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Metabarcoding the Antarctic Peninsula biodiversity using a multi-gene approach

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Marine sediment communities are major contributors to biogeochemical cycling and benthic ecosystem functioning, but they are poorly described, particularly in remote regions such as Antarctica. We analysed patterns and drivers of diversity in metazoan and prokaryotic benthic communities of the Antarctic Peninsula with metabarcoding approaches. Our results show that the combined use of mitochondrial Cox1, and 16S and 18S rRNA gene regions recovered more phyla, from metazoan to non-metazoan groups, and allowed correlation of possible interactions between kingdoms. This higher level of detection revealed dominance by the arthropods and not nematodes in the Antarctic benthos and further eukaryotic diversity was dominated by benthic protists: the world's largest reservoir of marine diversity. The bacterial family Woeseiaceae was described for the first time in Antarctic sediments. Almost 50% of bacteria and 70% metazoan taxa were unique to each sampled site (high alpha diversity) and harboured unique features for local adaptation (niche-driven). The main abiotic drivers measured, shaping community structure were sediment organic matter, water content and mud. Biotic factors included the nematodes and the highly abundant bacterial fraction, placing protists as a possible bridge for between kingdom interactions. Meiofauna are proposed as sentinels for identifying anthropogenic-induced changes in Antarctic marine sediments.

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INTRODUCTION

Under the current global climate crisis, there is a pressing need to understand how changing environmental conditions impact future biodiversity, particularly in those areas experiencing rapid climate change. One such area is the Western Antarctic Peninsula (WAP). Over the past 50 years the seas around the WAP have experienced, and are still experiencing, very rapid rates of regional warming resulting in glacier retreat and reductions in annual sea-ice extent [1]. These changes drive habitat and biome shifts with potentially significant impacts for biodiversity and ecosystem functioning. Local consequences of warming are already evident in data from long-term marine monitoring studies, such as the regular oceanographic and biological observations carried out at Rothera Research Station on the Antarctic Peninsula. Observations include a reduction in sea-ice cover, leading to decreased water stratification, impacting the magnitude, duration and composition of phytoplankton blooms [2, 3]. Furthermore, the reduction in sea-ice appears to affect reproductive capacity of some benthic invertebrates and led to increased iceberg scour, with consequent destructive impacts on benthic communities [4, 5]. Whilst these studies have highlighted directional trends beyond inter-annual variability, they do not include analyses of marine sediment communities. This is a significant knowledge gap as these communities play key roles in food webs, biogeochemical cycling and contribute significantly to ocean health and functioning [6, 7].

The limited analyses of sediment meiofaunal biodiversity around Rothera demonstrated levels of biodiversity similar to temperate regions, with grain size as a major driver of diversity [8, 9]. Meiofauna are critical elements of marine ecosystems and macrofauna predation accounts for 75% of meiofaunal production transferred to higher trophic levels [10]. Since many macrofaunal larvae are a similar size to adult meiofauna they are potential meiofauna prey items, and this has implications for macrobenthic recruitment and higher-level community structures [11]. However, meiofauna are one part of sediment communities and bacteria typically dominate marine sediment biomass [12]. Benthic bacteria play a major role in the remineralization of organic matter and represent a significant store, and source of, carbon in our oceans [13]. This provides the living foundation for sediment ecosystems and biogeochemical cycling, with the dominant metabolic pathway resulting from a range of biotic and abiotic factors [14]. Bacteria rapidly metabolise dissolved organic matter to fuel growth and the resulting bacterial biomass is a major food source for larger organisms. Bacteria are also important in the processing of detrital material and faecal pellets, thus exerting a strong influence on the availability of key nutrients for primary producers [15]. In fact, detritivores derive more energy and nutrients from detritus-associated bacterial communities than from the detritus itself, a process often called 'Trophic Upgrading' [16].

There is strong interdependence between the micro- and meiofauna in sediments [17]. For example, bioperturbation by

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larger animals (e.g. nematode and bivalve burrowing) and cryptobioperturbation of surface sediments by meiofauna can redistribute nutrients and alter oxygen fluxes, creating fertile microniches for microbial communities [18]. Furthermore, mucus secretions by caudal and oesophageal glands of nematodes attract bacteria as food sources and as producers of organic matter [19] with nematode richness having a positive effect on microbial activity [20]. Thus, the different elements (i.e. size classes, life cycle, ecology) of marine sediment organisms should not be considered separately as they are inextricably linked. Such combined studies are particularly important as the micro- and meiobenthic fauna in sediments, respond rapidly to changing environmental conditions and have been proposed as efficient environmental sentinels [7, 21, 22].

Hence, the aim of this study was to address the paucity of data on Antarctic sediment communities and provide a baseline for future monitoring and understanding of how regional climate change impacts biogeographic shifts of microbial and meiofaunal assemblages in this region. We metabarcoded the mitochondrial cytochrome c oxidase subunit I (Cox1) and the ribosomal 16S and 18S (rRNA) gene region, to produce the first multi-gene analysis of shallow-water sediment microbial and meiofaunal biodiversity communities around Rothera Research Station on the Antarctic Peninsula. Biodiversity patchiness was characterised spatially across three sites, with high levels of endemicity, community composition correlated with environmental variables, such as sediment characteristics and associations between Eukaryotes and Bacteria are discussed.

MATERIAL AND METHODS
Sample collection and processing
During March 2013 sediment samples were collected in triplicate every 100 m at 15–20 m depth from three sites: Bambay (S67° 34.00333 W68° 8.955 and S67° 33.98167 W68° 9.245), South Cove (S67° 34.12167 W68° 7.95333 and S67° 34.23833 W68° 7.91833) and Hangar Cove (S67° 33.91333 W68° 7.81333 and S67° 33.82333 W68° 7.56) near the British Antarctic Survey Rothera Research Station, West Antarctic Peninsula (Fig. 1). The substratum in South Cove and Bambay is a mixture of bedrock and cobbles with patches of soft sediment, though Bambay is smaller in area than

South Cove and patches of sediment are smaller in area. Conversely, Hangar Cove primarily has a base of compacted cobbles covered with a thin layer of fine sediment (2–3 cm) interspersed with patches of, deeper, fine sediment. In all three coves the thickness of the soft sediment ranges from 0 to ~20 cm with a gently sloping topography. The sediment in these coves is primarily silt and fine sand ranging from 0.06–0.1 mm [23] and all three bays receive sediment from glaciers at the head of the bay. During summer icebergs impact the benthic communities in these coves and in the winter the sea surface is covered by fast ice for 3 to 8 months [24]. From each location triplicate samples (total $N = 9$) were taken from undisturbed sediment, using a hand-held plastic corer; 8 cm diameter × 20 cm length and the collected sediments were stored in the cores at −80 °C until processing.

DNA extraction from sediments
For each sample the top 10 cm of sediment was cut while still frozen, mixed with a sterile metal spatula for homogenisation and during this process any larger organisms detected were discarded from the 10 g of starting material used for DNA extraction. DNA extraction was performed using the PowerMax® Soil DNA Isolation Kit (MO-BIO) following the manufacturer's instructions. DNA extracts were visualised by agarose gel electrophoresis, quantified using a NanoDrop2000 spectrophotometer and extracted DNA diluted to 10 ng/µl and stored at −20 °C for downstream molecular analysis.

