmicutes* in ornithogenic soils from occupied sites (CH9 and CB), where EC > 10 mS/cm (Table 1 and Fig. 3). Both salts and ammonium contribute to osmotic stress in these soils. Salts originate from guano and penguin nasal excretions as well as seawater (Janes 1997). Such salty conditions could be expected to favour halotolerant bacteria. Because soils will be exposed to cyclic wetting and drying following snowmelt, the resident bacteria must periodically tolerate extremely saline conditions. Many of the *Firmicutes* ribotypes (e.g., M, N, O, Q, R, A4, and A5) were most closely related to heterotrophic endospore-forming bacteria including *Oceanobacillus profundus*, *C. acidurici*, and *S. aquimarina* (Tables 3 and 4). As endospore production confers on a bacterium the ability to survive inhospitable conditions, it may aid the ability of bacteria to survive in and colonize soils occupied by penguins. Among the closest cultured relatives of 6 *Firmicutes* CD-ribotypes (M, N, O, R, A4, and A5), belonging to the *Bacillaceae*, was a cluster of moderately halotolerant bacilli including the genera *Paucisalibacillus*, *Salinibacillus*, *Oceanobacillus*, *Lentibacillus*, and *Virgibacillus* (Fig. 2), suggesting the halotolerance of organisms corresponding to these ribotypes. *Oceanobacillus profundus*, for example, the closest relative of ribotypes M, N, O, A4, and A5, is a salt and alkali-tolerant bacterium (Kim et al. 2007). Halotolerance is also characteristic of *Sporosarcina* (Yoon et al. 2001) and the gammaproteobacterium *Psychrobacter* (Bowman et al. 1996). Halotolerant bacteria accumulate organic solutes to provide the necessary osmotic balance. These organic compatible solutes include ectoine, trehalose, proline, and glycine betaine (Galinski and Trüper 1994). It has recently been demonstrated that ectoine production in *Virgibacillus pantothenticus* is triggered by both high salinity and low growth temperature (<30 °C), leading to the proposition that ectoine offers protection from salt and low-temperature stress (Kuhlmann et al. 2008). Whether this is an important mechanism for survival of the *Firmicutes* CD-ribotypes (Kuhlmann et al. 2008) in occupied soil from Cape Hallett and Cape Bird is worthy of investigation.

Sources of bacteria in ornithogenic soils

Bacteria resident in ornithogenic soils may be derived from penguin guano or nearby mineral soils. To our knowledge there are no publications describing the phylogenetic diversity of guano from penguins. However, investigations of the feces of gulls (Lu et al. 2008) and the intestines of chickens (Lu et al. 2003) and turkeys (Scupham 2007) revealed a predominance of *Firmicutes*, particularly *Clostridiales* and *Bacilli*, and few *Bacteroidetes*. The clones retrieved from the soils in this study, however, shared no closest relatives with those from the gull guano and chicken or turkey intestines. The dominant *Clostridiales* detected in the bird samples belong to *Clostridium* groups IV, IX, and XIVa (Scupham 2007), whereas those detected in this study cluster most closely to *C. acidurici* and *C. purinilyticum* of group XII. Whereas *C. acidurici* and *C. purinolyticum* are validly described species, they fall outside the genus *Clostridium* sensu stricto, and along with other group XII *Clostridium* species have been proposed as members of the *Peptostreptococcaceae* (Wiegel et al. 2006).

The most dominant clone retrieved from gull feces was related to *Catellicoccus marimammalium* (Lu et al. 2008). This bacterium belongs to the *Bacilli*, specifically the *Enterococcaceae*. No clones belonging to this family dominated the clones retrieved from Cape Bird or Cape Hallett soil. Zdanowski et al. (2004) examined the diversity of culturable bacterial in penguin guano during decomposition with in situ incubation adjacent to a penguin rookery of King George Is-

land, Antarctica. Seventy-two isolates were assigned to 3 major phylogenetic groups, namely *Moraxellaceae*/*Pseudomonadaceae* of the *Gammaproteobacteria*, the *Bacteroidetes* family *Flavobacteriaceae*, and the *Actinobacteria* family *Micrococcaceae*. As with this study, *Psychrobacter* species were prevalent, indicating their fitness for guano decomposition. Interestingly, many of the clones retrieved from the previously colonized soil (both *Gammaproteobacteria* and *Bacteroidetes*) were most closely related to clones from Antarctic lake sediment receiving penguin droppings. This indicates that when environmental conditions are favourable, *Gammaproteobacteria* and *Bacteroidetes* are able to metabolize organic compounds in guano such as chitin and uric acid.

Soil clones retrieved from mineral soils of the Ross Sea region are commonly assigned to the bacterial phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, *Gemmatimonadetes*, and *Proteobacteria* (Smith et al. 2006; Shravage et al. 2007; Aislabie et al. 2008*a*; Niederberger et al. 2008). Compared with occupied ornithogenic soils, *Firmicutes* were rare in mineral soils. We have retrieved clones assigned to *Sporosarcina* from mineral soil from Scott Base and Marble Point (Aislabie et al. 2008*a*), but their prevalence in the clone libraries was <2%. Clones assigned to *Proteobacteria*, including members of the alpha, beta, gamma and delta subphyla, have been retrieved from mineral soils. *Gammaproteobacteria* were prevalent in mineral soil from Cape Evans (Shravage et al. 2007) on Ross Island, Bull Pass in the McMurdo Dry Valleys (Aislabie et al. 2008*a*), and Luther Vale in Northern Victoria Land (Niederberger et al. 2008). Hence the prevalence of *Gammaprotebacteria* in soils from Cape Hallett does not appear to be unique to ornithogenic soils. In common with *Gammaproteobacteria* from Cape Hallett, those from Luther Vale have been assigned to the *Xanthomondaceae* and some were closely related to the genus *Lysobacter* (Niederberger et al. 2008). The dominant bacterial clones from Bull Pass were affiliated with *Ectothiorhodospiraceae* and are loosely related to ribotype K (Aislabie et al. 2008*a*). Although *Psychrobacter* spp. appear to be prevalent in ornithogenic soils (Table 3 and Bowman et al. 1996), they have also been detected from C-enriched soil from under a dead seal at Cape Evans (Shravage et al. 2007) and in a cyanobacterial mat (Shivaji et al. 2005), indicating their preference for Antarctic habitats with elevated C levels.

In conclusion, despite high levels of nutrients and biomass, the bacterial communities of Antarctic ornithogenic soils were not more diverse than Antarctic mineral soils. The bacterial diversity of occupied nest sites differs from that of the previously colonized sites. Occupied sites were dominated by *Firmicutes* and the gammaproteobacterium *Psychrobacter*, whereas *Gammaproteobacteria* belonging to the family *Xanthomonadaceae*, and related to the genera *Rhodanobacter*, *Lysobacter*, and *Dokdonella*, dominated previously colonized sites. Culturing and ecophysiological characterization of representatives of the dominant bacteria in these soils will most aid elucidation of factors structuring the bacterial communities and thus controlling soil function. We have isolated representatives of the *Gammaproteobacteria* (e.g., members of *Rhodanobacter*, *Lysobacter*, and *Alkanindiges*), and isolation of the dominant endospore-forming *Firmicutes* is continuing.

Acknowledgements

This research was supported by funding from the Foundation of Research, Science and Technology, New Zealand (C09X0307). M.R. Balks of the University of Waikato assisted with soil sampling. Antarctica New Zealand provided logistic support. R. Brown and G. McAllister of Antarctica New Zealand provided field support. Soil analyses were carried out at the Environmental Chemistry Laboratory, Landcare Research, New Zealand.

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