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Regional consistency in microbial community responses to hydrocarbon pollution in maritime Antarctic soils

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Abstract

Antarctica, though remote and sparsely inhabited, faces significant ecological risks due to human activities and settlements that generate, among others, fuel leaks. In particular, maritime ice-free soils are becoming increasingly vulnerable to environmental disturbances, particularly hydrocarbon (HC) contamination, which represents a significant ecological threat. Low temperatures and limited nutrients reduce microbial degradation rates, allowing contamination to persist for decades. Despite their ecological importance, the structure and environmental drivers of Antarctic soil microbial communities under chronic HC exposure, such as at research stations, remain poorly understood. In this study, we characterized the bacterial/archaeal and eukaryotic communities across 106 soils sampled near research stations in three regions of the Antarctic Peninsula and the South Shetland Islands over two consecutive years, encompassing a wide range of HC pollution levels. Using 16S and 18S rRNA gene sequencing, we assessed amplicon sequence variants (ASVs) and explored spatial and environmental variables that influence microbial diversity, structure, and functional potential. While bacterial/archaeal beta-diversity was primarily influenced by geographic distance, functional profiles and eukaryotic diversity were shaped mainly by environmental factors, particularly HC concentration, pH, and conductivity. We identified consistent shifts in community composition, with HC and conductivity negatively correlated with alpha-diversity, and pH positively correlated. Hydrocarbon pollution consistently reduced microbial alpha-diversity and enriched specific taxa and functions. Notably, an Actinobacterium (*Williamsia*) and a Leotiomycetes fungus, largely dominated in heavily polluted sites. These taxa emerged as consistent indicators – or sentinel taxa – of HC pollution at a regional scale. Microbial communities in Antarctic soils are shaped differently by a dynamic interplay between space and environment, but chronic pollution can drive consistent community shifts across geographically distant sites.

Keywords Microbial communities, Antarctic soil, Hydrocarbon pollution, Phylogenetic beta-diversity, Sentinel taxa, *Williamsia*, Leotiomycetes.

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Background

Antarctica is one of the most extreme and isolated ecosystems on Earth. Permanently ice-free soils cover less than 0.5% of the continent's surface and are typically characterized by harsh abiotic conditions, including low temperatures and limited nutrients [1, 2]. The absence or scarcity of vegetation, invertebrates, and herbivores results in a simplified food web. Here, microbial detritus trophic pathways account for most biomass breakdown and nutrient cycling, which are crucial for ecosystem functioning [3, 4]. However, these communities are also highly vulnerable to disturbances, including anthropogenic impacts such as pollution or temperature shifts [5, 6]. Although limited to tourism and scientific research, Antarctic human activities generate environmental risks due to pollution caused by waste mismanagement, sewage discharge, air pollutant emissions, and, especially, fuel spills from research stations [6]. Soils chronically polluted with hydrocarbons (HC) are commonly found near these stations and have been used to study the effects of HC on soil biota, potential bioremediation strategies, and the isolation of psychrophilic hydrocarbonoclastic bacteria [6–8]. Low temperature, oligotrophy, and nutrient imbalances constrain microbial metabolism, leading to long-term persistence of hydrocarbons in soils [6]. HC degradation rates in Antarctic soils can be as slow as 60 mg kg^{-1} over 60 days [9], or 1,500 mg kg^{-1} over 5 years [10]. These rates are significantly lower than those observed in temperate environments or under stimulated conditions, such as the 1,300 mg kg^{-1} y^{-1} reported in the bioremediation of the emblematic Exxon-Valdez oil spill in Alaska [11] or other studies in subantarctic or temperate soils [12, 13]. Biodegradation rates are highly variable, site-specific, and depend on abiotic and biotic factors. HC is a selective pressure: microorganisms that can degrade or tolerate it perform better and are crucial to the degradation process. Several genera have successfully evolved to utilize HC as a carbon source [14]. In Antarctic soils, hydrocarbonoclastic bacteria such as *Rhodococcus*, *Polaromonas*, *Pseudomonas*, *Rhodanobacter*, *Alkanindiges*, *Arthrobacter*, *Dietzia*, *Williamsia*, and *Bacillus*, as well as fungi such as *Mortirella*, *Exophiala*, *Phenoliferia*, and *Penicillium*, have been reported and frequently isolated from polluted sites [9, 15–18]. Due to HC's carbon input, a pattern emerges in which oligotrophs are inhibited and copiotrophs are stimulated during biodegradation [15, 17]. However, microbial community (MC) responses are context-dependent and influenced by site-specific conditions such as previous community composition, pollution age, bioremediation treatment, and niche features [13, 14]. Environmental drivers of the MC structure and diversity in Antarctic soils include classical edaphic features such as pH, organic matter, and moisture [19, 20], as well as the nutrient contribution, particularly nitrogen and phosphorus, from marine fauna [19]. Spatial distance also contributes to MC beta-diversity at regional and continental scales, with lower richness at higher latitudes [20]. Despite the recognized impacts of hydrocarbons on Antarctic microbiota, the long-term consequences on taxonomic and functional diversity remain underexplored. This is partly due to microbial functional redundancy – many phylogenetically diverse taxa can perform similar ecological roles [21] – and the complex interplay between environmental filters and microbial traits. In fact, it remains unclear whether HC pollution leads to convergence at the taxonomic level or primarily selects functional traits that allow survival in contaminated niches. Different taxa degrade HC, allowing several MC members to buffer the effect of pollution [14]. In addition, soil MC tends to stabilize after long-term exposure to HC [22].

The assessment of MC responses to HC pollution is essential for understanding and predicting the long-term effects of this disturbance on MC ecological functions. In this study, we sought consistent patterns of MC in response to HC pollution across ice-free soils located at kilometer-scale distances (regional scale) in the maritime Antarctic. We focused on three geographically separated regions: the Antarctic Peninsula (AP), Greenwich Island (GI), and King George Island (KG), next to research stations, where unpolluted and chronically polluted soils coexist. Through a combination of spatially explicit sampling, high-resolution community profiling using 16 S and 18 S rRNA gene amplicon sequencing, and robust environmental characterization, we aimed to identify key drivers of microbial diversity and function. By examining taxonomic shifts, we aimed to determine whether specific microbial taxa could serve as consistent bioindicators of hydrocarbon contamination across the region and to gain a deeper understanding of the ecological processes underpinning microbial resilience in Antarctic soils.