NGS libraries
Two ribosomal (16S and 18S rRNA) and the mitochondrial Cox1 gene were used in the PCR amplifications. The universal primers 'TAReuk454FWD1' (5′-CCAGCASCYGCGGTAATTCC-3′) and 'TAReukREV3' (5′-ACTTTCGTTCTTG ATYRA-3′) were used to amplify ~400 bp of the 18S V4 region [25]. The forward 515Fw (5′-GTGCCAGCMGCCGCGGTAA-3′) and reverse 806Rv (5′-G GACTACHVGGGTWTCTAAT-3′) primers were used to amplify ~300 bp of the 16S V4 region, recommended for microbial biodiversity studies [26]. The mitochondrial Cox1 313 bp gene region was amplified using the primers forward 'mlCOlintF' (5′ GGWACWGGWTGAACWGTWTAYCCYCC-3′) and reverse 'jgHCO2198' (5′-TAAACTTCAGGGTGACCAAARAAYCA-3′) [27]. All primer combinations were designed to include the Illumina MiSeq 8nt index-tags (i5/i7) and Adaptors (P5/P7). Each replicated sample site had these primer-adaptor tags to allow differentiation of sequenced samples. PCR amplification of the specified rRNA and Cox1 gene sequence was performed with a 2-step PCR approach using Pfu DNA polymerase (Promega). The first PCR involved 5 min denaturation at 95 °C, then
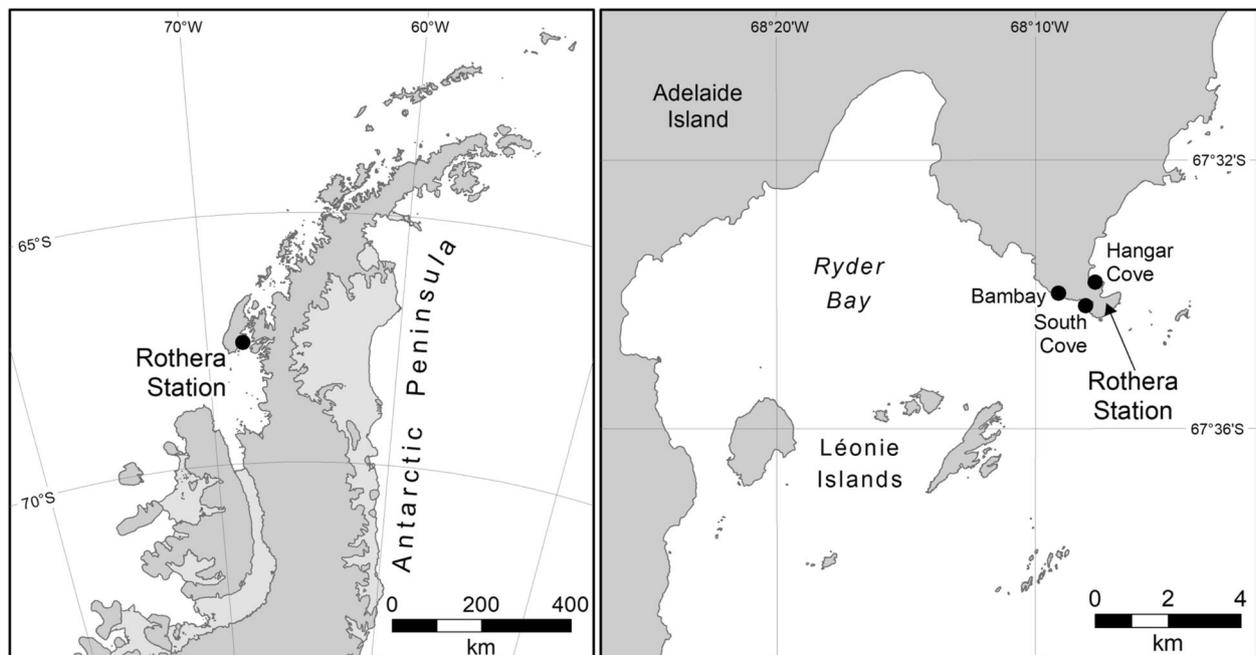
Fig. 1 Sediment sampling locations in the Antarctic Peninsula. Location of Rothera Research Station on the Antarctic Peninsula and sample sites around Rothera (Hangar Cove, South Cove and Bambay).

20 cycles with 30 s at 98 °C, 30 s at 50 °C (rRNA) or 45 °C (Cox1), 30 s at 72 °C and a final extension of 10 min at 72 °C. To add the Illumina index tag adaptors a second PCR was performed using the same conditions reported above but with 10 cycles and an annealing temperature of 55 °C. Negative controls were included for all amplification reactions. The second PCR products were visualised and purified from agarose gels (QIAquick Gel Extraction Kit, Qiagen) and quantified using an Agilent Bioanalyser. All PCR products were diluted to the same concentration, pooled to create one metagenetic sample/library and pair-end sequenced on a MiSeq platform using v2 Illumina chemistry (2 × 250bp).

Meta-data analysis

The 16S, 18S and Cox1 raw sequences from the Illumina MiSeq platform were pre-filtered by removing primer sequences from the forward and reverse reads using the default settings in cutadapt version 1.18 [28]. Forward and reverse reads were trimmed simultaneously, and were discarded if either the forward or reverse read pair was not within an error tolerance of 10% over a minimum overlap of ten nucleotides. Trimmed sequences were analysed using the dada2 plugin within QIIME2 [29]. This plugin produces amplicon sequence variants (ASVs), resolving differences as small as a single nucleotide [29]. Filtered and chimera-free ASVs were then assigned a taxonomy using the 'feature-classifier' plugin and the 'classify-consensus-blast command' within QIIME2 [30]. Taxonomy assignment was performed three times using different identity thresholds (95%, 97%, and 99%). Optionally, all redundant BLAST matches were merged using the 'filter-seqs' and 'collapse' commands of the qiime2 'taxa' plugin [29] to remove redundancy in the taxonomy assignments based on a specific taxonomic level (species). To identify taxon relative abundance and direct ecological comparisons, the sequencing data was normalised using the phyloseq R package [31], using the lowest sequencing depth per sample (62,322 reads for 18S, 118,465 for Cox1 and 241,418 for 16S) and randomly resampled reads (rarefy_even_depth) in order to equalise the sampling effort between samples [31] (Supplementary Information Figs. S1, S2, S3).

Reference databases

For the 16S dataset, a pre-compiled QIIME2 compatible reference dataset from SILVA release 132 was used [32], which was made non-redundant by clustering at 99% similarity and linking majority-based taxonomy strings, resulting in a total reference database of 369,953 sequences. For the 18S and mitochondrial Cox1 markers, reference sequences and taxonomies were downloaded from GenBank [33] and brought into a QIIME2 compatible format using in-house Perl scripts. During this process downloaded sequences were length filtered (18S: 500–2500 bp; Cox1: 500–2000 bp) and further processed, by discarding entries from environmental sequences, with missing taxonomic assignments from phylum to genus level. The resulting 18S reference database consisted of 669,693 entries, whilst the Cox1 reference database encompassed 2,857,567 entries.

Sediment characterisation: All sediment samples were characterised as follows: *Water content:* Sediment water content was determined before and after drying at 40 °C. Water content (%) was determined as water weight (Ww) divided by dry sediment weight (Wds) and multiplied by 100 [(W = Ww/Wds)*100]. *Grain size:* Sediment samples were submitted to wet separation of mud (silt and clay) from the sand fraction with a 63 µm sieve. The mud fraction (<63 µm) was recovered by sequential decantation of percolated material, which was dried and weighed. Sediments retained in the 63 µm sieve were subsequently dried and passed through a further sieve stack in a vibratory sieve shaker (5 min, amplitude 50%), to quantify the coarser fractions. The classes analysed, adapted from Wentworth 1922 [34] were: coarse gravel (>8 mm), medium gravel (8–4 mm), fine gravel (4–2 mm), very coarse sand (2–1 mm), coarse sand (1–500 µm) medium sand (500–250 µm), fine sand (250-125 µm) and very fine sand (125–63 µm). The contribution of the distinct fractions was calculated as a percentage. *LOI (loss on ignition):* The organic matter content of sediments was quantified through LOI [35]. About 200 mg of dry sediment, ground with a mortar and pestle, was burned at 450 °C for 2 h (ash weight). The organic matter content (ash-free dry weight) was estimated by the ratio: [(dry weight) − (ash weight)] × 100/ (dry weight). *Carbonates:* Carbonate content was determined using a calcimeter (Model 08.53, Eijkelkamp, Netherlands) which measures the volume of carbon dioxide produced during the reaction of the sample with hydrochloric acid [36]. The carbonate content was expressed as equivalent calcium carbonate content.

