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Proteobacteria, Acidobacteria, and Chloroflexi bacteria from Antarctic soils survive under simulated tropical conditions

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Abstract: The human movement to and from Antarctica has increased significantly in recent decades, particularly to the South Shetland Islands, King George Island (KGI), and Deception Island (DCI). Such movements may result in unintentional soil transfer to other warmer regions, such as tropical countries. However, the ability of Antarctic bacteria to survive in tropical climates remained unknown. Hence, the objectives of this work were (i) to determine the bacterial diversity of the soils at the study sites on the two islands, and (ii) to determine if simulated tropical-like growth climate conditions would impact overall diversity and increase the abundance of potentially harmful bacteria in the Antarctic soils. KGI and DCI soils were incubated for 12 months under simulated tropical conditions. After 6 and 12-months, samples were collected and subjected to metagenomic DNA extraction, 16S rDNA amplification, sequencing, and alignment analysis. The 12-month denaturing gradient gel electrophoresis (DGGE) analysis revealed changes in fingerprinting patterns and bacterial diversity indices. Following that, bacterial diversity analyses for KGI and DCI soils were undertaken using V3-V4 16S rDNA amplicon sequencing. Major bacterial phyla in KGI and DCI soils comprised Actinobacteria, Proteobacteria, and Verrucomicrobia. Except for Proteobacteria in KGI soils and Acidobacteria and Chloroflexi in DCI soils, most phyla in both soils did not acclimate to simulated tropical conditions. Changes in diversity were also observed at the genus level, with *Methylobacterium* spp. predominating in both soils after incubation. After the 12-month incubation, the abundance of potentially pathogenic bacteria such as *Mycobacterium, Massilia,* and *Williamsia* spp. increased. Overall, there was a loss of bacterial diversity in both Antarctic soils after 12 months, indicating that most bacteria from both islands sampling sites cannot survive well if the soils were accidentally transported into warmer climates.

Keywords: South Shetland Islands, soil bacterial diversity, simulated tropical conditions.

Introduction

Antarctica is one of the most remote regions on Earth, and the Protocol on Environmental Protection to the Antarctic Treaty designated it as a natural reserve. Despite this, Antarctic biodiversity is becoming increasingly vulnerable to perturbations such as climate change, biological invasions, and human movement in and out of the continent (Curry *et al.* 2002; Tin *et al.* 2009; Stark *et al.* 2015). This increases the likelihood of accidental transfer of soils between Antarctica and other geographical regions (Curry *et al.* 2002; Hughes *et al.* 2010) due to the movement of scientists, tourists, and migratory mammals and birds. It remains unclear how bacteria in Antarctic soils will respond if they are transported out to other regions with different and warmer climates such as the tropical countries.

Besides low temperatures and humidity that are inherent to Antarctica (Wynn-Williams 1990), soil microbes in Antarctica are also exposed to geothermal heat flux in several areas such as Deception Island (DCI) (De Rosa *et al.* 1995). DCI is a member of an archipelago known as the South Shetland Islands, which is located north of the Antarctic Peninsula. DCI exists as a caldera of a stratovolcano consisting of thermal springs and hydrothermal systems (Amenábar *et al.* 2013). This led to the proliferation of microbial communities in DCI that are distinct from other parts of Antarctica (Logan *et al.* 2000; Amenábar *et al.* 2013). In contrast with DCI, King George Island (KGI) lacks geothermal activity and has environmental conditions that are typical for Antarctica (Bölter 2011). The differences observed between these islands are expected to harbour different compositions of soil bacterial communities that may respond differently to environmental changes.

To monitor changes in the bacterial composition of KGI and DCI soils subjected to simulated tropical conditions, denaturing gradient gel electrophoresis (DGGE), and amplicon sequencing targeting hypervariable regions of bacterial 16S rDNA were used (Case *et al.* 2007; Pereira *et al.* 2010). The V3-V4 hypervariable region of 16S rDNA is suitable as a target for diversity studies because primers targeting this region showed less bias at phylum and genus levels, and higher coverage of genera can be achieved compared to primers targeting other hypervariable regions (Klindworth *et al.* 2013). The use of DGGE and amplicon sequencing targeting V3-V4 16S rDNA will therefore provide a comprehensive and reliable representation of bacterial communities in KGI and DCI soil samples throughout this study (Shokralla *et al.* 2012; Klindworth *et al.* 2013; Yu *et al.* 2015).

With increasing concerns of a pathogenic outbreak due to temperature anomalies (Golovnev 2017), the effects of drastic environmental changes on polar microbial diversity need to be determined. Currently, there is a lack of information on the effects of temperature changes on soilborne pathogens, which are difficult to detect and control using practical measures (Pritchard 2011). By identifying bacterial groups in KGI and DCI soils that can thrive outside of Antarctica, more specific screening for potentially pathogenic bacteria can then be carried out. This study, therefore, aims to determine the initial bacterial composition in KGI and DCI soils and their response to one year of simulated tropical conditions using PCR-DGGE analysis and V3-V4 16S rDNA amplicon sequencing. We demonstrated that soil bacterial diversity from the two islands responded differently to simulated tropical conditions.

Materials and methods

Soil sample collection. — Soil samples were collected during the austral summer in 2008 from sites in KGI (62°11'35.3"S 58°56'6.2"W) and volcanic soils in the DCI (62°59'023"S 60°40'51.7"W). Three soil replicates with a distance of 2 m were collected from both sites and stored in sterilized plastic bags at –20°C upon arrival to the station and transported back under similar conditions to the laboratory until used. The soil sample locations at KGI and DCI were indicated in Fig. 1c and Fig. 1d, respectively. Three soil replicates from the three KGI and DCI sites were used for the simulation experiment, and they were designated as KG0-R1, KG0-R2, and KG0-R3, and DC0-R1, DC0-R2, and DC0-R3, respectively. They were incubated in a growth chamber to simulate tropical conditions. The set parameters were 81% humidity, 12 hours of daylight exposure, and a constant temperature of 25°C. Sterilized distilled water (15 ml) was added to each soil sample every week. The conditions such as daily temperatures, rainfall, humidity, and light intensity within the growth chamber mimicked environmental tropical conditions in 2016. Those data were obtained from the Sabah Meteorological Department in Kota Kinabalu. The KGI and DCI soils were incubated for 1 year. A total of ten grams of soil samples were collected twice, with a 6-month interval between each collection. KGI and DCI soil replicates were labelled as KG6-R1, KG6-R2, and KG6-R3, and DC6-R1, DC6-R2, and DC6-R3, respectively, at 6-month sampling, while 12-month sampling of KGI and DCI soil replicates were labelled as KG12-R1, KG12-R2, and KG12-R3, and DC12-R1, DC12-R2, and DC12-R3.

