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Article

# Metagenomic Insights into the Adaptations of Antarctic Microorganisms to Extreme Environments

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## Abstract

Metagenomic analysis of Antarctic soils provides a key perspective on the microbial diversity of one of the most extreme ecosystems on Earth, where microorganisms dominate ecological processes, although their diversity and distribution remain less understood than those of macroscopic eukaryotes. This study aimed to identify bacteria present in Antarctic soils using a metagenomic approach. Soil samples were obtained from the sample bank of the Escuela Superior Politécnica Agropecuaria de Manabí and were collected during an expedition conducted in 2014 on four islands: Greenwich, Dee, Barrientos, and Torre. The analysis included DNA extraction, amplification of the 16S rRNA gene by polymerase chain reaction (PCR) using specific primers, and sequencing on the Illumina MiSeq platform (Macrogen©). These techniques enabled reliable identification of the microbial populations present. The results revealed the predominance of several bacterial phyla across all four islands, with Actinomycetales (36.60%), Proteobacteria (20.03%), Firmicutes (17.53%), and Bacteroidetes (6.91%) being the most abundant. In addition, Antarctic soil metagenomes indicated the potential access to novel genes encoding enzymes with possible biotechnological and industrial applications. Overall, these findings highlight Antarctic ecosystems as reservoirs of valuable genetic resources and underscore their importance for the long-term development of biotechnological applications, without extrapolating conclusions beyond the results obtained.

**Keywords:** genes; metagenomes; porkhooks; sequencing

## 1. Introduction

The Given the high responsiveness of polar ecosystems to global climate change, research on Antarctic microorganisms has become a subject of intense research. Terrestrial Antarctica represents one of the most extreme environments in which a living organism can survive (Pudasaini et al., 2017; Yin Wong et al., 2019). The complex topography provides polar deserts and meltwater lakes that vary in salinity from near-fresh to near-saturated (Bowman, 2018). This creates an ecosystem with harsh and extreme characteristics, where temperatures can reach -80 to -93.2 ° C (Parker, 2014).

While historical observational studies indicated that few microorganisms exist in terrestrial Antarctica, subsequent molecular studies have discovered rich microbial communities, especially in the ice-free regions of the continent (Ortiz et al., 2020). These communities contain phyla frequently observed in soils of temperate regions, such as Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes (Bottos et al., 2013; Cary et al., 2010a). These ice-free regions of Antartica contain a rich and dynamic ecosystem that represents a significant source of microbial diversity (Ahmed et al., 2018; Mocali & Benedetti, 2010). However, knowledge about the composition and functionality of these microorganisms remains limited compared to other, more well-studied ecosystems (Arora & Panosyan, 2019).

To adapt to the harsh physicochemical conditions of the continent, most members of this microbial community exhibit extremely slow growth or enter dormant states (Leung et al., 2020). Even in dormancy, cells require energy for maintenance, repair and membrane potential (Rittershaus et al., 2013). Consequently, this largely unexplored biodiversity represents a potentially valuable resource for natural products used in agriculture and biotechnology (Bonomo et al., 2022).

Antarctic microbial communities, including bacteria and archaea, have developed unique metabolic strategies that allow them to survive in harsh conditions, such as the production of psychrophilic enzymes, which can catalyze reactions at low temperatures without losing catalytic efficiency, making them ideal for industrial applications such as enzymatic biotransformation and the food industry (Bisaccia et al., 2023; Ramirez et al., 2006). Furthermore, halophilic and acidophilic microorganisms in Antarctica have shown great potential in environmental biotechnology, particularly in bioremediation processes for the degradation of pollutants in extreme environments (Ashaolu et al., 2025; Margesin & Schinner, 2001).

The exploration of Antarctic ecosystems can also provide clues about the limits of life on Earth and other planets. It has been suggested that microorganisms found in Antarctic permafrost and subglacial lakes could be similar to possible life forms on celestial bodies such as Europa and Enceladus, moons of Jupiter and Saturn, respectively (Mancinelli & Klovstad, 2000; Schuerger et al., 2003; Smedile et al., 2024). This line of research could have applications in astrobiology and space exploration, contributing to the development of technologies for the detection and analysis of life in extreme environments.

Most soil microbial communities represent an invaluable source of genetic and metabolic diversity, with untapped potential for biotechnology and microbial ecology. However, it is estimated that less than 1% of soil bacteria can be cultured under laboratory conditions (Dai et al., 2025; Mocali & Benedetti, 2010; Schloss & Handelsman, 2003). This challenge has greatly limited our knowledge of their ecological interactions and functions.

To overcome this limitation, metagenomics has emerged as a powerful tool. It allows direct access to the collective genome of these communities (Courtois et al., 2003; Delmont et al., 2011; Demanè et al., 2008; Hiraoka et al., 2016), making it possible to recover individual genomes directly from complex microbiomes (Nayfach et al., 2019; Tully et al., 2018). Specifically, using simulated community data, Latorre-Pérez et al. (2020) demonstrated that full-length biosynthetic gene clusters (BGCs) could be successfully recovered from long-read metagenomic sequencing.