Methods

Soil sampling

Samples were collected from sites located in the Antarctic Peninsula (AP, $n = 21$), Greenwich Island (GI, $n = 38$), and King George Island (KG, $n = 47$), 106 surface soil (0–20 cm depth) (Fig. 1). Sampling included non-polluted sites ($n = 67$) and sites with different levels of hydrocarbon pollution ($n = 39$), collected near the stations Bernardo O'Higgins (Chile, AP), Arturo Prat (Chile, GI), Eduardo Frei (Chile, KG), Great Wall (China, KG), and Bellingshausen (Russia, KG). The distance between samples in each area varied by location. For instance, in AP, the ice-free soils were in a 200 m x 200 m area (the ice-free soil next to the station), so the distance between each pair of samples ranged from 18 m to 144 m. On the

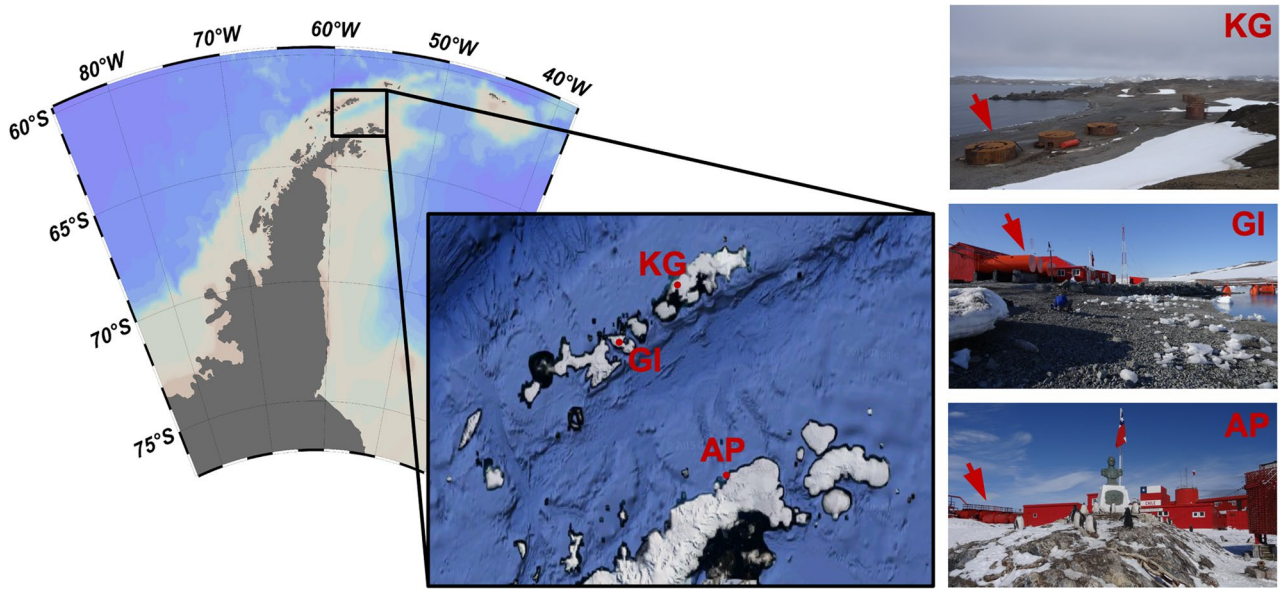

Fig. 1 Sampling sites of microbial communities from Antarctic soils contaminated with hydrocarbons during two consecutive summers (2016 and 2017). Antarctic Peninsula (AP), Greenwich Island (GI) and King George Island (KG). The red arrows indicate the diesel storage tanks. AP is located ca.129 km from GI and ca. 136 km from KG, separated by the Bransfield Strait, while GI and KG – both part of the South Shetland Islands – are ca. 50 km apart

other hand, distances on King George Island ranged from 4 to 5351 m because the ice-free area is larger. The criteria used to determine the polluted sites were information from the station crew and visual inspection. The pollution was confirmed on-site with the Oil in Soil Screening kit (Pine Environmental Inc., USA). Using these criteria, we classified the samples as "polluted" or "non-polluted". The final quantification was achieved in the laboratory by GC-FID. Sample collection was achieved twice, the first during February-March 2016 ($n = 66$) and the second during January-February 2017 ($n = 40$). The complete list and description are in Additional file 1. Each sample consisted of 5 subsamples, randomly collected within a 5×5 m square. This composite sample (single analytical unit) comment was used for DNA extraction, physicochemical analyses, and for the determination of total petroleum hydrocarbons (TPH). Samples were stored at −80 °C until processing.

Physicochemical characterization of soils

For physicochemical soil analyses, 300 g of each soil sample was collected and stored at 4 °C until analysis. Soil analyses were performed by the Soil Laboratory at the Agronomy Faculty, Universidad Austral de Chile (Valdivia, Chile). One measurement per sample was performed because this is a certified laboratory that follows protocols established by the Chilean National Institute of Agriculture and Livestock Research (INIA [23]). Briefly, soil electrical conductivity and pH were determined in aqueous soil suspension with a conductivity meter and pH meter, respectively. Available phosphorus was extracted with calcium bicarbonate and determined by the molybdenum blue method. Organic matter was determined by weight loss at 360 °C. Organic carbon was determined by oxidation with sodium dichromate and sulfuric acid. Total nitrogen was determined by Kjeldahl digestion.

Determination of total petroleum hydrocarbons

Total petroleum hydrocarbons (TPH) were quantified by gas chromatography coupled to flame ionization detector (GC-FID) as described previously [13]. Briefly, 1 g of soil was spiked with the internal standard 1-chlorooctadecane (Supelco analytical standard, Sigma-Aldrich) and dispersed in 25 mL of saturated NaCl solution. Analytical grade *n*-hexane (5 mL) was added, and the mix was homogenized by shaking and sonication. Each extract (1 µL of the organic phase) was injected into a DB5 column with helium as the carrier gas in a PerkinElmer Clarus 680 machine as described previously [13]. The identification of peaks was achieved with the DRO-1 standard (Dr. Ehrenstorfer GmbH, Augsburg, Germany), which includes C10–C25 *n*-alkanes in accordance with EPA 8015D method. Moisture was measured in parallel in a moisture meter (Sartorius MA35) to determine and correct the dry weight. The detection limit of the method was 65 ng in the standard curve, corresponding to 325 mg kg^{-1} for a soil sample.

DNA extraction, small sub-unit rRNA gene amplification and sequencing

Total community DNA was prepared from 0.5 g of soil using the PowerSoil DNA Isolation Kit (QIAGEN) following the manufacturer's instructions. The mechanical disruption step was aided by a bead-beater machine (2 pulses at 7 m s^{-1}, 1 min each. BeadBug 6, Benchmark). Blank extractions were included to control handling and kit components. Each purified DNA was amplified by PCR with the same primers used by the sequencing facility to ensure that DNAs are free of inhibitors and meet quality requirements for sequencing. Purified DNAs were used as templates to amplify the 16 S and 18 S rRNA genes separately, which were then sequenced according to the Earth Microbiome Project protocols (www.earthmicrobiome.org/protocols-and-standards). The 16 S rRNA V4-V5 region was amplified by PCR with the primers 515 F (GTGYCAGCMGCCGCGGTAA) and 926R (CCGYCAATTYMTTTRAGTTT) [24]. The 18 S rRNA V9 region was amplified by PCR with the primers 1391 F (GTACACACCGCCCGTC) and EukBr (TGATCCTTCTGCAGGTTCACCTAC) [25]. Amplification products were multiplexed and sequenced in the Illumina MiSeq platform (2 × 250 bp for 16 S gene, 2 × 150 bp for 18 S gene) at Argonne National Laboratory (Lemont, IL, USA) as described previously [26]. Sequences were submitted to the NCBI BioProject database (see "Availability of data and material" section).