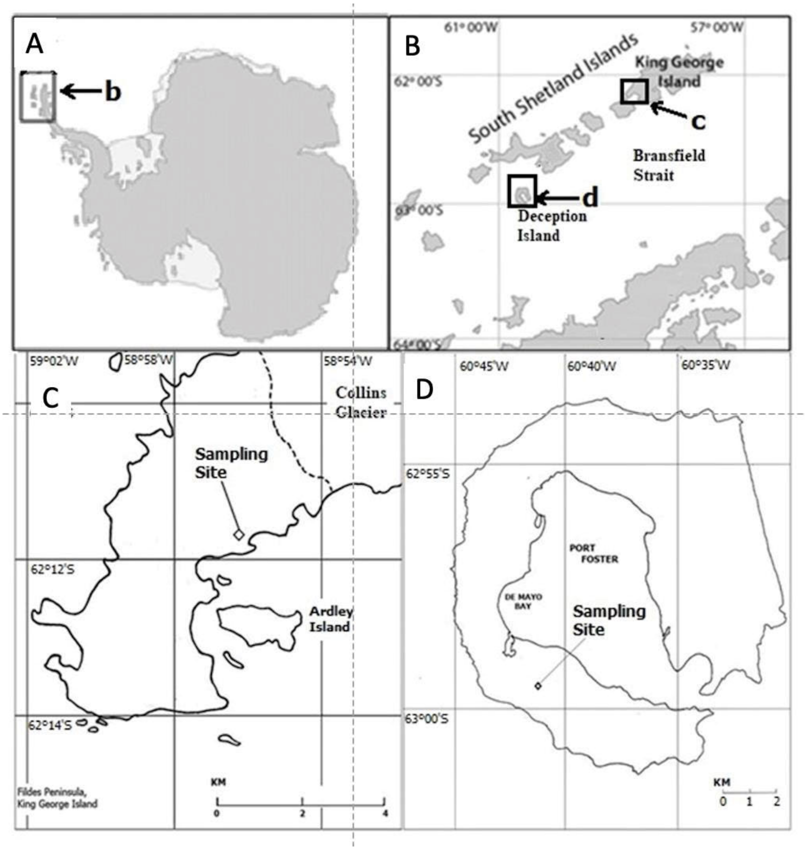

Fig. 1. Map of Antarctica (**A**); South Shetland Islands (**B**) and sampling locations at King George Island (**C**) (62°11'35.3"S 58°56'6.2"W) and Deception Island (62°59'023"S 60°40'51.7"W) (**D**).

Soil DNA extraction. — Total genomic DNA was extracted from the 0, 6 and 12-month sampling of KGI and DCI soil replicates using a previously described method (Zhou *et al.* 1996) with several modifications. The weight of soil used for extraction was reduced to 0.5 g and an additional nucleic acid purification step using 1:1 vol of phenol:chloroform:isoamyl alcohol (25:24:1) was applied to the supernatant before the addition of 1:1 vol of chloroform: isoamyl alcohol (24:1). The final elution volume for genomic DNA was 50 µL of sterile deionized water instead of 500 µL. The concentration and purity of genomic DNA obtained were measured using NanoDrop™ 2000 spectrophotometer (Thermo Scientific Inc., Waltham, MA, USA).

V3-V4 16S rDNA PCR amplification. — Genomic DNA extracted from KGI, and DCI soil replicates were subjected to PCR-DGGE analysis to provide an initial analysis of microbial community profiles. V3-V4 region of bacterial 16S rDNA was amplified using PCR primers GC-S-D-Bact-0341-b-S-17 (5'-GC*CCT ACG GGN GGC WGC AG-3') and S-D-Bact-0785-a-A-21 (5'- GAC TAC HVG GGT ATC TAA TCC -3') with an expected product size of 464 bp

(Klindworth *et al.* 2013). GC* represents a 40 bp GC clamp (5'- CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G) that was added to the 5' end of the forward primer. PCR amplifications were carried out in a 25 µl mixture containing 2 ng template DNA, 0.2 mM deoxynucleotide triphosphates, 2.5 mM magnesium chloride, 0.2 µM forward and reverse primers, 1.0 U GoTaq® G2 Flexi DNA Polymerase, and 5 µL 5X Green GoTaq® Flexi buffer (Promega, Wisconsin, USA). PCR conditions were as follows; 94ºC for 5 min, 35 cycles of 94ºC for 1 min, 50ºC for 25 sec, 72ºC for 50 sec, and a final extension of 72ºC for 7 minutes. PCR amplicon size obtained was verified by agarose gel electrophoresis.

Denaturing Gradient Gel Electrophoresis (DGGE). — PCR amplicons obtained were subjected to DGGE using D-Code Universal Mutation Detection System (Bio-Rad, USA). A total of 15 µL PCR amplicons were loaded into each lane of a 6% (wt/vol) 16 cm X 16 cm polyacrylamide gel (ratio of acrylamide to bis-acrylamide, 37:1) submerged in 0.5 X TAE (Tris–acetate-EDTA) buffer (40 mM Tris, 20 mM acetic acid and 1 mM EDTA, pH 8.0) containing 35–75% denaturant (100% denaturant consisted of 7 M urea and 40% formamide). Gel electrophoresis was conducted at 60°C at a constant voltage of 100 V for 14 h. The gel was stained with 1 X SYBR® Gold Nucleic Acid Gel Stain (Molecular Probe, Invitrogen, Carlsbad, USA) for 20 min. The stained gel was visualized using ultraviolet (UV) illumination with Alpha Imager System (Alpha Innotech, San Leandro, CA).

DGGE analysis. — DNA banding patterns obtained from DGGE were analyzed using GelCompar II software (Applied Maths, Belgium). Bands with an intensity of less than 5% were excluded from the analysis. Hierarchical cluster analysis was carried out using the unweighted pair group method with mathematical averages (UPGMA, Dice coefficient of similarity). The Shannon-Weaver index was calculated using the method described by Gafan *et al.* (2005). Briefly, DNA band strengths (peak height) were estimated visually, where weak, intermediate, and strong bands were assigned with values of 1, 2, and 3, respectively. Shannon-Weaver Index (H') was calculated using the formula $H' = -\sum(Ni/N) \times \ln(Ni/N)$ where H' represents Shannon diversity based on the natural logarithm (base e), Ni represents peak height and N represents the sum of all peak heights (Fromin *et al.* 2002). One-way repeated-measures analysis of variance (ANOVA) was carried out to determine significant changes in Shannon-Weaver indices of KGI and DCI soils after 12 months.