Diversity and community analysis

To analyse meiobenthic community (di) similarities among sites, a similarity profile ('SIMPROF') permutation test was performed and significant differences in community assemblages, were tested using a permutational multivariate analysis of variance ('PERMANOVA'). Analyses were based on Sørensen's similarity coefficient on untransformed data of an ASV presence/absence matrix over the 9 sampled sites, with 1000 permutations. To investigate associations between community composition and environmental variables (sediment parameters) a BIOENV ('biota-environment') analysis was conducted using Spearman's rank correlation method. The BIOENV analysis searches for the best explanatory variables between meiofaunal communities and the abiotic parameters measured. Six environmental sediment related parameters were tested, as percentages of: water content, carbonates, organic matter, gravel (2–8 mm), sand (<1 mm) and mud (<63 µM). To further test and visualise the relationships between the environmental variables and community composition a principal Coordinates Analysis (PCoA) was performed using the software Primer 6 (v6.1.16) to explore community dissimilarities between samples (beta diversity). Phylum-specific sample-size-based R/E curves with extrapolations of Hill numbers for presence-absence data were prepared using the R-package iNEXT at default settings (40 knots, 95% confidence intervals generated by the bootstrap procedure (50 bootstraps)) (Supplementary Fig. S4). To test for phylum specific associations a non-parametric Mantel-type test ('RELATE') based on phylum community composition (presence–absence data) was performed using Primer 6 (v6.1.16). Rarefaction and accumulation curves (sequencing depth and sampling effort) were performed on presence–absence matrices for each marker using a 99% sequence identity threshold in the R package iNEXT [36, 37] (Supplementary Fig. S5).

RESULTS

The normalised sequencing data did not affect the diversity patterns (Supplementary Figs. S1, S2, S3) and overlying conclusions, thus the results presented are based on non-normalised raw datasets analysed with dada2 in QIIME2. After filtering and quality control, the mean range of total raw-reads per sample was between 1.9M (18S) to ca. 2.5M (16S, Cox1). The analyses were run individually for each gene and after denoising (trimming, quality filter, chimera removal) the mean number of chimera-free reads per sample was 638,144 (18S), 922, 186 (16S) and 1,073,044 (Cox1) (Supplementary Table S1), which were then assembled into amplicon sequence variants (ASVs). Taxonomic assignments using 16S retrieved a total of 5791 prokaryotic ASVs, of which 5630 were bacteria and 161 were Archaea ASVs. The three most abundant bacterial groups comprised the Proteobacteria, Bacteroidetes and Planctomycetes (Fig. 2). The more ASV rich metazoan groups identified by 18S were the Nematoda (77 ASVs) followed by the Arthropoda (50 ASVs), Annelida (24 ASVs), Mollusca (22 ASVs) and Platyhelminthes (21 ASVs). The Arthropoda were the dominant group (143 ASVs) identified by Cox1 followed by the Mollusca (61 ASVs), Annelida (47 ASVs) and Echinodermata (14 ASVs), other metazoan groups were identified by both markers (18S and Cox1) with less than ten ASVs (Fig. 2). Non-metazoan groups mainly identified with 18S were dominated by diatoms (510 ASVs) followed by the group SAR (Stramenopiles, Alveolata, Rhizaria) (490 ASVs). Non-metazoan groups further identified using 18S were the dinoflagellates (250 ASVs), Labyrinhulomycetes (protists) (111 ASVs), algae (107 ASVs) and Obazoa (a clade consisting of the Opisthokonta, Apusomonadida and Breviatea) (76 ASVs). The Cox1 gene also identified non-metazoan eukaryotes including diatoms (32 ASVs), algae (23 ASVs) and others (<10 ASVs) (Fig. 2). Additionally, eleven (55%) of the phyla found were identified by both the Cox1 and 18S markers but only 10 (6%) and 4 (3%) of the family and genus were common to both markers (Fig. 3).

Both markers (Cox1 and 18S) showed similar meiobenthic structure for the most dominant (ASV rich) meiobenthic metazoans. In general, the most abundant phyla were the Arthropoda, and Nematoda, followed by the Annelida, Mollusca and Platyhelminthes (Fig. 4a, Supplementary Fig. S1). The Cox1 amplicon sequencing results for South Cove identified double the number
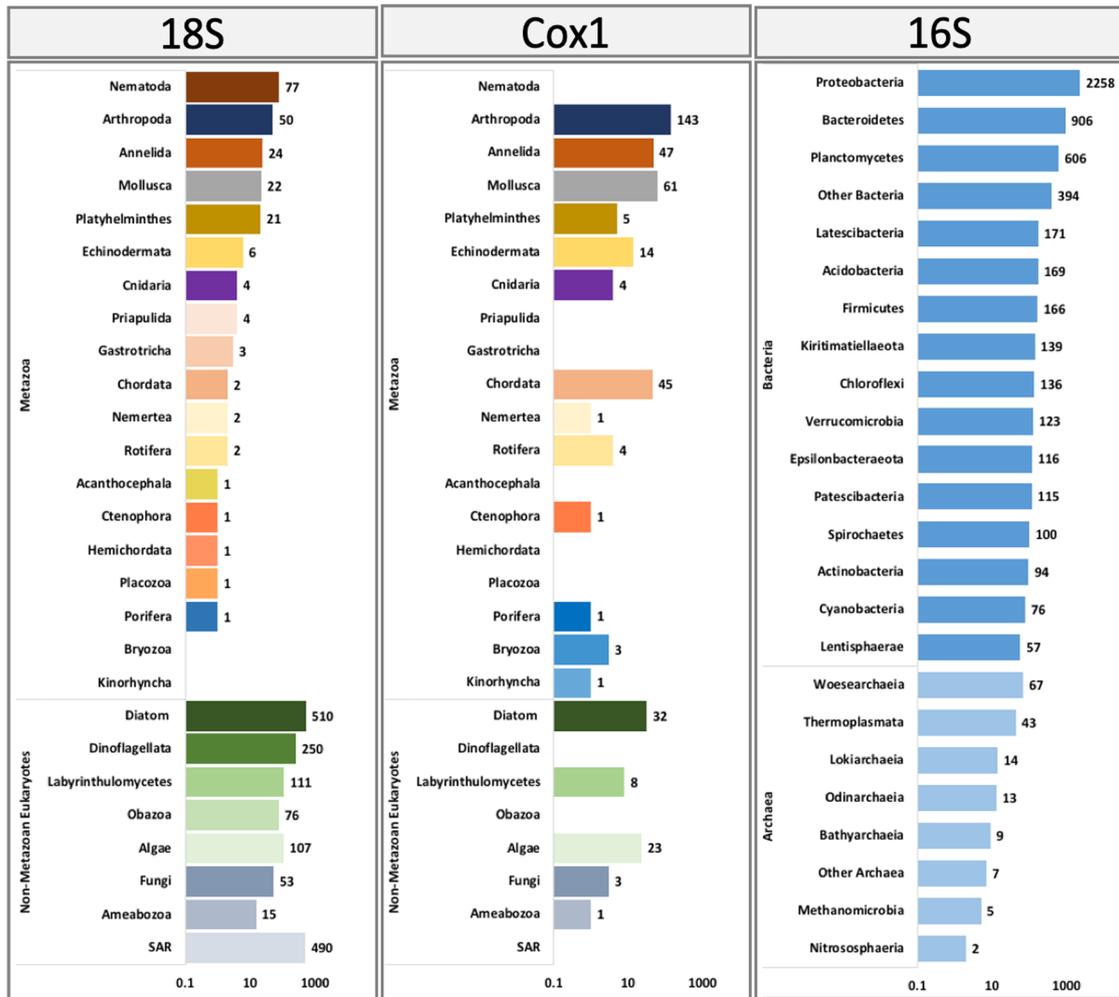
Fig. 2   Taxonomy assignments obtained with the 18S rRNA, Cox1 and 16S rRNA found in Rothera, West Antarctic Peninsula. Main metazoan and non-metazoan taxa assigned to 18S and Cox1 are shown as well as the main bacteria and Archaea groups assigned to the 16S. The scale was transformed logarithmically and the total number of ASVs are indicated for each taxa.
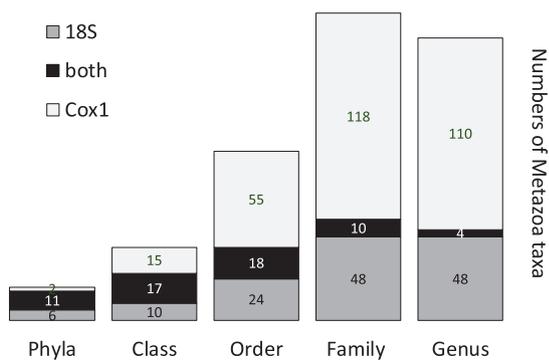
Fig. 3   Number of Metazoa taxa. Unique to each taxonomic ranking for Cox1 and 18S markers and shared between markers.