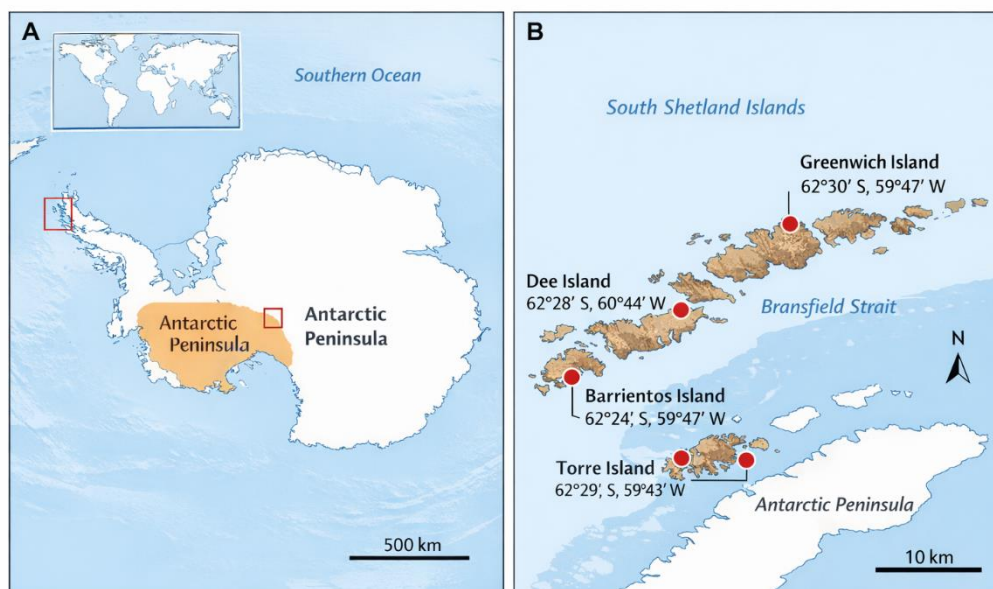
Despite these technological advances (Kwong et al., 2015), key questions remain regarding how Antarctic soil microbial communities respond to environmental variations and how their genetic structure relates to their ecological function (Chauhan et al., 2023; Grenni et al., 2023). In this context, the present study aims to perform a detailed metagenomic analysis of Antarctic soil microorganisms, identifying the main taxa present and their adaptation mechanisms to the extreme environment. The findings of this research will contribute to a better understanding of ecological processes in Antarctica and their implications in a context of global climate change.

## 2. Materials and Methods

### *Obtaining Samples*

Soil samples were obtained from the sample bank of the Escuela Superior Politécnica Agropecuaria de Manabí “Manuel Félix López” (ESPAM MFL), originally collected during an Antarctic expedition conducted in 2014. Sampling was carried out on four islands of the South Shetland Archipelago, located north of the Antarctic Peninsula: Greenwich Island (62°30' S, 59°47' W), Dee Island (62°28' S, 60°44' W), Barrientos Island (62°24' S, 59°47' W), and Torre Island (62°29' S, 59°43' W) (Figure 1). These islands are characterized by polar climatic conditions, with approximate altitudes ranging between 10 and 80 meters above sea level. The mean annual temperature in this region ranges from -2 °C to 2 °C, with strong seasonal variability and frequent freeze-thaw cycles. The soils are generally classified as poorly developed polar soils, typically composed of sandy and

rocky substrates with low organic matter content and sparse vegetation, mainly influenced by glacial processes and marine inputs. Soil samples were collected from the surface layer at an approximate depth of 0–10 cm using sterile sampling tools (Cowan et al., 2014). At each island, sampling followed a zig-zag transect design, collecting 25 subsamples that were subsequently homogenized to obtain a composite sample, resulting in four composite soil samples in total.



**Figure 1.** Geographic location of the sampling's sites in the South Shetland Islands, Antarctica indicating the study area (red box). (B) Detailed map on the South Shetland Islands showing the sampling sites on Greenwich Island, Barrientos Island, Dee Island, and Torre.

#### DNA Extraction

DNA was extracted from 100–250 mg of each composite soil sample using the Mag-Bind Soil DNA Kit (Omega Bio-tek, USA) following the manufacturer's protocol. Briefly, samples were lysed in a buffer containing RNase to remove RNA contamination. After consecutive washes with DS Buffer, DNA was purified using Mag-Bind RQ magnetic particles. The eluted DNA was transferred to a sterile 96-well microplate and stored at  $-20^{\circ}\text{C}$ . DNA integrity was verified by 1% agarose gel electrophoresis stained with GelRed (Biotium, USA).