V3-V4 16S rDNA Amplicon Sequencing. — For each sampling time of KGI and DCI soils, the genomic DNA of a representative soil replicate was subjected to V3-V4 16S rDNA amplicon sequencing. KGI and DCI samples used were KG0-R2, KG6-R2 and KG12-R2, and DC0-R2, DC6-R2, and DC12-R2, respectively. The primer pair used was S-DBact-0341-b-S-17 (5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3') and S-D-Bact-0785-a-A-21 (5'-GTC TCG TGG GCT CGG AGA TGT

GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3') with Illumina overhang adapter sequences attached to 5' end of both primers (Klindworth *et al.* 2013). Library construction for each DNA sample was carried out based on the 16S rDNA sequencing library preparation guide (Illumina, San Diego, CA). The libraries were normalized and pooled before V3-V4 16S rDNA amplicon sequencing using MiSeq Reagent Kit v2 (Illumina® Inc., San Diego, CA).

Data processing: demultiplexing, quality filtering, and merging of reads. — Demultiplexing of raw reads obtained for V3-V4 16S rDNA amplicon sequencing was carried out using MiSeq Reporter software (Illumina® Inc., San Diego, CA). Demultiplexed raw reads were trimmed using ConDeTri v2.2 software (Smeds and Künstner 2011). High (hq) and low (lq) quality thresholds were set at 25 and 10 respectively, with a fraction (frac) value of 0.8. The mh and ml values for trimming of reads were set at 5 and 1, respectively. After quality filtering, forward and reverse reads of each sample were merged into single contigs using PEAR 0.9.10 software (Zhang *et al.* 2014).

V3-V4 16S rDNA Amplicon Analysis. — Taxonomic classification of merged reads obtained for 0-month, 6-month, and 12-month KGI and DCI samples were carried out using Basic Local Alignment Search Tool (BLAST) with an e-value of e^{-10} against NCBI's 16S Microbial database. BLAST output files were normalized to 247884 reads per sample and visualized at phylum and genus levels using MEGAN5 software (Huson *et al.* 2011) with default LCA (lowest common ancestor) and analysis parameters. The GI mapping file used for taxonomy identification was updated in February of 2016.

Relative changes in abundance of bacterial phyla and genera at 0, 6, and 12-month sampling were calculated using the following formula:

$$\frac{\begin{bmatrix}\text{Relative abundance of bacterial group} \\ \text{at x} - \text{month } (\%)\end{bmatrix} - \begin{bmatrix}\text{Relative abundance of bacterial group} \\ \text{at 0} - \text{month } (\%)\end{bmatrix}}{\text{Relative abundance of bacterial group at 0} - \text{month } (\%)} \text{ x } 100\%$$

$$= \text{Relative change in abundance of the bacterial group } (\%)$$

Shannon-Weaver diversity index was calculated for all samples at each sampling time using MEGAN5 software (Huson *et al.* 2011), where all taxonomic nodes up to genus level were included in calculations for each sample.

Nucleotide sequence accession number. — Sequence data obtained from KGI and DCI soils are available in NCBI's Sequence Read Archive (SRA) database under BioProject PRJNA471123. BioSample accession numbers for 0-, 6-, and 12-month KGI samples are SRX4075963, SRX4075958, and SRX4075959 respectively. BioSample accession numbers of 0-, 6-, and 12-month DCI samples are SRX4075960, SRX4075961, and SRX4075962 respectively.

Results

Soil DNA extraction. — The concentration of genomic DNA extracted from the 0, 6, and 12-month sampling of KGI and DCI soil replicates was between 2.19 and 6.98 µg per gram of soil used while A_{260}/A_{280} ratios ranged from 1.75 to 2.00 (Table 1). The quantity and purity of genomic DNA were sufficient for PCR amplification.

DGGE analysis. — DGGE profiling of KGI and DCI soil replicates showed that fingerprint patterns were similar among replicates at each sampling time (Fig. 2a). The total number of DGGE bands per lane varied from 6 to 20, where each band represents a dominant bacterial species (Muyzer *et al.* 1993). Samples KG6-R2 and KG6-R3 have the highest number of DNA bands (20 bands), while samples DC0-R3 and DC12-R1 have the least with 6 DNA bands. Based on Shannon-Weaver indices (H') obtained from DGGE fingerprinting patterns, there was an increase from 0 to the 6-month sampling of both KGI and DCI soils, indicating an increase in overall bacterial diversity (Table 1). This was followed

Table 1

DNA concentration, A_{260}/A_{280} ratio, number of bands and diversity index for all soil replicates at 0-, 6- and 12-month sampling. Standard deviations (SD) for Shannon-Weaver index are indicated in parentheses.

Sampling Time (months)	Sample	DNA concentration (µg per g of soil)	A_{260}/A_{280} ratio	Number of bands	Shannon – Weaver index (H')
0	KG0-R1	2.83	1.81	13	2.57 (0.10)
	KG0-R2	4.15	1.82	16	
	KG0-R3	4.12	1.84	15	
6	KG6-R1	3.15	1.88	18	2.88 (0.06)
	KG6-R2	2.19	1.89	20	
	KG6-R3	3.09	1.83	20	
12	KG12-R1	3.83	1.86	12	2.45 (0.05)
	KG12-R2	3.60	1.88	13	
	KG12-R3	2.88	1.76	13	
0	DC0-R1	2.77	1.76	7	1.80 (0.09)
	DC0-R2	2.40	1.83	7	
	DC0-R3	3.57	1.75	6	
6	DC6-R1	6.57	1.94	8	1.94 (0.13)
	DC6-R2	6.98	1.93	9	
	DC6-R3	6.92	2.00	7	
12	DC12-R1	5.11	1.91	6	1.78 (0.09)
	DC12-R2	6.38	1.90	7	
	DC12-R3	3.84	1.96	7	

by decreases in diversity indices for both KGI and DCI samples after 12 months of incubation.