of Arthopoda ASVs (106) compared to the Nematoda (53 ASVs). The Annelida were more abundant in Hangar Cove and South Cove with ~25 ASVs whereas in Bambay there were fewer identifications (17 Annelida ASVs). The Mollusca were more abundant in Bambay (34 ASVs) when compared to the other locations (11–23 ASVs). Platyhelminthes, Echinodermata and Cnidaria were present in all locations but with fewer ASVs (Fig. 4a,

Supplementary Information Fig. S1). The 18S gene also identified non-metazoans, listed in overall descending numbers of ASVs, the diatoms, Dinophyceae, Labyrinthulomycetes, SAR (Stramenopiles, Alveolata, Rhizaria), Obazoa, algae, fungi and Chlorophyta (Fig. 4b). In general, for all non-metazoans the Bambay site always had around half the ASVs compared with Hangar and South Coves whereby diatoms and SARs were the most dominant with ca. 300 and 250 ASVs, respectively (Fig. 4b, Supplementary Information Fig. S1).

The analysis of metazoan ASVs found per sampling location showed that only 4% (Cox1) to 12% (18S) of the ASVs were common to all sampling sites (Fig. 5). Independently of the marker, the number of unique ASVs in each site was higher (from 37 to 182) when compared with shared ASVs (from 5 to 73). More unique metazoans were found at South Cove (127 ASVs), followed by Hangar Cove (60 ASVs) and Bambay (59 ASVs). Bambay and South Cove shared more metazoan ASVs than with Hangar Cove. The analysis of 16S showed that 31.3% (1762 ASVs) of bacterial ASVs were common to all locations, with 48% of bacterial ASVs unique to a particular site, from which ca. 30% (1694 ASVs) were exclusively found in Hangar Cove and the remaining 9% (523 ASVs) and 8.4% (472 ASVs) unique to South Cove and Bambay, respectively (Fig. 5). The remaining 21% of bacterial ASVs were shared between two sites only. Hangar Cove shared more ASVs with South Cove (556 ASVs) and Bambay (440 ASVs) and the least
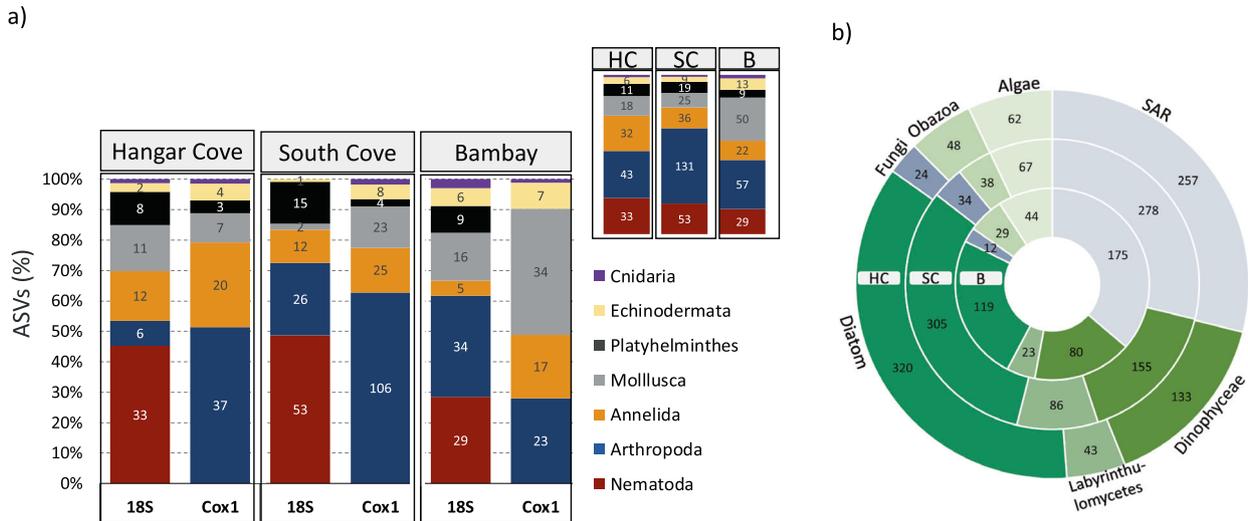
Fig. 4 Eukaryotic diversity found in Hangar Cove, South Cove and Bambay. ASVs proportions for the most dominant Metazoa (**a**) and non-metazoa (**b**) found in the 3 sampling locations in the Antarctic Peninsula using 18 S and Cox1 genes. **a** unique ASVs proportions per marker and for the combined markers per sample (top right-hand graph). **b** non-metazoan ASVs obtained only with 18S per sampling site. HC-Hangar Cove outer circle, SC-South Cove middle circle, B-Bombay inner circle. Number of ASVs are shown in numbers.
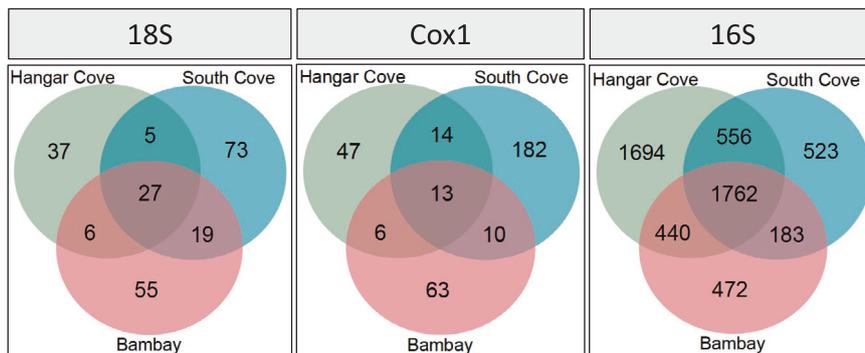
Fig. 5 Shared and unique ASVs between the three sampling locations in the Antarctic Peninsula. The Venn diagrams present the number of site unique ASVs, those shared between two sites and those shared between the three sites for the 18S and Cox1 (eukaryotes) and 16S (prokaryotes) genes.

shared numbers of bacteria were between Bambay and South Cove (183 ASVs). From a total of 15 main bacterial phyla, three were most dominant, the Proteobacteria, Bacteroidetes and Planctomycetes, with more than 500 ASVs in at least one sampling location. Higher numbers of bacteria for most phyla were always identified in Hangar Cove when compared to South Cove and Bambay. Other bacterial phyla were found with ca. 100 ASVs per sampling location, which included the Latescibateria, Acidobacteria, Firmicutes, Kiritimatieallaeota and Chloroflexi. The remaining bacterial phyla identified had less than 100 ASVs (Supplementary Fig. S2).