#### Amplification of 16S rDNA by PCR

The V3–V4 region of the bacterial 16S rDNA gene was amplified using universal primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') (Klindworth et al., 2013). PCR was performed in a 25  $\mu\text{L}$  reaction volume containing 12.5  $\mu\text{L}$  of 2 $\times$  DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, USA), 0.5  $\mu\text{M}$  of each primer, 2  $\mu\text{L}$  of template DNA, and nuclease-free water. Thermal cycling conditions were: initial denaturation at  $94^{\circ}\text{C}$  for 3 min, followed by 30 cycles of denaturation at  $94^{\circ}\text{C}$  for 45 s, annealing at  $54^{\circ}\text{C}$  for 30 s, and extension at  $72^{\circ}\text{C}$  for 90 s, with a final extension at  $72^{\circ}\text{C}$  for 10 min. PCR products were visualized on a 1% agarose gel in 1 $\times$  TAE buffer stained with GelRed, using a 300–1000 bp molecular weight marker (Axygen, USA). Bands were excised and purified using PureLink Quick Gel Extraction Kit (Invitrogen, USA).

#### DNA Quantification and Quality Assessment

DNA concentration and purity were quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA). Absorbance was measured at 260 nm ( $A_{260}$ ) for concentration, and purity was assessed using  $A_{260}/A_{280}$  (target:  $\sim 1.8$ ) and  $A_{260}/A_{230}$  (target:  $\sim 2.0$ ) ratios. Only samples meeting quality thresholds ( $A_{260}/A_{280} > 1.7$ ,  $A_{260}/A_{230} > 1.8$ ) were used for sequencing.

### Library Preparation and Sequencing

Sequencing libraries were constructed by attaching Illumina adapters (P5: 5'-TCGTCGGCAGCGTGAGATGTGTATAAGAGACAG-3'; P7: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3') and unique barcodes to the 16S rDNA amplicons in a secondary PCR reaction. Libraries were sequenced on the Illumina MiSeq platform (Macrogen, South Korea) using a 2 × 300 bp paired-end configuration, targeting a minimum of 100,000 reads per sample. A negative control (DNA-free ultrapure water) was processed alongside experimental samples to detect potential contamination.

### Bioinformatics Analysis

Raw reads were quality-filtered and trimmed using Trimmomatic v0.39 (Bolger et al., 2014) to remove adapters, low-quality bases (Phred score < 20), and reads shorter than 200 bp. Paired-end reads were merged using PEAR v0.9.11 (Zhang et al., 2014). Chimeric sequences were identified and removed with VSEARCH v2.15.0 (Rognes et al., 2016) using the UCHIME algorithm. Denoised sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using QIIME2 v2022.8 (Bolyen et al., 2019). Taxonomic assignment was performed against the SILVA 138 database (Quast et al., 2013) with a 99% identity threshold. OTU tables were rarefied to the lowest read depth across samples to normalize sequencing effort. Alpha diversity (Shannon and Simpson indices) was calculated using QIIME2 to assess microbial richness and evenness.

## 3. Results

### 3.1. Microbial Diversity and Sequencing Output

Metagenomic analysis of soil samples from four Antarctic islands (Greenwich, Dee, Barrientos, and Torre) was conducted using 16S rDNA sequencing. After quality filtering and chimera removal, a total of 674,266 high-quality reads were obtained across all samples, with an average of 168,567 ± 15,432 reads per island (Table 1). These reads were clustered into operational taxonomic units (OTUs) at 97% similarity, yielding 2,134 unique OTUs. Taxonomic classification was achieved from kingdom to species level, with classification rates ranging from 98.47–99.39% at the kingdom level to 57.39–65.88% at the species level (Table 2). Alpha diversity analysis revealed moderate microbial richness, with Shannon indices ranging from 4.8 (Torre) to 5.4 (Greenwich) and Simpson indices from 0.85 (Torre) to 0.91 (Greenwich), indicating varied evenness across islands.

**Table 1.** Environmental characteristics of the sampling sites located in the South Shetland Islands, Antarctica.

Island	Coordinates	Altitude (m a.s.l.)	Mean Annual Temperature	Soil Type	Environmental Characteristics
Greenwich Island	62°30' S, 59°47' W	10–80 m	–2 to 2 °C	Poorly developed polar soils, sandy and rocky	Glacial influence, freeze–thaw cycles, low organic matter content
Barrientos Island	62°24' S, 59°47' W	5–60 m	–2 to 2 °C	Sandy soils with marine influence	High exposure to winds and UV radiation
Dee Island	62°28' S, 60°44' W	10–70 m	–2 to 1 °C	Poorly developed volcanic and sedimentary soils	Marine and glacial influence
Torre Island	62°29' S, 59°43' W	10–75 m	–2 to 2 °C	Rocky soils with low organic matter content	Sparse vegetation and strong environmental stress

**Table 2.** Classification Rates by Taxonomic Level Across Four Antarctic Islands.

Taxonomic level	Tower		Barrientos		Greenwich		Dee	
	Read	%	Read	%	Read	%	Read	%
Kingdom	207,567	98.97	163,184	98.47	129,703	99.39	173,812	99.2
Phylum	196.83	93.85	160,255	96.7	126,407	96.87	167,781	95.76
Class	192,444	91.76	158,849	95.86	124,881	95.7	166,627	95.1
Order	190,144	90.66	157,649	95.13	123,447	94.6	164,426	93.84
Family	184.85	88.14	152,131	91.8	120,288	92.18	158,967	90.73
Genus	169,088	80.62	135,176	81.57	113,214	86.76	148,661	84.85
Species	121,641	58	96,242	58.08	85,974	65.88	100,558	57.39