Based on the results obtained for one-way repeated measures ANOVA, the assumption for Mauchly's test of sphericity was met for both KGI ($\chi^2(2) = 2.958$, $p = 0.228$) and DCI soils ($\chi^2(2) = 0.293$, $p = 0.864$). There was a significant main effect of incubation time on Shannon-Weaver index of KGI soils ($F(2,4) = 6.96$, $p = 0.0001$, $\eta_p{}^2 = 0.988$). Bonferroni post hoc tests showed that bacterial diversity in KGI soils were significantly higher in 6-month sampling (mean = 2.88; SD = 0.06) compared to 0-month sampling (mean = 2.57; SD = 0.10; $p = 0.022$) and 12-month sampling (mean = 2.45; SD = 0.05; $p = 0.001$). This provided evidence that 12 months of incubation under simulated tropical conditions affected overall bacterial diversity in KGI soils. As for DCI soils, that there was no significant main effect of incubation time on Shannon-Weaver index of DCI soils ($F(2,4) = 2.63$, $p = 0.186$, $\eta_p{}^2 = 0.568$). Bonferroni post hoc tests also showed that no significant differences in Shannon-Weaver index of DCI soils between 0-month sampling (mean = 1.80; SD = 0.09), 6-month sampling (mean = 1.94; SD = 0.13) and 12-month sampling (mean = 1.78; SD = 0.09). This suggests that the effects of incubation under simulated tropical conditions were less apparent on overall bacterial diversity in DCI soils.

Based on the dendrogram generated for KGI and DCI samples (Fig. 2b), the replicates for each sample were highly similar and formed clusters together, indicating the similar composition of bacterial communities among soil replicates. All triplicates for the 0, 6 and 12-month sampling of KGI and DCI soils showed a similarity of more than 77.00%. The triplicate with the highest similarity (96.00%) was the 12-month sampling of KGI soils, while the triplicate with the least similarity (77.09%) was the 6-month sampling of DCI soils. Overall, the dendrogram provided an overview of bacterial composition in KGI and DCI soil replicates after 0, 6, and 12 months under simulated tropical conditions.

V3-V4 16S rDNA amplicon sequencing. — Samples KG0-R2, KG6-R2 and KG12-R2, and DC0-R2, DC6-R2, and DC12-R2 were selected for V3-V4 16S rDNA amplicon sequencing of KGI and DCI soils respectively because they showed the highest number of DGGE bands (Fig. 2a) and gave a good representation of bacterial composition for both soils. Fig. 3 depicted baseline bacterial diversity at the phylum level for KGI and DCI soils before the incubation. The phylum Actinobacteria was predominant in KGI soils (49.23%), while Proteobacteria was the most classified bacterial phylum (38.52%) in DCI soils. Other major bacterial phyla such as Verrucomicrobia, Bacteriodetes, Cyanobacteria, and Acidobacteria classified in the 0-month sampling of KGI, and DCI soils were similar in terms of relative abundance.

After 6 months of incubation, the relative abundance and diversity of major bacterial phyla were affected in both KGI and DCI soils. Fig 4a showed consistent decreases in the relative abundance of Actinobacteria, Verrucomicro-

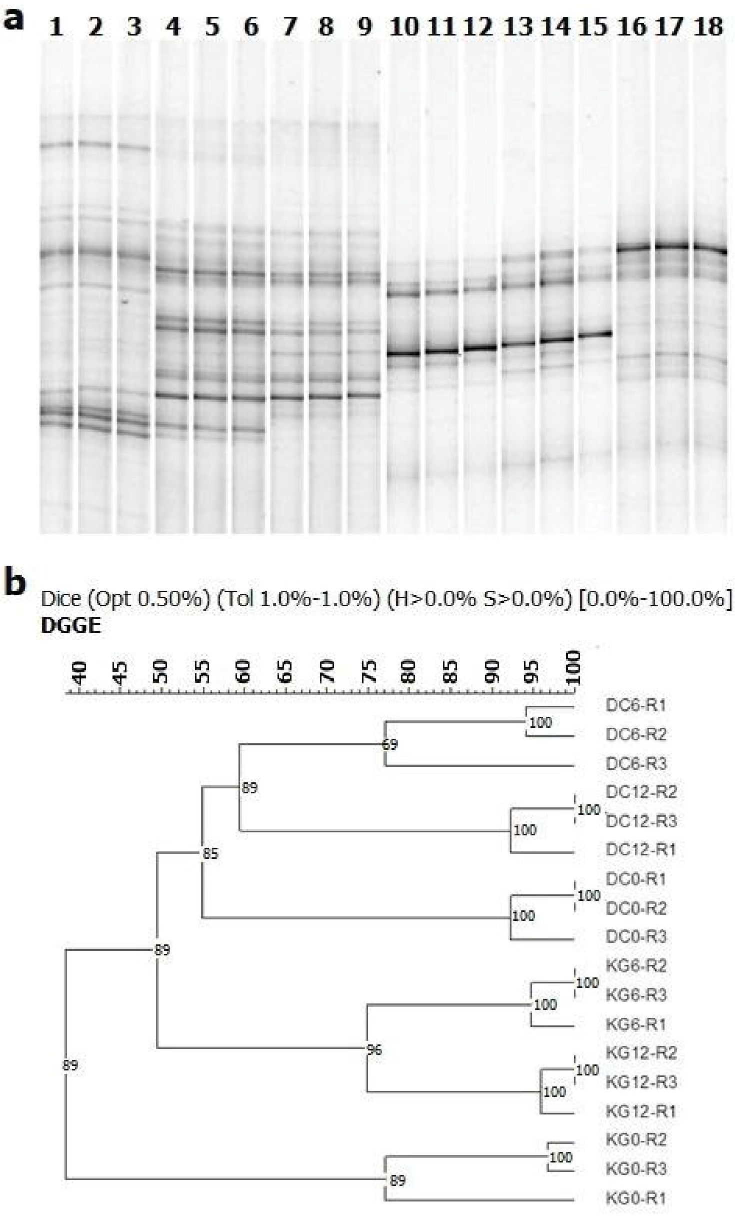

Fig. 2 **(a)** DGGE banding patterns of V3-V4 16S rDNA. Lanes 1 to 18 represents KG0-R1, KG0-R2, KG0-R3, KG6-R1, KG6-R2, KG6-R3, KG12-R1, KG12-R2, KG12-R3, DC0-R1, DC0-R2, DC0-R3, DC6-R1, DC6-R2, DC6-R3, DC12-R1, DC12-R2 and DC12-R3 respectively. **(b)** Dice coefficient–UPGMA dendrogram constructed using GelCompar II cluster analysis. Figures next to branches are cophenetic correlations value that indicates consistence of a cluster. Bootstrap analysis was carried out with 1,000 repetitions, and values greater than 50 % are displayed.