Processing of the 16 S and 18 S rRNA sequence data

The processing of both 16 S and 18 S datasets, from raw sequences to contingency tables, was performed separately with QIIME2 v2020.2 [27]. Each dataset was demultiplexed into per-sample sequences, which were quality-checked, assembled, and chimera-filtered with DADA2 [28] using the default parameters of the version in QIIME2. With the DADA2 strategy, we obtained unique amplicon sequence variants (ASV). Bacteria/Archaea and Eukarya ASVs were assigned using the SILVA138 database (November 2019 [29]), with vsearch (query alignment coverage and identity < 80%) [30]. 3.2% of reads in the 16 S dataset were classified as chloroplasts and mitochondria and were discarded. 42.6% of reads in te 18 S dataset were classified as 16 S rRNA sequences or had low-quality taxonomic assignments and were discarded, which is normal for amplicons generated with this primer pair. To calculate beta-diversity indexes (i.e. Bray-Curtis and weighted UniFrac), the 16 S and 18 S ASV tables were first rarefied to 7,810 and 9,057 reads per sample, respectively. This value corresponds to the sample with the lowest depth, thereby minimizing data loss. For weighted UniFrac calculation, sequences were first aligned with MAFFT [31], and the trees were constructed using Fasttree [32] separately for 16 S and 18 S datasets. All steps were performed with the software versions implemented in QIIME2.

The taxonomy-based functional assignment was performed using the Functional Annotation of Prokaryotic Taxa (FAPROTAX v1.1) pipeline, which converts the rarefied ASV table (16 S only) into a table of prokaryotic functions associated with biogeochemical processes, including carbon, nitrogen, sulfur, and other major metabolic features [21].

Spatial and environmental descriptors of community beta-diversity

The geographical coordinates (latitude and longitude, Additional file 1) were transformed into a pairwise great-circle (geodesic) distance matrix (in meters) with the R geosphere v1.5–10 package. Starting from here, all spatial and environmental analyses of communities were performed in the R vegan v2.5–6 package [33]. The geodesic distance matrix (Additional file 2) was further converted into principal coordinates of neighbor matrices (PCNM), which were used as spatial descriptors [34]. The environmental/contextual descriptors assessed were: TPH, conductivity, pH, phosphorous, C:N ratio, tidal effect (i.e., yes/no; where "yes" corresponded to intertidal sites inundated during high tides and "no" to sites out of the intertidal zone), penguin presence (i.e., yes/no; where "yes" corresponded to sites where penguins were nesting and raising their chicks and "no" to sites without nests), location (i.e., AP, GI, KG), and sampling year (i.e., 2016 and 2017). All were first checked for collinearity using the vegan *vif.cca* function as a diagnostic tool to identify useless constraints. The C: N ratio, penguin presence, and location had variance inflation factors > 5 and were removed from further analysis. The selection of significant explanatory descriptors of microbial communities was accomplished by automatic forward selection with vegan's *ordiR2step* function [35]. For this, environmental and spatial descriptor matrices were used separately as explanatory matrices of the beta-diversity matrix in a distance-based redundancy analysis (db-RDA) [36]. The significance of descriptors, RDA axes, and the whole model was tested using the *anova* function. Only those with adjusted $p < 0.01$ (Bonferroni corrected) were kept. Finally, the contribution of each variable to explain the model was computed by several partial db-RDA analyses where the contribution of each variable or group of variables was assessed separately with the *RsquareAdj* function, i.e., the effect of a single environmental descriptor was assessed by controlling the contribution of the other variables.

Identification of ASVs and functions associated with hydrocarbon pollution and other environmental drivers

The identification of ASVs and functions with different relative abundance across TPH levels was performed by the analysis of composition (ANCOM [37]), implemented in QIIME2. First, the low abundance ASVs (i.e. <0.01% of the total reads and present in < 10 samples) were filtered out from non-rarefied 16 S and 18 S ASV tables. Second, the environmental descriptors were collapsed into groups of increasing levels, defined by the range of each variable, aiming to include enough samples in each range to balance the groups as much as possible (ANCOM can analyze unbalanced groups). Five groups were defined for TPH: 0, 1–5, 5–10, 10–15, and > 15 g kg^{-1}. For pH: 5.9–6.5, 6.7–6.9, 7.0–7.4, 7.5–7.9, 8.1–8.5, and 8.6–9.5. For conductivity: 0.01–0.07, 0.1–0.2, 0.3–0.9, 1.0–1.8, 2.1–5.3, and 9.4–12.6 mS cm^{-1}. For phosphorous: 2.6–3.8, 4.0–5.4, 6.2–9.8, 11–14, 42–64, and 85–620 mg kg^{-1}. For categorical descriptors, only two groups were defined: presence/absence of tidal influence and presence/absence of penguins. Third, samples were classified into each group, and ANCOM was applied to find significant differences in mean abundances of each Bacteria/Archaea ASV, each Eukarya ASV, and each bacterial/archaeal FAPROTAX-inferred function across the groups defined above. Several filters were applied to conserve ASVs/functions with different abundance exclusively for each descriptor. First, ASVs/functions with significant differences (ANCOM) in their mean relative abundance across groups were filtered into a new table. Second, if an ASV/function resulted significant value for more than one descriptor, it was removed from the tables. This was done because sites with the highest TPH levels also had basic pH, high phosphorous content, or high conductivity. Third, Spearman correlation was applied between the abundance of each ASV/function in the previous tables and the corresponding environmental descriptor wih *rcorr* function (R Hmisc v5.1-1 package). Only those with significant (adjusted $p < 0.01$, Bonferroni corrected) correlation coefficients (Spearman's ρ) were conserved. Only those with $\rho < -0.5$ or $\rho > 0.5$ were conserved to avoid meaningless correlations.

Results

Environmental and spatial characterization of Antarctic soils

Soil sampling was conducted during two consecutive austral summers, 2016 and 2017, across three areas: the Antarctic Peninsula (AP), Greenwich Island (GI), and King George Island (KG) (Fig. 1). All 106 soil samples were classified as sandy to sandy-loam in texture and covered a range of hydrocarbon pollution levels (Table 1), including sites near diesel storage tanks at AP (Bernardo O'Higgins Station, Chile), GI (Arturo Prat Station, Chile), and KG (Eduardo Frei Station, Chile; Great Wall Station, China; and Bellingshausen Station, Russia). Total petroleum hydrocarbon (TPH) content in the 39 polluted soils ranged from mild (970 mg kg^{-1}) to heavily polluted (37,430 mg kg^{-1}). GC-FID gas chromatography profiles confirmed the presence of weathered diesel, particularly at AP and GI (Additional file 3). In contrast, 67 unpolluted soils were collected either adjacent to or distant from polluted sites in the research stations (Fig. 1). Soil pH values ranged from 5.9 to 9.5 across all areas, with equal distribution among soils from AP, GI, and KG. The organic matter content varied between 0.09% and 10.7%, with the polluted sites having the highest values. A group of 21 AP samples, 17 from 2016 to 4 from 2017, were collected near a Gentoo penguin (*Pygoscelis papua*) colony and exhibited ornithogenic characteristics, such as elevated nitrogen content (700–3,400 mg kg^{-1}) and phosphorus (42–620 mg kg^{-1}) whereas the nitrogen content of non-ornithogenic soils ranged between 2.6 and 13.6 mg kg^{-1}. This is expected due to nitrogen and phosphorus contributions from penguin feces. The same pattern was observed for conductivity, which ranged between 0.01 and 12.6 mS cm^{-1}; however, the 17 samples with conductivity > 4 mS cm^{-1} were also the ornithogenic soils collected in 2016. This pattern was less evident in the 2017 ornithogenic samples, where conductivity was < 2 mS cm^{-1}. Additionally, 23 sites were located in intertidal zones of AP, GI, and KG (here included as a categorical variable). The complete data and sample description are provided in Additional file 1.