### 3.2. Taxonomic Composition

#### Kingdom

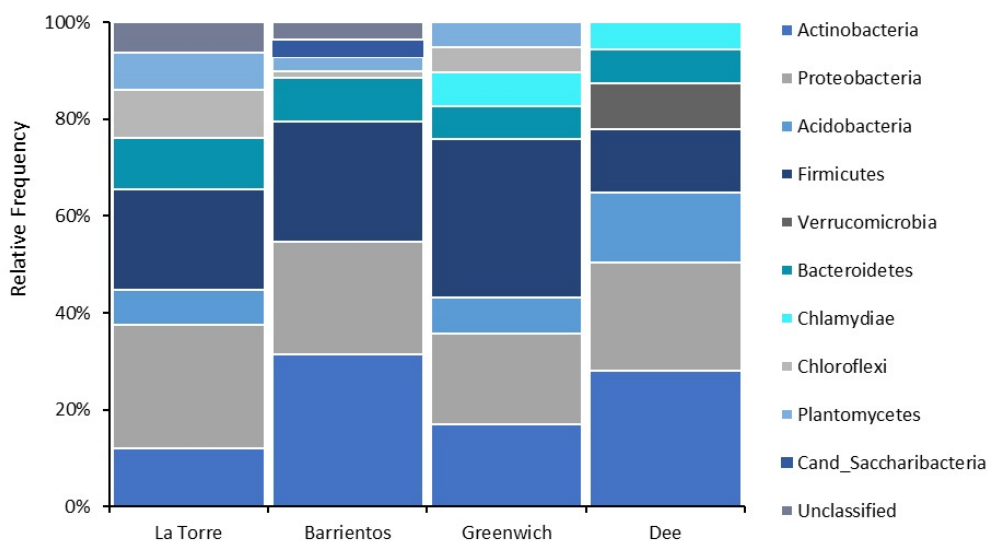
The microbial communities were predominantly bacterial ( $98.96 \pm 0.37\%$  of reads), with Archaea comprising a minor fraction (0.02–0.15%) across all islands (Table 3). Unclassified sequences at the kingdom level accounted for 0.61–1.46% of reads, likely representing novel or divergent taxa. The low archaeal abundance aligns with previous studies of Antarctic soils, where bacteria dominate due to their metabolic versatility in extreme conditions (34).

**Table 3.** Kingdom Classification Across Four Antarctic Islands.

Taxonomic level	Classified to taxonomic level (The Tower)		Classified to taxonomic level (Barrientos)		Classified to taxonomic level (Greenwich)		Classified to taxonomic level (Dee)	
	Read	%	Read	%	Read	%	Read	%
	Bacterium	207,533	98.96	131,168	98.49	129,675	99.37	173,549
Archaea	34	0.02	73	0.05	28	0.02	263	0.15
Unclassified at Kingdom level	2,155	1.03	1,941	1.46	795	0.61	1,399	0.8

#### Phylum

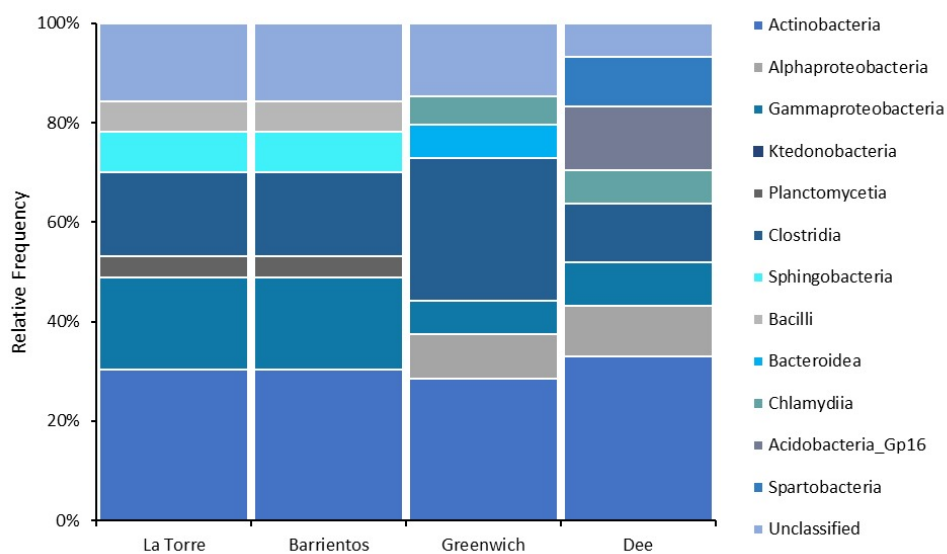
Four phyla dominated the microbial communities: Actinobacteria ( $36.60 \pm 9.12\%$ ), Firmicutes ( $17.53 \pm 6.22\%$ ), Proteobacteria ( $20.03 \pm 4.87\%$ ), and Bacteroidetes ( $6.91 \pm 2.34\%$ ) (Figure 2). Actinobacteria was the most abundant phylum across all islands, particularly on Barrientos (42.31%). Firmicutes showed the highest relative abundance on Greenwich (26.66%), while Proteobacteria peaked on Torre (24.15%). Bacteroidetes was consistently present but less abundant. Island-specific phyla included Verrucomicrobia (7.89%) on Dee and Chlamydiae (4.77–5.63%) on Greenwich and Dee, suggesting unique ecological niches (Yin Wong et al., 2019).