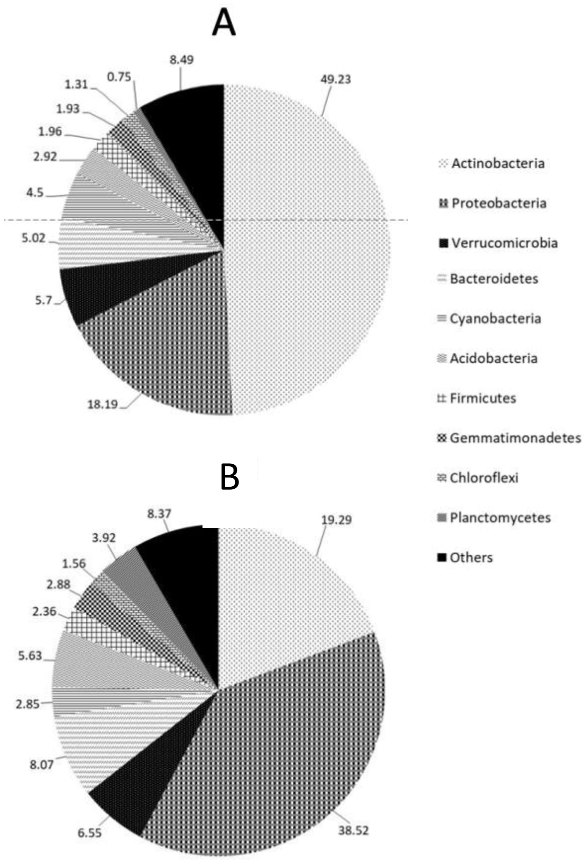

Fig. 3. Relative abundance (%) of major bacterial phyla classified in (**A**) King George Island and (**B**) Deception soils at 0 month sampling.

bia, Bacteroidetes, and Acidobacteria in the 6-month and 12-month sampling of KGI soils. This contrasted with Proteobacteria, which has an increase in relative abundance by more than 150% in both 6-month and 12-month sampling of KGI soils. There was an initial increase in relative abundance by almost one-fold for Firmicutes after 6 months of incubation, but it decreased in the 12-month sampling of KGI soils. This contrasted with what was observed for Chloroflexi and Planctomycetes in KGI soils, which decreased in relative abundance after the 6-month of incubation but was followed by an increase in relative abundance for the 12-month sampling of KGI soils.

As for DCI soils (Fig. 4b), the changes in the relative abundance of major bacterial phyla differed from that of KGI soils. The relative abundance of Actinobacteria decreased slightly in both 6- and 12-month sampling of DCI soils. There were also consistent decreases in the relative abundance of Verrucomicrobia, Gemmatimonadetes, and Planctomycetes throughout 12 months of incubation. There was an initial increase in relative abundance by 44.24 and

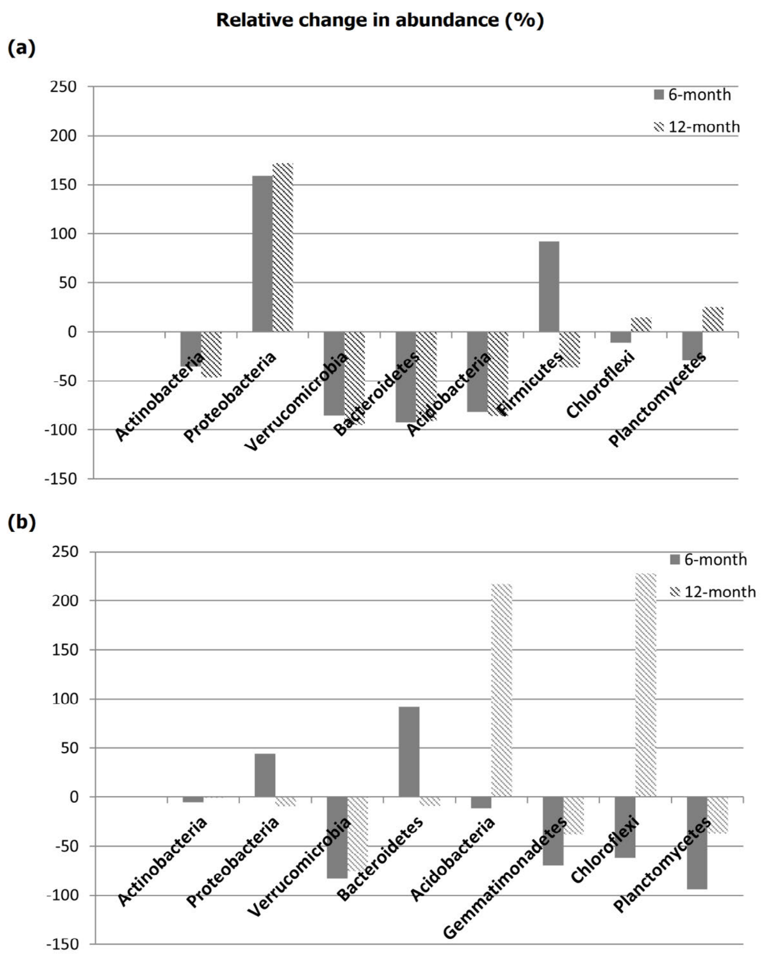

Fig. 4 Relative change in abundance (%) of major bacterial phyla classified in **(a)** King George Island and **(b)** Deception Island soil samples at 6^{th} and 12^{th} month sampling.

91.95% for Proteobacteria and Bacteriodetes respectively in the 6-month sampling of DCI soils, but the relative abundance of both bacterial groups in the 12-month sampling was lower compared to their initial abundance at 0-month. This differed from what was observed for Acidobacteria and Chloroflexi, where their relative abundance decreased in the 6-month sampling of DCI soils but increased by more than two-fold in relative abundance after 12 months of incubation.