Spatial and environmental drivers of microbial community diversity and composition

The MC was studied by sequencing the small sub-unit rRNA gene of Bacteria/Archaea (16 S) and Eukarya

Table 1 Summary of samples analyzed in this study. Total petroleum hydrocarbons (TPH) are in Mg kg^{-1} soil (dry weight)

Location	2016				2017			
	Total	No HC	Polluted	TPH	Total	No HC	Polluted	TPH
AP	17	6	11	1,150 – 11,000	4	0	4	970 – 37,430
GI	23	17	6	2,250 – 16,350	15	9	6	4,240–17,350
KG	26	19	7	4,620 – 25,620	21	16	5	4,690 – 13,920
TOTAL	66	42	24		40	25	15	

Table 2 Summary of spearman correlation between alpha-diversity and soil parameters. Only significant correlations (adjusted p-value < 0.01) are shown. OM: organic Matter, TPH: total petroleum hydrocarbons

Parameter	Bacteria/Archaea		Eukarya	
	Richness	Faith's PD	Richness	Faith's PD
Nitrogen	−0.600	−0.540	−0.678	−0.712
Phosphorus	−0.695	-	-	-
Conductivity	-	−0.594	-	-
OM	-	−0.674	−0.802	−0.794
TPH	−0.661	−0.670	−0.701	−0.665

(18 S). The Bacteria/Archaea dataset comprised 2,014,123 reads across 12,527 ASVs, with sequencing depths ranging from 7,810 to 38,423 reads per sample. The 18 S rRNA dataset (Eukarya) comprised 3,149,462 reads, distributed into 6,762 ASVs, with depths ranging from 9,057 to 71,088 reads per sample. A general trend was observed for bacterial/archaeal alpha-diversity, which decreased significantly in soils with higher nutrient content, salinity, and TPH (Table 2).

The db-RDA showed that the phylogenetic beta-diversity of the Bacteria/Archaea community was primarily explained by spatial distance (21.3%) rather than environmental factors (12.0%), with 30.3% that cannot be explained separately, i.e., a mixed effect depicted as the intersection (Fig. 2a). Among the environmental drivers, TPH and pH had the strongest effects, ca. 4-fold higher than conductivity or tidal influence (Fig. 2a). In contrast, Eukarya beta-diversity was more explained by environmental factors (19.1%) than spatial distance (13.4%), with a modest mixed effect (4.6%) (Fig. 2b). The most influential environmental descriptors were conductivity, tidal effect, and TPH, and a minor contribution of pH (Fig. 2b). Bacteria/Archaea functional diversity, inferred using FAPROTAX, was greatly influenced by the environment instead of space (29.7% vs. 5.7% with 9.3% of mixed effect), with pH, conductivity, and TPH having similar contributions (Fig. 2c), and supporting the notion that environmental filtering targets microbial traits rather than taxa.

The full taxonomy assignments for Bacteria/Archaea and Eukarya MC per sample are in Additional files 4 and 5. The dominant bacterial and archaeal phyla included Proteobacteria, Bacteroidota, and Actinobacteriota, followed by Acidobacteriota and Verrucomicrobiota in some sites. HC-polluted soils showed a dominance of Proteobacteria and Actinobacteriota, whereas non-polluted soils were enriched in Bacteroidota. The dominant eukaryotic phyla were Ascomycota and Basidiomycota (Fungi); Ochrophyta, Chlorophyta, Diatomea, and Dinoflagellata (marine algae); Euglenozoa, Arthropoda, Nematozoa, Rotifera, and Phragmoplastophyta (soil, fresh- and seawater metazoans); and the parasitic phyla

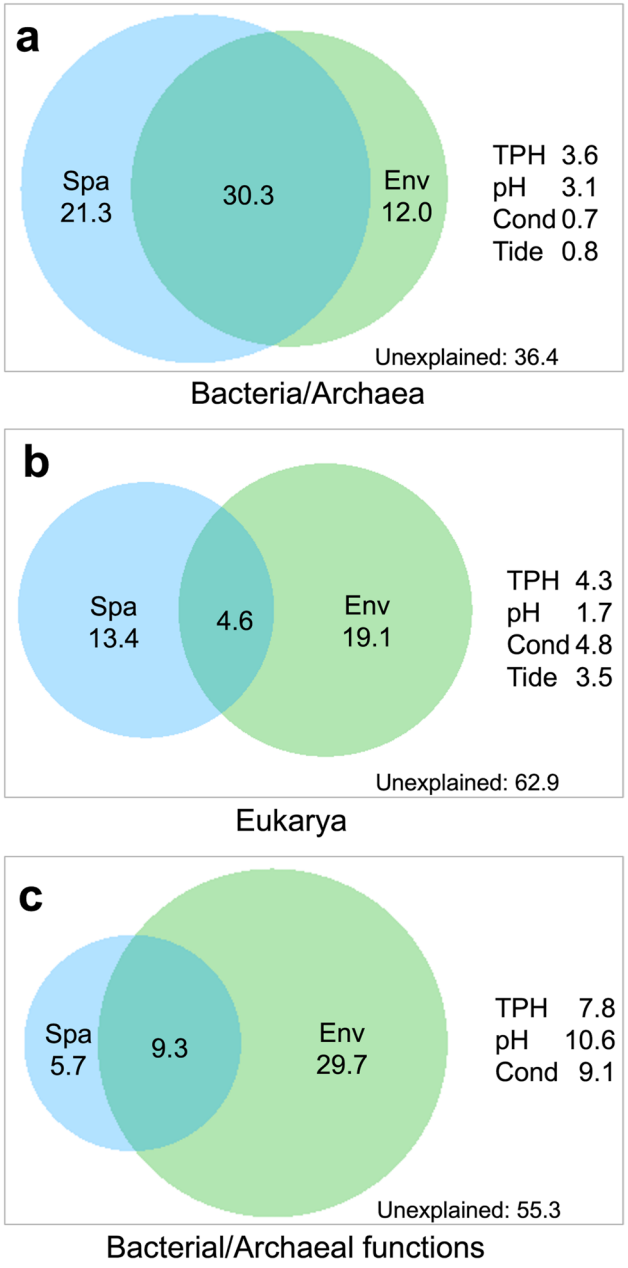

Fig. 2 Influence of spatial and environmental descriptors on the beta-diversity of microbial communities from HC-contaminated Antarctic soils. **a** Bacteria/Archaea community. **b** Eukarya community. **c** Bacterial/archaeal functions. In all cases, the db-RDA analysis was conducted using spatial (PCNM) and environmental (TPH, conductivity, pH, phosphorous, C:N ratio, tidal effect, penguin presence, location) data as explanatory variables. The beta-diversity for **a** and **b** was weighted UniFrac, which computes phylogenetic distance between each pair of samples. Bray-Curtis was used in **c**, which accounts for the number of functions in common between each pair of samples without considering phylogenetic information

Apicomplexa. Fungal phyla dominated in HC-polluted soils, while Apicomplexa dominated in non-polluted soils located out from the intertidal zone. The significance of these patterns was further tested.