**Figure 2.** Phylum-level composition of microbial communities in Antarctic soil samples from four South Shetland islands (Torre, Barrientos, Greenwich, Dee). Relative abundances were determined by 16S rDNA sequencing. Actinobacteria dominated across all islands ( $36.60 \pm 9.12\%$ ), with Firmicutes, Proteobacteria, and Bacteroidetes also prevalent. Island-specific phyla included Verrucomicrobia (Dee) and Chlamydiae (Greenwich, Dee). Minor taxa ( $<3.5\%$  abundance) are grouped as “Others.”

### Class

Twelve bacterial classes were identified, with Actinobacteria, Gammaproteobacteria, and Clostridia present across all islands (Figure 3). Actinobacteria (class) was the most abundant, ranging from 11.67% (Torre) to 25.91% (Barrientos). Gammaproteobacteria varied from 5.14% (Greenwich) to 15.95% (Barrientos), and Clostridia from 5.28% (Torre) to 21.63% (Greenwich). Island-specific classes included Ktedonobacteria (8.09%) on Torre, Sphingobacteria (6.84%) and Bacilli (5.22%) on Barrientos, Bacteroidia (5.14%) and Chlamydia (4.30%) on Greenwich, and Acidobacteria\_Gp16 (9.09%) and Spartobacteria (7.13%) on Dee. These distributions reflect adaptations to local environmental conditions, such as moisture and organic carbon availability.

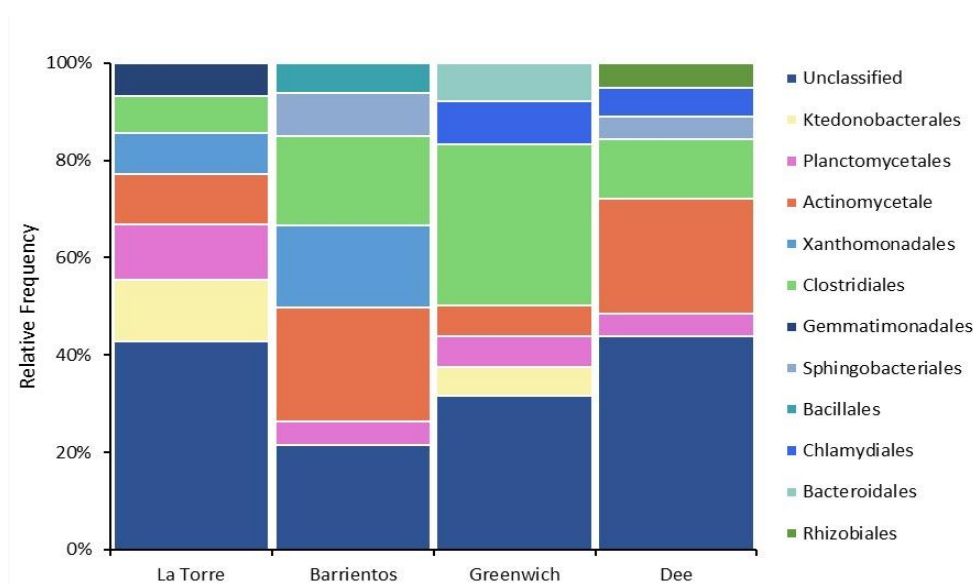


**Figure 3.** Class-level composition of bacterial communities in Antarctic soils from Torre, Barrientos, Greenwich, and Dee islands. Sequencing of the 16S rDNA V3–V4 region revealed Actinobacteria (11.67–25.91%),

Gammaproteobacteria (5.14–15.95%), and Clostridia (5.28–21.63%) as ubiquitous classes. Island-specific classes included Ktedonobacteria (Torre), Sphingobacteria (Barrientos), Bacteroidia (Greenwich), and Acidobacteria\_Gp16 (Dee). Taxa with <3.5% abundance are grouped as “Others.”.