Community assemblages using BIOENV analysis showed that the combined effect of most granulometric variables measured accounted for much of the variance observed and were significantly correlated with community structure for Eukaryotes in general (rho > 0.70, $P < 0.01$) (Table 1). The only granulometric parameter that had no effect on community structure was gravel particle size (rho = 0). Particularly high and significant correlations (rho = 0.75, $P < 0.001$) were obtained between metazoan assemblages and sediment water content, organic matter (OM) and mud. The Nematoda were the only metazoan group with strong correlations (rho = 0.73) with water content, carbonates and OM. Within the non-metazoa the bacteria had significantly high correlations (rho > 0.70, $P < 0.001$) with all granulometric

parameters, with the exception of gravel. In particular, the phylum Planctomycetes and the group SARs were very highly correlated (rho = 0.73, $P < 0.001$) with water content and mud, whilst the SARs also showed additional effects of carbonates and sand (Table 1).

Overall, there was a significant positive association between Bacteria groups, with minimum associations observed between Woseiaceae (rho = 0.57) and maximum associations between Proteobacteria (rho = 0.97), with average significant associations between all Bacteria groups of 0.83 ($p < 0.05$). Associations within eukaryotes were weaker, ranging from 0.5 between diatoms and Metazoa and 0.74 between Nematoda and Metazoa, with an average rho of 0.55 ($p < 0.05$). The eukaryotic phyla with the highest associations with Bacteria were the Platyhelminthes, diatoms and SARS. In particular, the Planctomycetes (rho = 0.74), Gammabacteria (rho = 0.68) and Woeseiaceae (rho = 0.64) were associated with the Platyhelminthes and also with diatoms and SARS (Fig. 6). Phylum-specific and sampling accumulation curves did not reach an asymptote showing incomplete sampling effort (Figures Sp and Sc) but rarefaction curves for all markers and samples reached an asymptote indicative of sufficient sequencing depth (Supplementary Figs. S4, S5).

Beta diversity analysis revealed a clear separation of sample site according to community composition with more than 70% similarity clusters between sites. These similarity profiles along the PCoA axis explained 33.7% of the variation found for

Table 1. Correlations found between the combined effect of environmental parameters and community composition for the main phyla. BIOENV analysis using Bray-Curtis index and BEST spearman correlation (rho) with 1000 permutations.

	Water	Carbonates	OM	Sand	Mud
Bacteria	0.718	0.697	0.700	0.718	0.718
Proteobacteria	0.650	0.640	0.630	0.650	0.650
Bacteroidetes	0.671	0.654	0.643	0.671	0.671
Planctomycetes	0.728	0.692	0.706	0.714	0.728
Eukaryotes	0.727	0.710	0.710	0.727	0.727
Diatom	0.575	0.567	0.567	0.575	0.575
SARs	0.733	0.733	0.697	0.733	0.733
Metazoa	0.750	0.702	0.750	0.704	0.750
Nematoda	0.729	0.729	0.728	0.720	0.729
Arthropoda	0.548	0.305	0.548	0.548	0.337
Annelida	0.514	0.479	0.479	0.479	0.000

rho values shown in grey gradients: dark grey (0.75 < rho < 0.73), grey (0.73 < rho < 0.71), light grey (0.71 < rho < 0.65) to white (<0.5). Water content, carbonates and organic matter (OM) are measures of percentage. Arthropoda and Annelida rho values from Cox1 presence–absence matrix. Non-significant and low rho values were excluded from the analysis (e.g. Gravel rho = 0, Archaea, Obazoa, Mollusca rho < 0.4; $p > 0.05$).
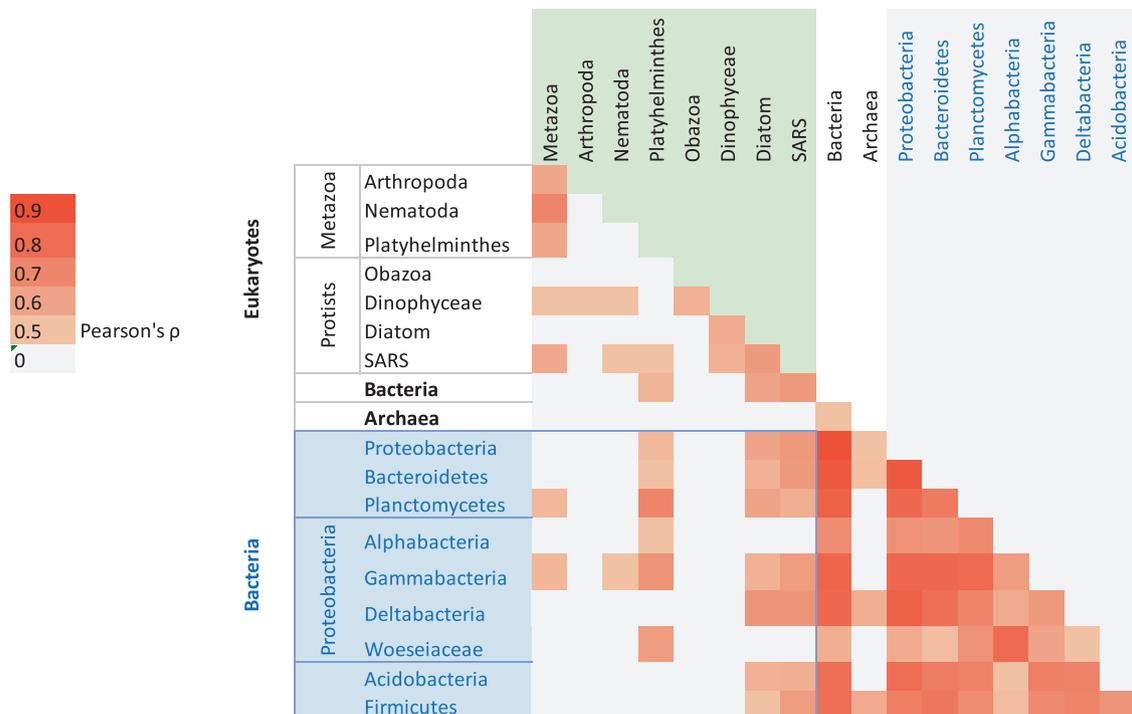
Fig. 6 Heatmap showing significant associations between the main groups of Eukaryotes, Bacteria and Archaea. Pearson correlation rho values range from 0 to 0.9, grey to dark red, respectively. Associations between prokaryotes (Bacteria/Archaea) and eukaryotes are highlighted in the blue shaded box and with blue letters. Associations within Eukaryotes are shaded in green and within Bacteria/Archaea are shaded in grey. Only dominant phyla that exhibited $p < 0.05$ and rho > 0.5 are represented. rho values of zero comprise 0 < rho < 0.45.
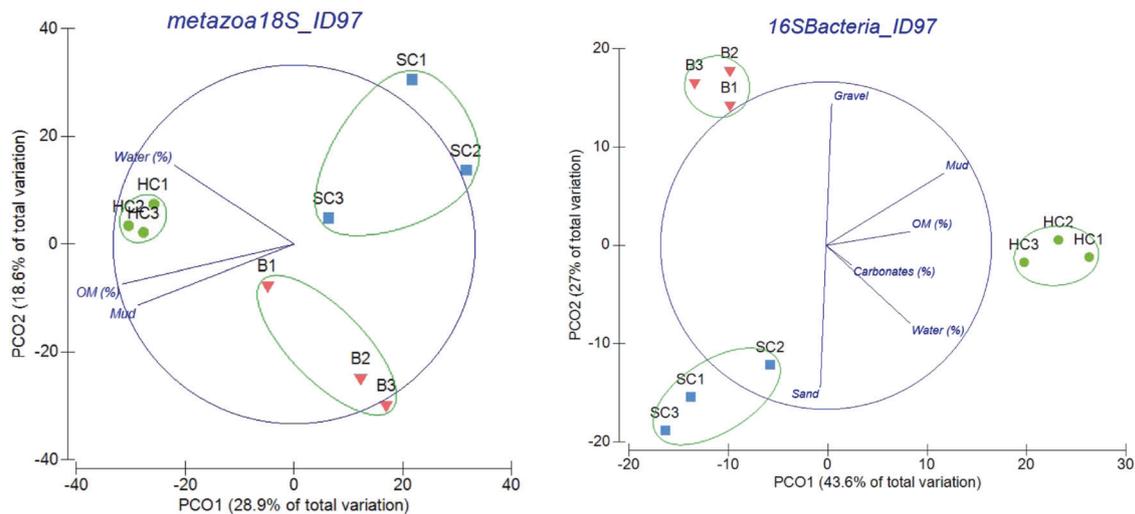
Fig. 7 Principal Coordinate analysis based on similarity profiles (green line > 70% similarity) between samples for Metazoan (18S) and Bacteria (16S). Analysis was based on Sorensen similarity coefficient using a presence-absence matrix. Environmental variables that showed the greatest individual effect (BIOENV, Pearson rho > 0.45) on each phyla community structure are also represented (blue lines). SC-South Cove; HC-Hangar Cove; B- Bambay.