Sentinel taxa and functions associated with Antarctic hydrocarbon-polluted soils

After identifying the environmental descriptors that best explained community beta-diversity, we assessed whether some specific taxa are associated with functional traits related to hydrocarbon-polluted Antarctic soils. ANCOM and Spearman correlation analyses were used to identify ASVs and functions significantly related to TPH, pH, and conductivity. These variables were grouped into incremental ranges, allowing for the identification of ASVs with significantly different relative abundances across groups. A total of 11 Bacteria/Archaea ASVs differed significantly across the six pH ranges, all enriched in slightly acidic to neutral soils (Fig. 3). For conductivity, 34 Bacteria/Archaea (Fig. 4a) and 2 Eukarya ASVs (Fig. 4b) showed significant differences among conductivity ranges. Regarding TPH, 10 Bacteria/Archaea ASVs (Fig. 5a), 9 Eukarya ASVs (Fig. 5b), and two bacterial/archaeal functions (Fig. 5c) varied significantly in relative abundance across five TPH categories.

All ASVs associated with pH were exclusively bacterial/archaeal. These ASVs were consistently more abundant in mildly acidic to neutral soils (pH 5.9–7.5) and depleted in mildly alkaline soils (pH 7.5–9.5) (Fig. 3). Enriched ASVs at neutral to mild acid were Acidobacteriota (*Bryobacter*, Holophagae), Actinomycetota (*Gaiella*), Bacteroidota (Chitinophagaceae), Chloroflexota (Ardenticatenales), Gemmatimonadota (Gemmatimonadaceae), and Verrucomicrobiota (Candidatus *Udaeobacter*). The five ASVs identified as Candidatus *Udaeobacter* and the ASV identified as Gemmatimonadaceae ASV represented > 10% of the MC in mildly acidic to neutral soils.

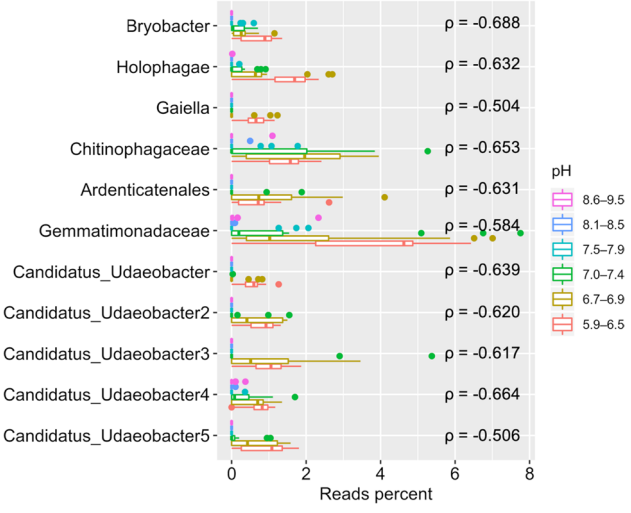

Fig. 3 ASVs with significantly different relative abundance among different pH ranges. Boxplots show the median and quartiles; points indicate the outliers. Spearman correlation (ρ) between relative abundance and pH is shown for each ASV

Conductivity positively correlated with 23 Bacteria/Archaea ASVs, belonging to a wide range of taxa (Fig. 4a), including the classes Alphaproteobacteria (NRL2, Altererythrobacter, Sphingomonadaceae, *Sphingorhabdus*), Gammaproteobacteria (*Luteimonas*, *Marinobacter*, *Psychrobacter*, *Thermomonas*, Xanthomonadales), Actinomycetota (*Rhodococcus*, *Tessaracoccus*), Planctomycetota (*Rhodopirellula*), and Bacteroidota (*Algoriphagus*, *Ferruginibacter*, *Flavimarina*, *Flavobacterium*, *Gelidibacter*, *Gillisia*). In contrast, 11 bacterial ASVs negatively correlated with conductivity, including Betaproteobacteria (*Limnobacter*, Nitrosomonadaceae), Gammaproteobacteria (*Rhizobacter*), Acidobacteriota (Acidobacteriaceae, *Bryobacter*), Gemmatimonadota (Gemmatimonadaceae), Verrucomicrobiota (Pedosphaeraceae) and Bacteroidota (Chitinophagaceae). *Thermomonas* was strongly favored in high-conductivity soils (ca. 8% of reads), whereas one Gemmatimonadaceae ASV was exclusive to low-conductivity soils. Within Bacteroidota, conductivity responses were taxon-specific: Flavobacteriaceae and Cyclobacteriaceae exhibited positive correlations, whereas Chitinophagaceae showed negative correlations. Among Eukarya, only one Ulvophyceae ASV was positively correlated with conductivity, while one Phragmoplastophyta ASV was negatively correlated (Fig. 4b).

TPH concentration showed only positive correlations with microbial abundance of ASVs belonging to Alphaproteobacteria (*Rhizorhapis*, Sphingomonadaceae, *Tardiphaga*), Betaproteobacteria (*Sulfuritalea*), and Actinobacteriota (Microbacteriaceae, *Microbacterium*, *Mycobacterium*, *Williamsia*). The *Williamsia* ASV had not only a strong correlation (ρ = 0.746) but also represented a major proportion of the Bacteria/Archaea community in polluted soils, exceeding 40% of reads at heavily polluted sites (Fig. 5a). A similar trend, albeit at lower abundance, was observed for bacterial/archaeal hydrocarbon degradation genes (Fig. 5c). Several Ascomycota ASVs also showed a positive correlation with TPH. One dominant Ascomycota ASV (at the top of Fig. 5b), affiliated with Pezizomycotina, was strongly correlated with TPH, although genus-level resolution was not achieved. An ASV identified as Leotiomycetes, likely within the Helotiales order (based on BLAST hits, not shown), reached more than 60% of reads in some heavily polluted samples. Two additional Helotiales ASVs (the second and third in Fig. 5b), also showed TPH enrichment, supporting the potential role of this group in hydrocarbon-contaminated environments. Other eukaryotes enriched in TPH soils include the Ascomycota family Sclerotiniaceae, the Basidiomycota genus *Bannoa*, and protists belonging to the phylum Cercozoa and the orders Glisssomonadida and Chrysophyceae (Fig. 5b). Remarkably, the enrichment of *Williamsia* and Leotiomycetes was consistently observed across all three regions (AP, GI, KG), indicating