## Order

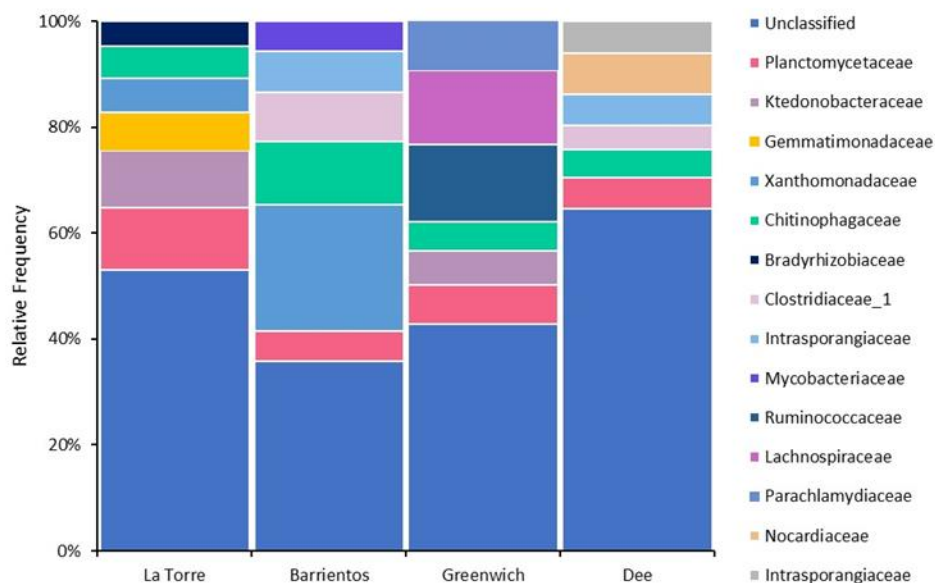
Eleven bacterial orders were detected, with Actinomycetales, Clostridiales, and Planctomycetales ubiquitous across all islands (Figure 4). Actinomycetales was most abundant on Barrientos (18.19%) and Dee (16.06%), Clostridiales on Greenwich (21.46%), and Planctomycetales on Torre (7.20%) and Dee (8.08%). Island-specific orders included Bacillales (4.79%) on Barrientos, Gemmatimonadales (4.37%) on Torre, Bacteroidales (5.14%) on Greenwich, and Rhizobiales (3.41%) on Dee, indicating specialized metabolic roles such as nitrogen fixation and organic matter degradation.



**Figure 4.** Order-level composition of bacterial communities in soil samples from four Antarctic islands. Illumina MiSeq sequencing identified Actinomycetales (4.06–18.19%), Clostridiales (4.78–21.46%), and Planctomycetales (3.64–8.08%) across all islands. Island-specific orders included Bacillales (Barrientos), Gemmatimonadales (Torre), Bacteroidales (Greenwich), and Rhizobiales (Dee), reflecting diverse ecological roles. Taxa with <3.5% abundance are grouped as “Others.”.

## Family

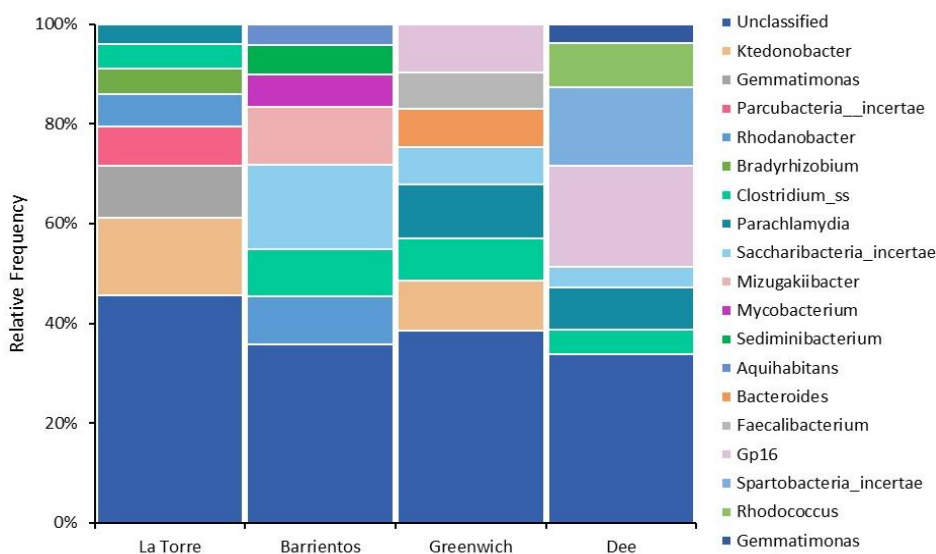
Fourteen bacterial families were identified, with Planctomycetaceae and Chitinophagaceae present across all islands (Figure 5). Planctomycetaceae ranged from 3.08% (Dee) to 7.20% (Torre), and Chitinophagaceae from 2.80% (Dee) to 6.61% (Torre). Island-specific families included Gemmatimonadaceae (4.37%) and Ktedonobacteraceae (6.61%) on Torre, Mycobacteriaceae (3.14%) on Barrientos, Ruminococcaceae (8.09%), Lachnospiraceae (7.77%), and Parachlamydiaceae (5.18%) on Greenwich, and Intrasporangiaceae (3.12%) and Nocardiaceae (4.04%) on Dee. These variations suggest distinct nutrient cycling dynamics across islands.



**Figure 5.** Family-level composition of microbial communities in Antarctic soils from Torre, Barrientos, Greenwich, and Dee islands. Planctomycetaceae (3.08–7.20%) and Chitinophagaceae (2.80–6.61%) were prevalent across all islands, based on 16S rDNA sequencing. Island-specific families included Gemmatimonadaceae (Torre), Mycobacteriaceae (Barrientos), Ruminococcaceae (Greenwich), and Nocardiaceae (Dee). Taxa with <3.5% abundance are grouped as “Others.”.