Major bacterial genera in KGI and DCI soils were also classified on the 0, 6, and 12-month of simulated tropical conditions. The bacterial genus *Gaiella* spp. was most predominant in KGI soils, while *Chthoniobacter* spp. was the most classified bacterial genera in DCI soils (Fig. 5). There were nine bacterial genera in KGI soils that were classified with a relative abundance of more than 0.5%, while

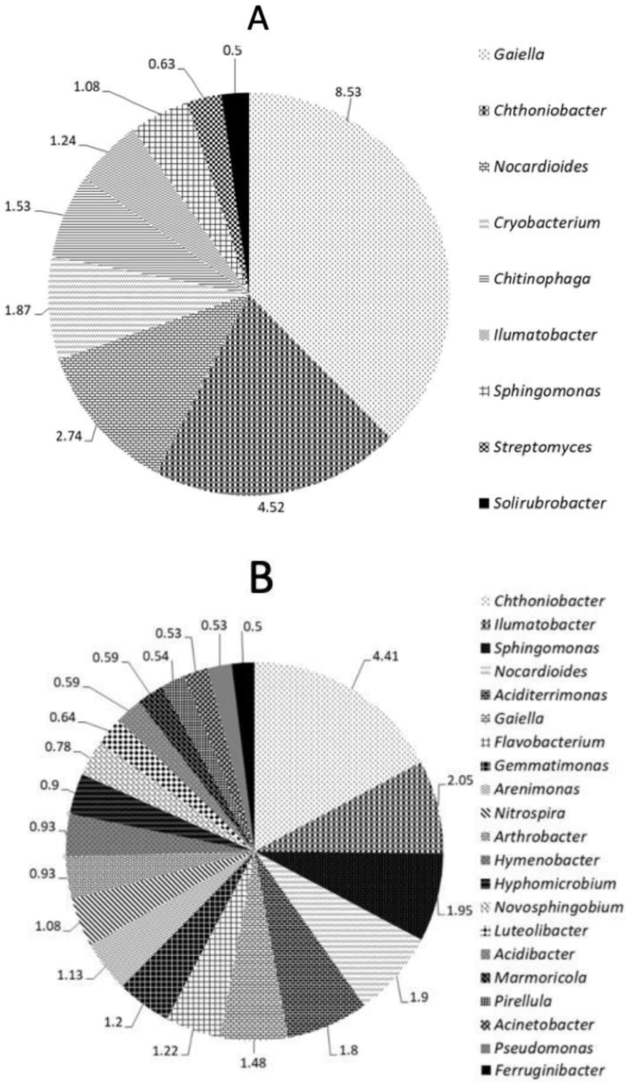

Fig. 5. Major bacterial genera classified in the (**A**) King George Island and (**B**) Deception Island at 0-month of simulated warming. Only bacteria genera classified with abundance > 0.5% were included.

21 bacterial genera were classified in DCI soils. After the 6-month of incubation under simulated tropical conditions, there were notable changes in the relative abundance of major bacterial genera classified for KGI soils (Table 2). Most major bacterial genera in KGI soils decreased in relative abundance, while the relative abundance of bacterial genera *Methylobacterium, Rhodococcus, Mycobacterium,* and *Deinococcus* spp. increased substantially after 6 months of incubation. Similar changes in bacterial composition were observed for the 12-month sampling of KGI

Table 2

Relative change in abundance (%) of major bacterial genera classified in King George Island and Deception Island soil samples after 6 and 12 months of simulated warming.

Bacterial genera in KGI				Bacterial genera in DCI			
Bacterial genus	Abundance (%) at 0 months	Relative change in abundance (%)		Bacterial genus	Abundance (%) at 0 months	Relative change in abundance (%)	
		6 months	12 months			6 months	12 months
Gaiella spp.	8.53	-99.43	-99.66	*Chthoniobacter* spp.	4.41	-75.84	-65.48
Chthoniobacter spp.	4.52	-81.87	-94.58	*Illumatobacter* spp.	2.05	-92.39	-96.22
Nocardioides spp.	2.74	14.54	-24.77	*Sphingomonas* spp.	1.95	94.19	-19.36
Cryobacterium spp.	1.87	-98.87	-99.49	*Nocardioides* spp.	1.90	-3.47	-65.15
Methylobacterium spp.	0.01	1.68×10^5	2.78×10^5	*Aciditerrimonas* spp.	1.80	-82.67	-76.01
Rhodococcus spp.	0.03	2.05×10^4	1.16×10^4	*Gaiella* spp.	1.48	-84.84	-76.22
Mycobacterium spp.	0.05	4.14×10^3	2.50×10^3	*Flavobacterium* spp.	1.22	-99.36	-99.99
Deinococcus spp.	0.04	1.15×10^3	1.16×10^4	*Gemmatimonas* spp.	1.20	-80.73	-70.92
Chitinophaga spp.	1.53	-98.42	-98.93	*Hymenobacter* spp.	0.93	1.17×10^3	-92.14
Illumatobacter spp.	1.24	-55.26	-48.55	*Arthrobacter* spp.	0.93	9.13×10^2	1.45×10^2
Sphingomonas spp.	1.08	57.99	-28.83	*Methylobacterium* spp.	0.03	2.09×10^4	6.51×10^4
Streptomyces spp.	0.63	-97.29	-98.98	*Massilia* spp.	0.20	2.99×10^3	1.12×10^2
Solirubrobacter spp.	0.50	-99.02	-98.29	*Williamsia* spp.	2.0×10^{-3}	3.22×10^3	2.42×10^5
				Deinococcus spp.	0.17	6.78×10^2	1.36×10^3

soils, where *Methylobacterium* spp. was predominant. Bacterial genera *Nocardioides* and *Sphingomonas* spp. in KGI soils showed initial increases in relative abundance by 14.54 and 57.99% respectively after 6 months, but their relative abundance decreased after 12 months of incubation.

As for DCI soils, *Hymenobacter, Arthrobacter,* and *Methylobacterium* spp. were predominant after 6 months of incubation. There were also substantial increases in the relative abundance of *Massilia, Williamsia, Deinococcus,* and *Sphingomonas* spp. as a result of simulated tropical conditions (Table 2). The drastic change in environmental conditions was however not favourable for *Chthoniobacter, Illumatobacter, Aciditerrimonas, Gaiella, Flavobacterium,* and *Gemmatimonas* spp., all of which decreased in relative abundance by more than 75%. Similar to what was observed in KGI soils, *Methylobacterium* spp. was predominant in DCI soils during the 12-month of incubation. Relative abundance of *Chthoniobacter, Illumatobacter, Aciditerrimonas, Gaiella, Flavobacterium,* and *Gemmatimonas* spp. after 12 months of incubation remained lower than their initial abundance at 0-month sampling. Although the relative abundance of *Sphingomonas* and *Hymenobacter* spp. increased in the 6-month sampling of DCI soils, an additional 6 months of incubation led to decreases in relative abundance for both genera by 19.36 and 92.14% respectively.