Eukaryotes, 28.9% for the Metazoa and 43.6% for the bacteria community structure (Fig. 7, Supplementary Information Fig. S6). Other similarity profiles analysed for eukaryotes, nematodes, diatoms, SARs, planctomycetes, Bacteria and Archaea also showed distinct location clusters with up to 45.1% of the variation found explained by the analysis. For the Nematoda, South Cove and Bambay communities overlapped and Hangar Cove formed a distinct cluster (Supplementary Fig. S6).

DISCUSSION

This study clearly shows the importance of using multiple gene markers in biodiversity surveys, with little overlap at the family and genus level between Cox1 and 18S. The use of both makers allowed identification across broader taxonomic groups when using the 18S, and better species-level resolution when using the Cox1, the latter is particularly good for targeting arthropods [38, 39] (Figs. 3, 4). Furthermore, the use of 16S enabled the investigation of possible interactions between kingdoms. The use of several markers provides better estimates of biodiversity [40] and improve taxonomic resolution by up to 10% [41]. This multi-barcode approach placed arthropods as the most dominant organisms of the Antarctic benthos, contrary to what was previously found when using just the 18S [8]. Likewise, the Platyhelminthes previously listed as the third most dominant taxon [8], when adding more power to the genetic analysis, annelids and molluscs were promoted to the third and fourth most dominant phyla in the Antarctic soft seabed benthos. Nonetheless, finding fewer Platyhelminthes might be due to sampling issues (e.g. crypsis and delicate body structures) and primer bias towards some taxa [42, 43]. Although, these new findings are not surprising since more markers will uncover more diversity and allow higher resolution levels [41], it acknowledges the importance of such approaches to better reflect local diversity levels. The phylum and sample (Supplementary Figs. S5 and S2.2) accumulation curves further evidence that the sampled Antarctic habitats are yet to fully describe local eukaryotic diversity and that more biological replicates and additional markers would be needed (including other gene regions if budget is not a constraint). However, this study adds new facts to the existing knowledge on Antarctic meiobenthic biodiversity in shallow

waters, placing arthropods as the most dominant in the meiobenthos, followed by nematodes and annelids [8, 9, 44, 45]. These findings are fundamental to identifying key species in the marine benthos, understanding trophic relationships and evaluating ecosystem dynamics that globally sustain many other marine life forms [46].

The Antarctic marine benthos harbours diverse communities with high levels of endemicity, including non-metazoan microorganisms [8]. The high prevalence of diatoms and SARs (stramenopiles, alveolates and rhizaria) found in this study was no exception. Diatoms are part of the SAR super group 'stramenopiles with chloroplasts' but were analysed separately since they are one of the most important autotrophs in marine habitats [47]. Benthic diatoms play key roles in biogeochemical cycles and can also be the main food source of nematode and copepod species [47, 48] and thus, are very species rich and most widespread across several habitats. Although benthic protists have received little attention compared to their planktonic counterparts, the SAR super group identified may well be part of one of the world's largest reservoirs of marine diversity [49]. The benthic protists are thought to be more diverse than planktonic protist communities [49] and in this study we found ~1000 benthic protist ASVs, which were poorly annotated and most are still not formally described (Supplementary Fig. S7).

Alpha diversity

Previously we showed that meiobenthic communities tend to have high alpha diversity in both temperate and Antarctic regions [8, 9, 50]. This study now extends these observations across kingdoms, from metazoans to bacteria. Despite the fact that microorganisms are reported as cosmopolitan species [51] the high levels of endemicity found in Antarctic meiobenthic communities suggests that these are partly niche-driven [8, 45, 52] (Fig. 5). Although the three sites are relatively close together (within 1–2 km) they all exhibited significant diversity in both metazoan and bacterial communities. This is the result of a complex mix of seabed topography, ocean currents and freshwater run-off from glaciers, producing individual and specialised habitats within each cove (Lloyd Peck, pers. obs.). South Cove and Bambay are most similar in terms of seabed topography, both comprising unconsolidated mixed aggregates of boulders,

pebbles and sediment, with the sediment present in lenses over solid rock, whereas Hangar Cove consists of layers of sediment including an anoxic layer [23, 24]. Hence, it was not surprising to find higher metazoan diversity in South Cove compared with Hangar Cove. The mixed aggregate nature of the South Cove seabed results in many small protected areas between boulders and stones, which can harbour the Metazoa, whereas the relatively homogeneous muddy areas in Hangar Cove are more suitable for bacteria. Here, the most dominant bacteria were Proteobacteria, followed by Bacteroidetes and Planctomycetes. Benthic bacterial assemblages in sediment show typical dominance by Proteobacteria, which are characteristic of deep-sea microbiomes [53], but also dominate the water column in the Antarctica, comprising the vast and vital bacterioplankton community [54, 55]. As also found here for sediments, the bacteria groups Alphaproteobacteria and Gammaproteobacteria dominate and are key colonisers of Antarctic surface and deep marine waters [54, 56, 57] which can thrive in areas with limited resources [58]. Other Proteobacteria such as the Planctomycetes contribute to benthic communities by increasing organic-matter in depleted subsurface sediments [59]. Although, both South Cove and Bambay are subject to similar currents, Hangar Cove experiences a different regime, with all three bays receiving different levels of fresh water run-off from glaciers. Habitat characteristics seem to be key for benthic and bacterial community structure. For example, South Cove, without a glacier near-by had more Metazoa than Bambay, which receives some glacial melt water from Reptile Ridge, whilst Hangar Cove on the opposite side of Rothera Point runs alongside the Wormald Ice Piedmont. This glacier is in retreat with regular melting and ice calving events and hence the fauna in Hangar Cove will experience regular and often considerable freshwater input. Nonetheless, meiofaunal organisms are clearly well adapted for living near to melting ice with osmoregulatory mechanisms that allow them to survive across a wide spectrum of salinities [60].