### Genus

The genus *Clostridium\_sensu\_stricto* was detected across all islands, with abundances of 2.08–4.55% (Figure 6). Barrientos hosted unique genera such as *Mizugakiibacter* (5.60%), *Mycobacterium* (3.12%), *Sediminibacterium* (2.79%), and *Aquihabitans* (2.07%). Greenwich featured *Bacteroides* (2.62%) and *Faecalibacterium* (2.54%), while Dee included *Spartobacteria\_genera\_incertae\_sedis* (7.05%), *Rhodococcus* (4.01%), and *Gemmatimonas* (1.68%). These genera reflect diverse ecological roles, from organic matter decomposition to potential symbiotic interactions.

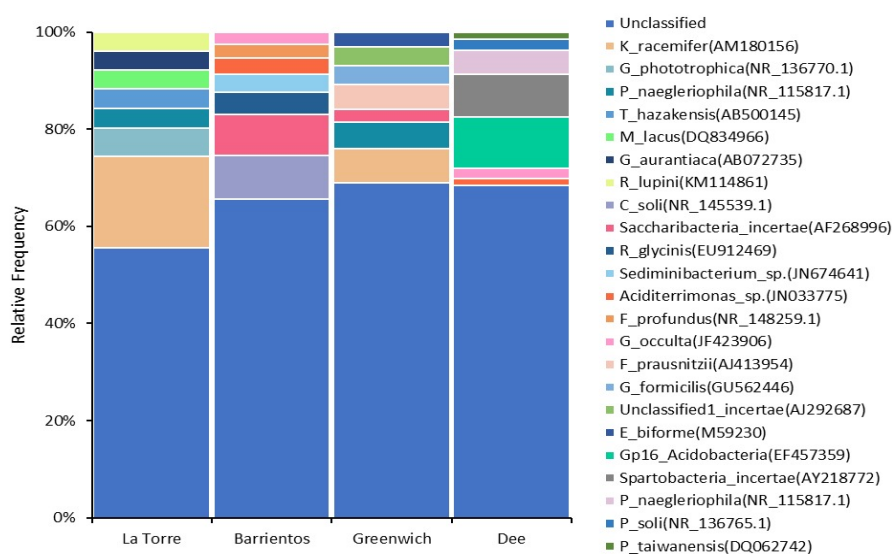


**Figure 6.** Genus-level composition of bacterial communities in Antarctic soil samples from four South Shetland islands. *Clostridium\_sensu\_stricto* (2.08–4.55%) was detected across all islands via 16S rDNA sequencing. Island-specific genera included *Mizugakiibacter* and *Mycobacterium* (Barrientos), *Bacteroides* and

Faecalibacterium (Greenwich), and Spartobacteria\_genera\_incertaines and Rhodococcus (Dee). Taxa with <3.5% abundance are grouped as "Others."

## Species

Fourteen bacterial species were identified, with notable distributions (Figure 7). *Ktedonobacter racemifer* (AM180156) was present on Torre (6.61%) and Greenwich (3.50%), indicative of its role in oligotrophic environments. *Protochlamydia naegleriophila* (NR\_115817.1) occurred on Torre (1.39%) and Greenwich (2.68%), suggesting endosymbiotic interactions. *Aciditerrimonas* sp. (JN033775) was found on Barrientos (2.07%) and Dee (2.08%), associated with acidic soils. *Faecalibacterium prausnitzii* (AJ413954) was detected on Greenwich (2.54%), potentially indicating anthropogenic influence or extreme condition tolerance.



**Figure 7.** Species-level composition of bacterial communities in Antarctic soils from Torre, Barrientos, Greenwich, and Dee islands. Sequencing of 16S rDNA identified *Ktedonobacter racemifer* (Torre: 6.61%, Greenwich: 3.50%) and *Protochlamydia naegleriophila* (Torres: 1.39%, Greenwich: 2.68%) as notable species. *Aciditerrimonas* sp. (Barrientos, Dee) and *Faecalibacterium prausnitzii* (Greenwich) were also detected, suggesting diverse ecological niches. Taxa with <3.5% abundance are grouped as "Others."

## 4. Discussion

This metagenomic study provides a comprehensive analysis of microbial diversity in Antarctic soils from four South Shetland islands (Torre, Barrientos, Greenwich, and Dee), revealing a highly specialized bacterial community dominated by Actinobacteria (36.60%), Firmicutes (17.53%), Proteobacteria (20.03%), and Bacteroidetes (6.91%). These findings align with previous research highlighting the prevalence of these phyla in polar ecosystems, where extreme conditions such as subzero temperatures, high UV radiation, and limited nutrient availability shape microbial adaptations (Albanese et al., 2021; Cary et al., 2010b; Koo et al., 2018). The low representation of Archaea (0.02–0.15%) is consistent with their minor role in Antarctic terrestrial environments, likely due to the dominance of bacterial metabolic versatility (Ortiz et al., 2021).