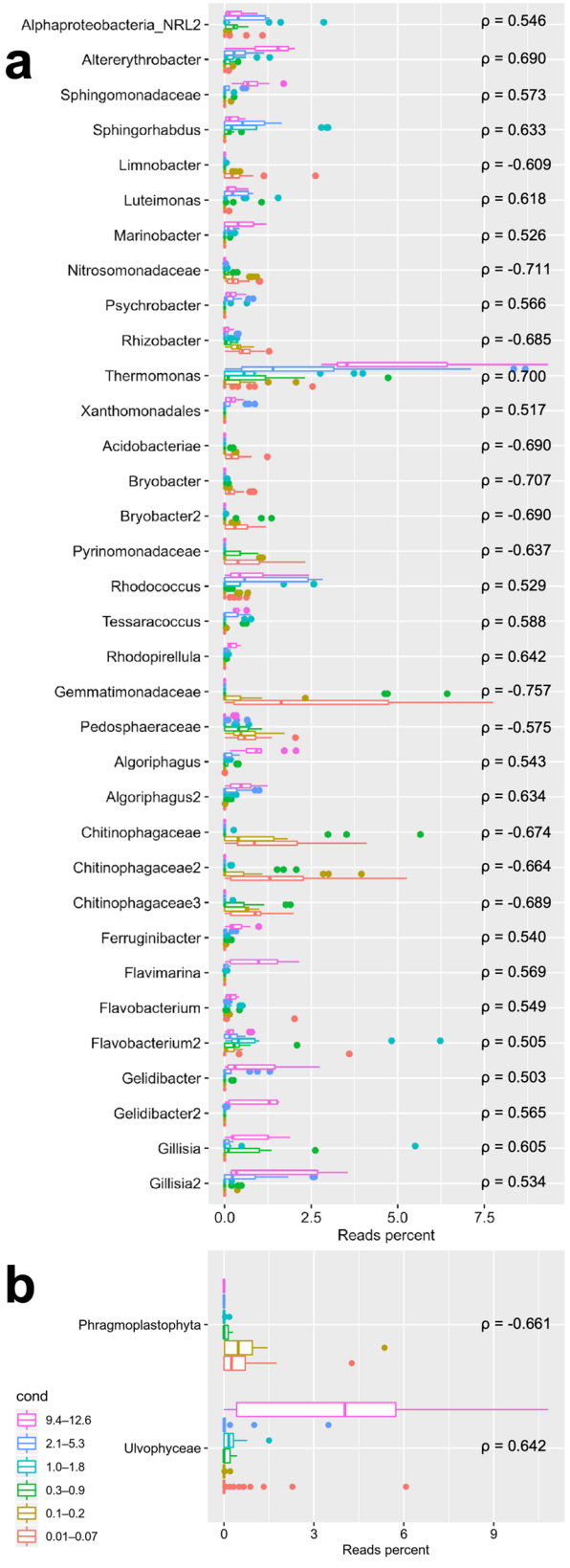

Fig. 4 ASVs with significantly different relative abundance among different conductivity ranges. Boxplots show the median and quartiles; points indicate the outliers. Spearman correlation (ρ) between relative abundance and conductivity is shown for each ASV. **a** Bacterial/Archaeal ASVs. **b** Eukarya ASVs

a non-site-specific, regionally consistent response to hydrocarbon pollution.

Discussion

Environmental and spatial drivers shape microbial communities differently

This study shows that Antarctic soil MCs are shaped by both environmental and spatial factors, with different effects on Bacteria/Archaea and Eukarya. Heavily polluted soils were found in the three areas at concentrations similar to other reported Antarctic soils (Table 1), which range from hundreds to a few thousand mg TPH kg^{-1}, e.g., 82–426 [9], 2,000 [10], 2,180 [15], 401–5,000 [38], 7,620 [7]. However, extreme cases up to 33,000 mg kg^{-1} have been reported [6]. Stations in these areas have been active for decades – Eduardo Frei (Chile, since 1969), Great Wall Station (China, since 1985), Bellingshausen Station (Russia, 1968), Arturo Prat (Chile, 1947), and Bernardo O'Higgins (Chile, 1948). While no specific pollution events could be confirmed, ongoing low-level leakage likely contributed to the persistence of recalcitrant hydrocarbons – fractions that are less bioavailable and biodegradable [39, 40]. GC-FID chromatograms, particularly from AP and GI, revealed profiles consistent with weathered diesel (Additional file 2). A fresh diesel chromatogram has a characteristic bell shape with well-defined peaks corresponding to the C10–C25 *n*-alkanes. On the other hand, the chromatogram of weathered diesel lacks these peaks, and the bell shape can be displaced towards higher retention times, i.e., a higher proportion of high-molecular-weight hydrocarbons, the recalcitrant fraction [39, 40]. This suggests a chronic pollution likely resulting from decades of station operation. Long-term exposure to HC likely contributes to the observed negative correlation between TPH and alpha-diversity, reflecting a strong selection for HC-tolerant or degrading taxa and a reduction of taxonomic complexity [13]. Functional inference further revealed that environmental filtering in polluted soils results in functional specialization, specifically an increase in HC-degrading functions (Fig. 5c). Thus, a trait-based filtering, rather than taxonomic filtering, probably governs MC assembly under intense stressors.

Edaphic factors, such as pH, conductivity, moisture, and nutrient availability, are known to significantly impact the MC composition of Antarctic soils [41–44], consistent with our results (Fig. 2). Importantly, spatial and environmental effects differed between domains: whereas spatial distance had a stronger influence on Bacteria/Archaea, soil properties were more significant for Eukarya. This contrasts with Antarctic marine environments, where environmental filtering exerts a stronger influence on Bacteria/Archaea than on Eukarya [45]. These differences highlight that community assembly

varies with habitat type, taxonomic groups, and spatial scale [46, 47]. However, a broader spatial sampling effort is necessary to confirm these patterns and fully describe the Antarctic microbial diversity.

This pattern likely reflects differences in ecological traits, dispersal mechanisms, and resilience to environmental stressors between prokaryotes and eukaryotes. The minimal effect of geographical distance on fungal beta-diversity aligns with previous findings [48] and is consistent with the fungal dominance within eukaryotic MC in this study (Additional file 5). Fungi may respond more directly to local edaphic conditions, whereas the broader taxonomic and functional redundancy among Bacteria/Archaea allows spatial separation to play a larger role in shaping community structure. Soil depth may also contribute to the observed heterogeneity, as reported in other studies [45]. However, this study focused on surface soil, and this was not assessed. Although the total variation explained was relatively low, TPH and salinity-related descriptors had a higher effect on Eukarya, while pH was the most significant descriptor for Bacteria/Archaea. These findings reflect differences in ecological traits between the two domains. In the eukaryotic community, the ability to degrade hydrocarbons is restricted mainly to fungi, which likely explains their enrichment in highly polluted soils [14]. Although some green algae and diatoms have been reported as HC degraders [49, 50], their ecological contributions under natural conditions remain poorly understood. Additionally, the presence of taxa with marine lifestyles and taxa found in both soil and aquatic habitats [51] may contribute to the phylogenetic differentiation observed in Eukarya communities.