What drives Antarctic benthic community structure?

Significant endemism is characteristic of polar ocean meiofauna [8, 61]. The environmental parameters measured that showed the highest correlation with Metazoa were water content and organic matter (Table 1). Sediment chemistry seems to be one of the drivers of meiobenthic communities along with the availability and composition of organic matter [62–64]. Usually, marine metazoan diversity is highest in wet, organic matter rich sediments [65] and both parameters showed the highest correlation across kingdoms, being two of the main drivers associated with microbial community structure (eukaryotes and prokaryotes) (Table 1). Similarly, Antarctic terrestrial bacteria were also reported to be quite site-specific and most affected by soil physical factors such as grain size and moisture [66]. Sediment grain size was also correlated with microbial community assemblages with the exception of gravel, which showed no correlation with any taxa. Most microbial benthic organisms live attached to sediment grains [67] and each sediment particle has different microenvironments that support several microbial taxa [68]. Ecological grouping in nematodes is often related to substrate type and feeding mode (e.g. microvores, predators) [69] and previous studies have further corroborated associations between sediment granulometry at local scales [70–72]. Usually coarser sediment like gravel, with larger pockets between particles can harbour rotifers and copepods, whereas finer sediments like muds are mainly occupied by nematodes and oligochaetes [69] but since the latter were dominant this could explain the absence of a correlation with gravel. This trend persisted within the Bacteria group, and bacteria more commonly found in coarser sediments were not identified in this study (e.g. *Gemmatimonadales*) whereas the most abundant taxa (e.g. Proteobacteria, Planctomycetes and Bacteroidetes) usually dominate muddy sediments, mostly comprised of fine silts and clay [68].

In general, seawater temperature can have the greatest impact on the diversity, abundance and structure of marine meiobenthic communities [50], and together with nutrient availability and chlorophyll-a it can impact significantly prokaryotic production and diversity [55, 73]. In the Antarctic Peninsula coastal systems, abiotic factors such as seawater temperature and oxygen availability are described as one of the drivers of eukaryotic community structure, whereas silicates and salinity tend to be more important for bacteria [57]. Local drivers of marine distributions in Antarctic benthic metazoa and marine bacteria are reported to be the result of many factors such as food availability, life-cycle strategies, season, depth, glaciers melting, substrate physical and chemical properties [8, 54, 74–76]. Among these factors, availability of organic matter and water content within the sediment, but also mud, had the highest correlations and seemed to have a strong effect shaping Antarctic benthic communities, both for bacteria and metazoans. Some authors suggest that organic matter is the main driver of Antarctic benthic community structure [74], and in fact it is also determinant in shaping prokaryotic communities in surface waters of the Southern Ocean [54]. Notably, Oztruk et al. [55] identified organic matter as one of the key factors shaping community structure in heterotrophs.

Key phyla driving meiobenthic community structure

Associations between the main taxa were the highest within bacterial groups showing how intricate relationships between microorganisms can be, especially for the Proteobacteria, Bacteroidetes and Planctomycetes. The Proteobacteria found in this study, dominated by the Gammabacteria had the highest correlations with Metazoa, namely with Platyhelminthes and nematodes (Fig. 6). These groups of bacteria are dominant in several marine habitats, including sediments in the Western Antarctic Peninsula [74] and the Antarctic Polar Front [77]. The microbial diversity in the Southern Ocean increases with depth [55] and Proteobacteria and Bacteroidetes seem to dominate both surface waters [55–57] and sediments [this study, 53]. Bacteria that dominate the benthos are crucial for driving such communities, not only as decomposers but also as a food source for meiofauna [78]. While there is a consensus that Proteobacteria, Bacteroidetes and Planctomycetes thrive in marine sediments [79], the role of lower taxonomic bacteria is still unknown, especially if they are part of the 'core microbiome [80]. In this regard, we describe here for the first time in the Antarctic Peninsula, the presence of the bacterial Woeseiaceae family. The Woeseiaceae have recently been highlighted due to their role in N-cycling [81, 82] and potential reduction of nitrous oxide (N_2O) into the atmosphere [81]. Platyhelminthes were the only metazoan group showing significant correlations with the Woeseiaceae, perhaps due to their physiology (e.g. soft body, diffusion to breathe etc.). The Woeseiaceae have a widespread distribution and it is possible that they could have significant implications for N_2O emissions from sediments [82]. Together with Platyhelminthes, they could potentially be used as sentinels of marine sediment changes, identifying shifts in biogeochemical cycles.

Within the meiofauna, nematodes have been shown to be inversely correlated with Platyhelminthes [70]. Although not observed in this study, nematodes were one of the main drivers of meiobenthic community structure since they showed the highest overall association values with the metazoa. Similarly, within the eukaryotic groups, diatoms and SARs can be the food of choice for most meiobenthic metazoans [83]. Benthic protists, one of the world's largest reservoirs of marine diversity, are the primary bacterivores in most aquatic systems [84] and could be the missing link between meiofauna–bacteria interactions as described in other habitats like Red Sea sediments [78]. The dominance of the microbial fraction and its high association with metazoan communities emphasises their vital role in marine ecosystems.

Local adaptation driving niche-specific metazoan and bacterial taxa

Overall PCOA explained up to 45% of community structure, suggesting that almost half the microbial patterns identified are factual despite incomplete sampling. Such incompleteness shows once more the immense benthic diversity of the Antarctic [8, 85, 86] and emphasises the importance of having enough biological replicates. The community profiles for bacteria and eukaryotes, including Metazoa were markedly dissimilar between locations. Although there was very little distance between the sampling sites, each site had distinct topographies promoting high levels of local diversity where 50% bacteria and 70% Metazoa were unique to each site. Each site also showed very clear separation in terms of community composition for both Bacteria and Metazoa, whereby each location clustered independently by 70% similarity, showing that such habitats harbour unique features for microbial communities (Fig. 7). Both Bacteria and Metazoa clearly adapt at the very local level (niche driven) observed by high ASV numbers per location, concomitant to species self-assembling into clusters based on their niche requirements [87]. A recent meiofaunal metacommunity analysis suggested that niche-based processes were the main structuring mechanism in both meiofauna and bacteria [88, 89]. This was substantiated in our study by the high alpha diversity found across kingdoms in each location. Both bacteria and eukaryotes were strongly associated with biotic and abiotic factors further supporting the species sorting paradigm whereby at local scales species occur in specific habitats where conditions are favourable [87, 89]. Considering that Antarctica contains some of the strongest environmental gradients on the planet [90], smaller spatial scale studies show how abiotic and biotic factors dictate diversity at habitat and community level [86]. This also highlights the high possibility for local extinctions and the importance of implementing strategies of site-specific management, as these will be key for sustaining unique levels of biodiversity and habitats in Antarctica.