The predominance of Actinobacteria across all islands, particularly on Barrientos (42.31%), underscores their ecological significance in cold, oligotrophic soils. Genera such as *Streptomyces* and *Arthrobacter*, common within this phylum, are known for producing psychrophilic enzymes and secondary metabolites, which are valuable for biotechnological applications like bioremediation and pharmaceutical development (Chauhan et al., 2023; Jansson & Taş, 2014). The high abundance of Firmicutes on Greenwich (26.66%), driven by Clostridiales and genera like

*Clostridium\_sensu\_stricto*, suggests their role in fermenting residual organic matter trapped in ice, contributing to carbon cycling in these ecosystems (Christner et al., 2014; Rivkina et al., 2000). Proteobacteria, peaking on Torre (24.15%), include Gammaproteobacteria, which are prevalent in psychrophilic habitats like subglacial lakes, indicating their adaptability to fluctuating moisture and oxygen levels (Waschulin et al., 2022).

Island-specific taxa provide insights into local environmental influences. For instance, *Verrucomicrobia* (7.89%) and *Acidobacteria\_Gp16* (9.09%) on Dee suggest nitrogen cycling and organic matter decomposition in nutrient-poor soils, consistent with their roles in permafrost ecosystems (Jansson & Taş, 2014). Similarly, the presence of *Chlamydiae* (*Protochlamydia naegleriophila*) on Greenwich and Torre points to potential endosymbiotic interactions with protists, a niche adaptation in cold environments (Maire et al., 2023). The detection of *Ktedonobacter racemifer* on Torre (6.61%) and Greenwich (3.50%) highlights its ability to form spores and recycle organic matter in arid, oligotrophic conditions, mirroring its role in other cold deserts (Chang et al., 2011; Yabe et al., 2017).

The unexpected presence of *Faecalibacterium prausnitzii* on Greenwich (2.54%) raises questions about its origin. While typically associated with human gut microbiota, its detection could indicate anthropogenic contamination from expedition activities, as suggested by (Khan et al., 2014). Alternatively, its survival in Antarctic soils may reflect previously unrecognized adaptations to extreme conditions, warranting further investigation through functional genomics or culturing studies. This finding underscores the need to monitor human impacts on pristine ecosystems, especially in the context of increasing Antarctic tourism and research activities.

The observed differences in microbial composition among islands, confirmed by PERMANOVA ( $F = 3.24$ ,  $p = 0.012$ ), likely reflect variations in environmental factors such as soil moisture, pH, and organic carbon content. For example, the higher abundance of *Bacteroidia* on Greenwich (5.14%) suggests elevated organic carbon availability, as this class is known to degrade dissolved organic compounds in cold aquatic systems (Wexler, 2007). In contrast, the prevalence of *Ktedonobacteria* on Torre (8.09%) indicates drier, more arid conditions, similar to cold desert soils in Mongolia (Yabe et al., 2017). These findings highlight the sensitivity of microbial communities to local abiotic factors, with implications for predicting ecosystem responses to climate-driven changes, such as increased meltwater from global warming (Waschulin et al., 2022).

From a biotechnological perspective, the identification of taxa like *Actinobacteria* and *Planctomycetaceae*, which are known to produce novel biosynthetic gene clusters (BGCs), emphasizes the potential of Antarctic soils as a source of enzymes and metabolites for industrial applications (Latorre-Pérez et al., 2020). For instance, psychrophilic enzymes from *Actinobacteria* could enhance processes in food preservation or bioremediation under low-temperature conditions (Ashaolu et al., 2025). Similarly, *Chitinophagaceae*'s ability to decompose chitin, observed on Barrientos (6.61%), suggests applications in waste management and biofuel production (Raimundo et al., 2021). These genetic resources underscore the importance of conserving Antarctic ecosystems to preserve their biotechnological potential.

The study also has implications for astrobiology, as the adaptations of Antarctic microbes to extreme conditions may mirror those of potential life forms on icy extraterrestrial bodies like Europa or Enceladus (Smedile et al., 2024). The presence of spore-forming taxa (*Ktedonobacter racemifer*) and endosymbionts (*Protochlamydia naegleriophila*) supports the hypothesis that microbial life could persist in subsurface or permafrost-like environments on other planets. Future studies combining metagenomics with metatranscriptomics could further elucidate the active metabolic pathways sustaining these communities, enhancing our understanding of life's limits.

Despite its strengths, this study has limitations. The reliance on 16S rDNA sequencing restricts functional insights, as it primarily provides taxonomic information. Integrating shotgun metagenomics or metatranscriptomics could reveal active genes and pathways, particularly those related to psychrophilic enzyme production or stress resistance. Additionally, the lack of detailed environmental data (e.g., soil pH, moisture) limits our ability to fully explain inter-island differences.

Future research should incorporate physicochemical analyses and experimental validation of microbial functions to complement taxonomic findings.

## 5. Conclusions

In conclusion, this study advances our understanding of Antarctic microbial ecology, highlighting the diversity and adaptability of bacterial communities in extreme environments. The identified taxa and their potential biotechnological applications underscore the value of these ecosystems as genetic reservoirs. As climate change continues to alter Antarctic habitats, ongoing metagenomic research will be critical for monitoring microbial responses and preserving these unique biological resources.

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## Abbreviations

The following abbreviations are used in this manuscript:

ESPAM MFL Escuela Superior Politécnica Agropecuaria de Manabí Manuel Felix López

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