In contrast, HC degradation capabilities are widespread across numerous bacterial taxa [14]. Here, the Bacteria/Archaea MC was primarily dominated by bacteria (> 95% of reads in all samples and up to 99.99% in some samples, see Additional file 4), suggesting that functional redundancy may buffer the effects of HC as a selective pressure. This buffering probably contributes to the stronger influence of spatial distance over TPH in shaping bacterial beta-diversity. Furthermore, previous work has shown that microbial communities can stabilize after prolonged HC exposure, reducing the impact of HC concentration on community structure over time [22, 52]. For Bacteria/Archaea MC, there was a decoupling between taxonomic and functional beta-diversity, with a strong environmental influence on functions (Fig. 2a and c). This supports the idea that environmental filtering acts primarily on microbial traits rather than on specific lineages [21]. Similar patterns have been observed for pH, a classical edaphic driver of soil MC diversity consistently reported in Antarctic, Arctic, and template soils [43, 53, 54]; and across local to global scales [55–57]. Except for some specific acidophiles or alkalophiles favored in highly acidic or alkaline environments, pH has a broad effect on the entire community and is an essential driving force in the phylogenetic assembly of bacteria [56].

Sentinel taxa in polluted soils are consistent across kilometers of distance

The identification of specific ASVs correlated with the diversity drivers offered a finer resolution into potential microbial indicators of environmental stress. Soil pH emerged as a significant factor influencing Bacteria/Archaea diversity. Soil pH is also a main driver of bacterial communities in different Andes Mountains glaciers of Chile and Argentina, where glaciers are subjected to anthropic input of aerosols and black carbon [58]. Among the ASVs enriched in mildly acidic to neutral soils there are common members of Antarctic soil microbiomes known to be sensitive to pH variations. For instance, *Bryobacter* has been frequently associated with the rhizospheres of Antarctic vascular plants (*Deschampsia antarctica* and *Colobanthus quitensis)* and with bulk soils [1, 59]. Similarly, Gemmatimonadaceae have been linked to mildly acidic soils [57]. The remaining taxa in Fig. 3 have been described in Antarctic soils; therefore, they are part of the soil microbiome and are influenced by pH to varying degrees [60].

Taxa positively correlated with conductivity – a proxy for salinity and seawater intrusion – were associated with marine habitats or, at the very least, exhibit some degree of halotolerance. For instance, Flavobacteriaceae is a marine family known to colonize the algal phycosphere and is a key player during Antarctic phytoplankton blooms [61, 62], but also exhibits plasticity, allowing colonization of terrestrial environments [63]. Cyclobacteriaceae species have been isolated from marine environments, including seaweeds [64, 65], and may be ecologically linked to the Ulvophyceae ASV detected in intertidal soils (Fig. 4b). Although Ulvophyceae have also been reported in arid soils of the AP [66], their presence in this study likely reflects seawater influence, particularly given the elevated conductivity values at the intertidal zones, which exceed those of typical intertidal beaches (1–3 mS cm^{-1}) [67], and approach marine values (30–60 mS cm^{-1}) [68]. The positive correlation of a *Thermononas* ASV with conductivity is unexpected, as this denitrifying genus typically inhabits a wide range of thermophilic and mesophilic environments but has been poorly described in marine or polar ecosystems [1, 69]. In contrast, ASVs negatively correlated with conductivity were predominantly associated with terrestrial habitats, such as temperate soils and rhizospheres or Antarctic vascular plants [70–72]. These patterns suggest that conductivity acts as a strong environmental filter, selecting for marine or halotolerant taxa while excluding more strictly terrestrial microorganisms [67]. Together, pH

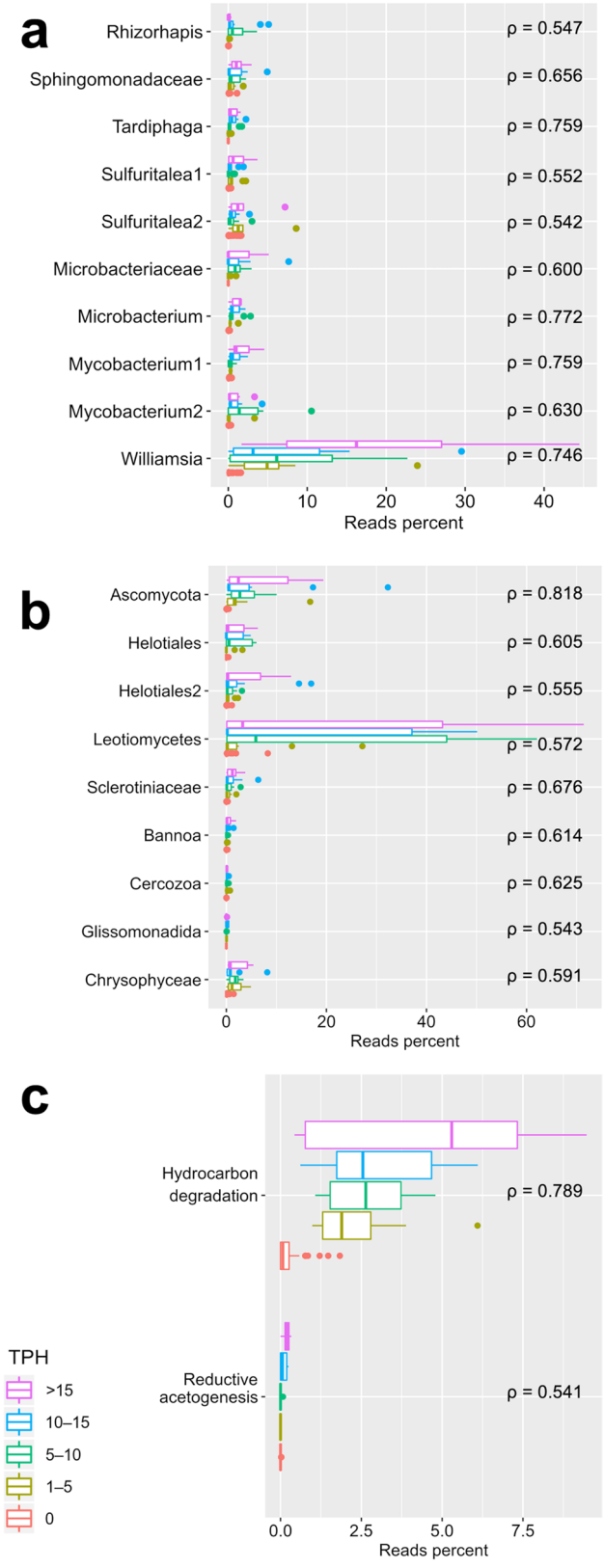

Fig. 5 ASVs with significantly different relative abundance among different TPH ranges. Boxplots show the median and quartiles; points indicate the outliers. Spearman correlation (ρ) between relative abundance and TPH is shown for each ASV. **a** Bacterial/Archaeal ASVs. **b** Eukarya ASVs. **c** Inferred bacterial functions

and conductivity descriptors were significantly correlated with the relative abundance of a wide range of taxa with currently unknown ecological functions.