Diversity indices of bacterial communities in KGI and DCI soils. — Shannon-Weaver indices obtained from amplicon sequencing of KGI, and DCI soils were shown in Table 3. Based on values obtained for the 0-month sampling of both soils, bacterial communities in DCI soils were more diverse (5.418) as compared to that of KGI soils (4.929). Six months of incubation under simulated tropical conditions led to decreases in overall bacterial diversity in KGI and DCI soils, with index values of 4.714 and 4.915 respectively. The Shannon-Weaver index obtained for the 12-month sampling of KGI soils was 4.640, which remained lower than its initial value of 4.929 before incubation. As for the 12-month sampling of DCI soils, the index value increased from the value at 6-month sampling to 5.156 and was comparable to its initial value of 5.418 at 0-month sampling.

Table 3

Shannon-Weaver Indices of bacteria classified in King George Island (KGI) and Deception Island (DCI) soil samples at 0-, 6-, and 12-month sampling.

Sampling time (months)	Shannon-Weaver Index	
	KGI	DCI
0	4.929	5.418
6	4.714	4.915
12	4.640	5.156

Discussion

Low genomic DNA concentrations extracted from KGI and DCI soils in this study suggest that there was a low abundance of microbes in soils from both islands. A similar low concentration was commonly observed in Antarctic soil, mainly due to extreme environmental conditions (Niederberger *et al.* 2008). Initial DGGE analysis indicated that bacterial composition and diversity in KGI samples were distinct from DCI samples. This can be attributed to the environmental conditions of both islands, which are two extremes. In contrast to the cold and arid environment in KGI, as well as in most parts of Antarctica, hydrothermal systems in DCI may have formed unique bacterial communities (Amenábar *et al.* 2013). In terms of overall diversity calculated using DGGE fingerprinting patterns, Shannon-Weaver indices obtained for DCI soils were comparable to those of Cumming Coves in Signy Island in Antarctica, which were 1.76 (Chong *et al.* 2009a). For KGI soils, the resulting indices were similar to those in Danum soils (2.56) collected from Darwin Mountains (Aislabie *et al.* 2013), and Thalla Valley soils (2.73) near Casey Station in the Windmill Islands (Chong *et al.* 2009b).

Shannon-Weaver indices obtained from DGGE analysis also demonstrated that only bacterial communities in KGI soils showed significant changes in overall diversity after 12 months of simulated tropical conditions. This suggests that soil bacteria in the DCI samples were better acclimated compared to the KGI samples. The DCI samples are volcanic soils that may harbour microorganisms that are more resistant to higher temperatures, which translates to better survivability in tropical-like conditions (Moussard *et al.* 2006; Amenábar *et al.* 2013). Due to the high similarity of triplicates for KGI and DCI soils (Fig. 4b), a single representative sample for KGI and DCI soils collected at 0, 6 and 12-month of incubation were subjected to V3-V4 16S rDNA amplicon sequencing for further analysis.

Bacteria from phyla Actinobacteria, Proteobacteria, Verrucomicrobia, Bacteroidetes, and Cyanobacteria were predominant in the 0-month sampling of KGI and DCI soils. These bacterial phyla represent the major bacterial community of Antarctic soils (Niederberger *et al.* 2008; Stomeo *et al.* 2012; Wang *et al.* 2015), as well as Southern and Northern Hemisphere soils (Araujo *et al.* 2020), which have adapted to harsh and extreme environmental conditions. This observation is congruent with earlier reports on soil bacterial diversity in Antarctic soils (Aislabie *et al.* 2006, 2008; Newsham *et al.* 2010; Teixeira *et al.* 2010; Ganzert *et al.* 2011; Chong *et al.* 2012). The predominance of Actinobacteria and Proteobacteria in both KGI and DCI soils was expected, given their role in essential ecological functions such as degradation of organic matter and various ecological functions (Babalola *et al.* 2009; Araujo *et al.* 2020). The relative abundance of Proteobacteria increased in both KGI and DCI soils by 158.93% and 44.24%, respectively, after 6 months of incubation. This may reduce soil carbon content as Proteobacteria are known to contain many bacterial groups that degrade recalcitrant carbon (Barret *et al.* 2011; DeAngelis *et al.* 2015).

Several bacterial phyla such as Chloroflexi and Planctomycetes in KGI soils and Acidobacteria and Chloroflexi in DCI soils demonstrated an initial decrease in relative abundance after 6 months but increased in the subsequent 6 months of incubation. This can be attributed to thermal acclimatization (Bradford *et al.* 2008; Rinnan *et al.* 2009) of these bacterial groups towards simulated tropical conditions in the growth chamber. Most of the other bacterial phyla in KGI and DCI soils, however, decreased in relative abundance throughout 12 months of incubation. Given that the Antarctic climate is harsh with low environmental temperatures and year-round humidity (Wynn-Williams 1990), the drastic change in environmental conditions was expected to be unfavourable for most bacterial groups in KGI and DCI samples that lack an acclimation response towards higher temperatures and humidity (Rinnan *et al.* 2009).

Initial bacterial diversity at the genus level differed between KGI and DCI soils, and higher diversity was observed in DCI soils. The unique landscape and environment at Deception Island allowed different types of bacteria to proliferate compared to typical cold and dry environments in KGI and other parts of Antarctica (Braun *et al.* 2012; Amenábar *et al.* 2013). After 6 months of simulated tropical conditions, *Methylobacterium* spp. was the most abundant bacterial genus in KGI soils, while *Hymenobacter* and *Arthrobacter* spp. were predominant in DCI soils. *Methylobacterium* spp. are pink-pigmented facultative methylotrophic (PPFM) bacteria that utilize one-carbon compounds such as methanol for energy and carbon source (Dworkin 1999). However, *Methylobacterium* spp. are not considered to assimilate methane, which is one of the most potent anthropogenic greenhouse gases in the atmosphere that contributes to global warming (Walter *et al.* 2006). The proliferation of *Methylobacterium* spp. in this study can be attributed to their ability to form biofilms and become resistant to high temperatures and drying conditions (Furuhata *et al.* 2006; Yano *et al.* 2013).

As mentioned above, *Hymenobacter* and *Arthrobacter* spp. were the most commonly recorded bacterial genera in DCI soils after 6 months of incubation. *Hymenobacter* spp. isolated from Antarctic soils had an optimal growth temperature that ranges from 10 to 27°C (Hirsch *et al.* 1998), which explains their surge in population in the growth chamber. *Arthrobacter* spp. were obligatory aerobic bacteria that utilize various organic compounds such as nucleic acids, nicotine, and pesticides for carbon sources and energy (Overhage *et al.* 2005; Arora and Sharma 2015). The nutritional versatility and hardiness of these bacterial genera allowed them to thrive in harsh environments such as Antarctica (Dsouza *et al.* 2015) and acclimatize to drastic changes in their environment (Fernández-González *et al.* 2017).

There were also consistent decreases in the relative abundance of other bacterial genera in KGI and DCI soils, which suggests that temperature does strongly influence the composition and abundance of soil bacteria in Antarctica, which is mostly covered with ice (Ruess *et al.* 1999; Deslippe *et al.* 2012). Although some bacterial groups such as *Methylobacterium* and *Deinococcus* spp. were able to thrive under simulated tropical conditions in the growth chamber, a decrease in overall diversity richness and bacterial abundance in KGI and DCI soils were expected due to the lack of labile substrates and increased microbial competition at higher temperatures (Castro *et al.* 2010; Yergeau *et al.* 2012; Liang *et al.* 2015).

There was a substantial increase in the relative abundance of *Mycobacterium* spp. in KGI soils after 12 months of incubation under simulated tropical conditions (Table 2). *Mycobacterium* spp. found in the environment are well-adapted to thrive in various natural habitats, despite their slower growth rate when compared to other microorganisms in soils (Falkinham 2009; Hruska and Kaevska 2012). Given that many bacterial species from this genus are human pathogens, it is important to monitor their population growth as a result of the tropical conditions simulated in this study. Identification of *Mycobacterium* spp. based on partial 16S rDNA is inconclusive and insufficient to discriminate between species that are of clinical relevance (Young *et al.* 2005; Hruska and Kaevska 2012). Further verification will therefore be required to determine whether true pathogenic *Mycobacterium* spp. such as *M. tuberculosis* and *M. leprae* are present in KGI soils.

There was also a surge in the population of bacterial genera *Massilia* and *Williamsia* spp. in DCI soils throughout 12 months of simulated tropical conditions. *Williamsia* spp. consist of mycolic acid-containing, opportunistic microorganisms (Kämpfer *et al.* 1999) that have been reported to cause infections in immune-compromised individuals (Murray *et al.* 2007; Yassin *et al.* 2010). As for *Massilia* spp., they consist of bacterial species such as *Massilia timonae* that are known to cause corneal infections in immunocompromised patients (Lindquist *et al.* 2003; van Craenenbroeck *et al.* 2011). Similar to *Mycobacterium* spp., further validation will be required to determine the pathogenicity of these bacteria genera in DCI soils.

The Shannon-Weaver index obtained from amplicon sequencing of KGI and DCI soils (Table 3) showed that although bacterial diversity in both soils changed after 12 months of incubation, soil bacteria in DCI soils were better acclimated to simulated tropical conditions compared to that of KGI soils. This also confirms the results from DGGE analysis, which showed that the simulated tropical conditions had less effect on the overall bacterial diversity in DCI soils. The resilience of bacterial communities in DCI soils to environmental disturbances can be attributed to the presence of thermal springs, hydrothermal systems, and unique geographical characteristics at Deception Island (Logan *et al.* 2000). Such conditions would allow the proliferation of metabolically diverse microbes that are more tolerant of higher temperatures (Moussard *et al.* 2006; Amenábar *et al.* 2013). Nonetheless, 12 months of incubation under simulated tropical conditions still led to significant changes in the diversity and composition of bacterial communities in both KGI and DCI soils. Overall, the results of this study have indicated how Antarctic bacteria would respond when soils were accidentally transported outside of Antarctica by attaching to the vehicle surfaces, boots, etc. However, this experiment only assessed the survivability of the Antarctic bacteria in their native soils under tropical climatic conditions. A more comprehensive evaluation will be required to elucidate the interaction between Antarctic bacteria and endemic tropical-terrestrial soil microorganisms.

Conclusion

The first part of this study reviewed bacterial diversity in soils from two different sites in the South Shetland Islands. Meanwhile, the second part, involving the incubation of Antarctic soils under simulated tropical conditions, demonstrated the survivability of bacteria in KGI and DCI soils when inadvertently transported outside Antarctica. It was found that 12 months under simulated tropical conditions had a significant effect on overall bacterial diversity in KGI soils, but not DCI soils. The major bacterial phyla in KGI and DCI soils are Actinobacteria, Proteobacteria, and Verrucomicrobia. Except for Proteobacteria in KGI soils and Acidobacteria and Chloroflexi in DCI soils, most phyla in both soils did not acclimate to simulated tropical conditions. Several bacteria species from KGI and DCI soils survived 12 months of simulated tropical conditions, with potentially pathogenic bacteria among the microorganisms that survived in both soils.

Although this study has shown that most Antarctic bacteria from KGI and DCI soils have a low overall survival rate in tropical-like climates *per se*, some bacteria communities from specific phyla can still survive. This part two results give us a general idea of how Antarctic bacteria fare in tropical climates *per se*, but this work does not address the major challenges that arise when Antarctic bacteria mix with tropical soil microbes, which are more complex and offer stiffer competition between Antarctic and tropical microbes. The overall results of KGI and DCI, however, show that some bacterial strains are unable to adapt, whereas others may adapt to higher temperatures and humidity levels, and the fate of those that are more adaptable can be further monitored. Using the above baseline data, more complex experiments involving the mixing of the Antarctic and tropical soils can be carried out in the future to determine how Antarctic bacteria fare in complex competitions between the Antarctic and tropical soils with high biodiversity on top of the challenging tropical climates. While the chances of Antarctic bacteria outcompeting tropical microbes are low, it is not impossible, particularly for DCI bacteria. As a result, more efforts to conduct more studies like this will help in understanding the ability of Antarctic bacteria to survive outside the continent.

This work is a first step in highlighting the importance of preventing the unintentional introduction of Antarctic soils to other geographical regions. As Antarctica becomes more accessible to researchers and tourists, effective mitigation procedures must be implemented and maintained to prevent the accidental transfer of Antarctic soils outside of the continent.

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