In Bacteria/Archaea communities, TPH concentrations were positively correlated with the relative abundance of specific ASVs belonging to taxa commonly found in Antarctic soils [73]. The *Williamsia* ASV stands out as the dominant ASV in soils contaminated with TPH (Fig. 5a). This ubiquitous genus has been found in a variety of environments, including marine and lake sediments, as well as phyllospheres [74–76]. One species isolated from a site contaminated with oil – *W. serinedens* – has the ability to degrade compounds such as *p*-hydroxybenzoate and paraffin as the sole carbon source [77]. Similar to our results, a metagenomic study of oil-contaminated soils on KG found a positive correlation between *Williamsia* abundance and TPH concentration [16], reinforcing its relevance as a functional indicator of HC degradation in Antarctic soils. Among Eukarya, ASVs enriched in TPH-contaminated soils belonged to Ascomycota, which is the predominant phylum of filamentous fungi in Antarctic terrestrial environments [78]. Notably, the enrichment with Leotiomycetes and Helotiales orders (Fig. 5b) could be explained by being involved in the degradation of TPH and hydrocarbon-based plastics [79, 80]. Their consistent presence in Antarctic soils – including those associated with *D. antarctica* – supports their ecological adaptability and bioremediation potential [81]. The ability of Leotiomycetes to colonize the soils of this continent, together with their capacity to degrade hydrocarbons, suggests their potential role in restoring TPH-contaminated Antarctic soils. The other eukaryotic taxa associated with TPH-rich soils included Sclerotiniaceae, *Bannoa*, Cercozoa, Glissomonadida, and Chrysophyceae (Fig. 5b). While these taxa have been previously reported in Antarctic soils [66, 81], this study, to our knowledge, is the first to link them to HC pollution.

Functional redundancy and trait filtering in Antarctic microbial communities under HC

Notably, the enrichment of both *Williamsia* and Leotiomycetes ASVs was consistently observed across all three geographically distant sites (AP, GI, KG), suggesting a regionally conserved microbial response to hydrocarbon contamination. These taxa were also associated with the abundance of hydrocarbon degradation genes (Fig. 5c), reinforcing their potential role as functional indicators of HC pollution. The correlation between HC degradation gene abundance and TPH serves as a positive control for the validity of our functional inference. However, due to taxonomic and functional decoupling [21], the high rates of horizontal gene transfer in bacteria, and the large number of HC-degrading bacterial taxa, traits are not necessarily associated with a specific taxon

[82]. Therefore, other analyses, such as shotgun metagenomic sequencing, are necessary to assess whether the HC degradation pathways are associated with the *Williamsia* ASV or other less abundant species. Our analysis between ASVs and environmental descriptors was particularly restrictive (i.e., ASVs with low abundance and prevalence, with significantly different proportions for more than one descriptor in ANCOM, or with $-0.5 > \rho < 0.5$, were filtered out from the analysis), thus avoiding meaningless correlations. In addition, all samples were handled, sequenced and analyzed in exactly the same way, thus discarding methodological biases. Despite this, the robustness of the *Williamsia* and Leotiomycetes signals across sites emphasizes their potential as sentinels for monitoring HC pollution. Still, broader sampling across additional Antarctic regions is required to determine whether these patterns are consistent across the continent and to further disentangle the taxonomic and functional dynamics of microbial responses to hydrocarbon stress.

A key finding of this study is the decoupling between taxonomic and functional diversity in the Bacteria/Archaea domain. While taxonomic beta diversity was more strongly influenced by spatial distance, functional profiles were more strongly shaped by environmental factors, particularly TPH, pH, and conductivity. This supports the concept of functional redundancy in microbial ecosystems, in which phylogenetically distinct taxa perform similar ecological functions, thereby buffering ecosystem functional capacity despite taxonomic turnover. This observation aligns with previous research suggesting that HC pollution selectively favors functional traits (e.g., hydrocarbon degradation) rather than specific microbial taxa. It also highlights the need to integrate both taxonomic and functional assessments in studies of microbial responses to environmental stress, particularly in extreme and vulnerable ecosystems such as Antarctica.

Conclusions

This study provides a comprehensive understanding of how chronic hydrocarbon pollution is a major driver of microbial communities in maritime Antarctic soils. There is a consistent pattern in which HC favors specific community members at sites located several kilometers away. The identification of sentinel microbial taxa consistently enriched across distant sites reinforces the importance of long-term monitoring and the potential development of bioindicators for pollution. Surprisingly, the influence of nutrients and bacteria introduced by penguin feces appears to be minor, indirect, or masked by the other drivers. While our approach relied on high-resolution amplicon sequencing and functional inference, future studies incorporating metagenomic, metatranscriptomic or metaproteomic analyses could elucidate the active metabolic pathways and confirm the role of dominant taxa in HC degradation. Additionally, broader sampling across other Antarctic regions would allow us to evaluate the generalizability of our findings and to refine predictive models of microbial ecosystem responses to environmental stressors. In conclusion, microbial communities in Antarctic soils are shaped differently by a dynamic interplay between space, environment, and pollution. Chronic exposure to hydrocarbons continues to influence microbial assemblages for decades after initial exposure, underscoring the long-term ecological footprint of anthropogenic activity in one of the planet's most sensitive ecosystems.

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Supplementary Material 5.

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Authors' contributions

SFA: Conceptualization, Formal analysis, Data curation, Funding acquisition, Investigation, Validation, Writing – Original draft, Writing – review & editing. MJV: Validation, Writing – Original draft, Writing – review & editing.MS: Resources, Writing – review & editing.BD: Conceptualization, Funding acquisition, Resources, Validation, Writing – Original draft, Writing – review & editing.

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Data availability

The 16S rRNA gene amplicon sequence dataset is available in the NCBI BioProject repository. It can be accessed via the following URL: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1293543, which includes BioSamples SAMN50145876 to SAMN50145981. Raw datasets are available in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) with SRA numbers SRR34671997 to SRR34672102.
The 18S rRNA gene amplicon sequence dataset is also stored in the NCBI BioProject repository. It is accessible through the URL: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1294992, covering BioSamples SAMN50146187 to SAMN50146292. Raw datasets are available in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) with SRA numbers SRR34672363 to SRR34672468.
The soil physicochemical and full sample metadata is provided in Additional file 1.
The matrix of geodesic distances (in meters) is available in Additional file 2.
The diesel chromatograms are available in Additional file 3.
The Bacteria/Archaea heatmap and taxonomic assignment is available in Additional file 4.

The Eukariotic heatmap and taxonomic assignment is available in Additional file 5.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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