DATA SHARING
The raw data from this study are available through NCBI's Sequence Read Archive under Project Accession Number SUB8868490 (Bioproject PRJNA692247).

REFERENCES
1. Meredith M, Sommerkorn M, Cassotta S, Derksen C, Ekaykin A, Hollowed A. IPCC special report on the ocean and cryosphere in a changing climate In: Pörtner H-O, Roberts D, Masson-Delmotte V, Zhai P, Tignor M, Poloczanska Eea, editors. 2022; chapter 3: https://doi.org/10.1017/9781009157964 (in press).
2. Rozema PD, Venables HJ, van de Poll WH, Clarke A, Meredith MP, Buma AGJ. Interannual variability in phytoplankton biomass and species composition in northern Marguerite Bay (West Antarctic Peninsula) is governed by both winter sea ice cover and summer stratification. Limnol Oceanogr. 2017;62:235–52.
3. Venables HJ, Clarke A, Meredith MP. Wintertime controls on summer stratification and productivity at the western Antarctic Peninsula. Limnol Oceanogr. 2013;58:1035–47.
4. Barnes DKA, Souster T. Reduced survival of Antarctic benthos linked to climate-induced iceberg scouring. Nat Clim Change. 2011;1:365–8.
5. Grange L, Tyler P, Peck L, Cornelius N. Long-term interannual cycles of the gametogenic ecology of the Antarctic brittle star Ophionotus victoriae. Mar Ecol Prog Ser. 2004;278:141–55.
6. Schratzberger M, Ingels J. Meiofauna matters: The roles of meiofauna in benthic ecosystems. J Exp Mar Biol Ecol. 2018;502:12–25.
7. Mayor D, Thornton B, Jenkins H, Felgate S. Microbiota: the living foundation. In: Beninger P, editor. Mudflat ecology. Switzerland AG: Springer Nature 2018. p. 43–61.
8. Fonseca VG, Sinniger F, Gaspar JM, Quince C, Creer S, Power DM, et al. Revealing higher than expected meiofaunal diversity in Antarctic sediments: a meta-barcoding approach. Sci Rep. 2017;7:6094.
9. Vause BJ, Morley SA, Fonseca VG, Jazdzewska A, Ashton GV, Barnes DKA, et al. Spatial and temporal dynamics of Antarctic shallow soft-bottom benthic communities: ecological drivers under climate change. BMC Ecol. 2019;19:27.
10. Danovaro R, Scopa M, Gambi C, Fraschetti S. Trophic importance of subtidal metazoan meiofauna: evidence from in situ exclusion experiments on soft and rocky substrates. Mar Biol. 2007;152:339–50.
11. Watzin MC. The effects of meiofauna on settling macrofauna: meiofauna may structure macrofaunal communities. Oecologia. 1983;59:163–6.
12. Schmidt JL, Deming JW, Jumars PA, Keil RG. Constancy of bacterial abundance in surficial marine sediments. Limnol Oceanogr. 1998;43:976–82.
13. Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: the unseen majority. Proc Natl Acad Sci USA. 1998;95:6578–83.
14. Burdige DJ. Preservation of organic matter in marine sediments: controls, mechanisms, and an imbalance in sediment organic carbon budgets? Chem Rev. 2007;107:467–85.
15. Zou K, Thébault E, Lacroix G, Barot S. Interactions between the green and brown food web determine ecosystem functioning. Funct Ecol. 2016;30:1454–65.
16. Anderson TR, Pond DW, Mayor DJ. The role of microbes in the nutrition of detritivorous invertebrates: a stoichiometric analysis. Front Microbiol. 2016;7:2113.
17. Lacoste E, Piot A, Archambault P, McKindsey CW, Nozais C. Bioturbation activity of three macrofaunal species and the presence of meiofauna affect the abundance and composition of benthic bacterial communities. Mar Environ Res. 2018;136:62–70.
18. Bonaglia S, Nascimento FJ, Bartoli M, Klawonn I, Bruchert V. Meiofauna increases bacterial denitrification in marine sediments. Nat Commun. 2014;5:5133.
19. Riemann F, Helmke E. Symbiotic relations of sediment-agglutinating nematodes and bacteria in detrital habitats: the enzyme-sharing concept. Mar Ecol. 2002;23:93–113.
20. dos Santos GAP, Derycke S, Fonseca-Genevois VG, Coelho LCBB, Correia MTS, Moens T. Differential effects of food availability on population growth and fitness of three species of estuarine, bacterial-feeding nematodes. J Exp Mar Biol Ecol. 2008;355:27–40.
21. Zeppilli D, Sarrazin J, Leduc D, Arbizu PM, Fontaneto D, Fontanier C, et al. Is the meiofauna a good indicator for climate change and anthropogenic impacts? Mar Biodivers. 2015;45:505–35.
22. Moens T, Beninger PG. Meiofauna: an inconspicuous but important player in Mudflat ecology. In: Beninger P, editor. Mudflat ecology aquatic ecology series. 7. Switzerland: Springer; 2018.
23. Webb AL, Hughes KA, Grand MM, Lohan MC, Peck LS. Sources of elevated heavy metal concentrations in sediments and benthic marine invertebrates of the western Antarctic Peninsula. Sci Total Environ. 2020;698:134268.
24. Brown KM, Fraser KP, Barnes DK, Peck LS. Links between the structure of an Antarctic shallow-water community and ice-scour frequency. Oecologia. 2004;141:121–9.
25. Stoeck T, Bass D, Nebel M, Christen R, Jones MDM, Breiner H-W, et al. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol Ecol. 2010;19:21–31.
26. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012;6:1621–4.
27. Leray M, Yang JY, Meyer CP, Mills SC, Agudelo N, Ranwez V, et al. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Front Zool. 2013;10:34.
28. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J. 2011;17:10–2.
29. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581–3.
30. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST +: architecture and applications. BMC Bioinform. 2009;10:421.
31. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One. 2013;8:e61217.
32. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41:D590–6.
33. Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. GenBank. Nucleic Acids Res. 2005;33:D34–8.
34. Wentworth CK. A scale of grade and class terms for clastic sediments. J Geol. 1922;30:377–92.
35. Dean WE. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition; comparison with other methods. J Sediment Res. 1974;44:242–8.
36. Tatzber M, Stemmer M, Spiegel H, Katzlberger C, Haberhauer G, Gerzabek MH. An alternative method to measure carbonate in soils by FT-IR spectroscopy. Environ Chem Lett. 2007;5:9–12.

37. Hsieh CH, Reiss CS, Hunter JR, Beddington JR, May RM, Sugihara G. Fishing elevates variability in the abundance of exploited species. Nature. 2006;443:859–62.

38. Elbrecht V, Braukmann TWA, Ivanova NV, Prosser SWJ, Hajibabaei M, Wright M, et al. Validation of COI metabarcoding primers for terrestrial arthropods. Peer J. 2019;7:e7745–e.

39. Kirse A, Bourlat SJ, Langen K, Fonseca VG. Unearthing the potential of Soil eDNA metabarcoding—towards best practice advice for invertebrate biodiversity assessment. Front. Ecol. Evol. 2021;9:630560.

40. Zhang GK, Chain FJJ, Abbott CL, Cristescu ME. Metabarcoding using multiplexed markers increases species detection in complex zooplankton communities. Evolut Appl. 2018;11:1901–14.

41. Marquina D, Andersson AF, Ronquist F. New mitochondrial primers for metabarcoding of insects, designed and evaluated using in silico methods. Mol Ecol Resour. 2019;19:90–104.

42. Leasi F, Sevigny JL, Laflamme EM, Artois T, Curini-Galletti M, de Jesus Navarrete A, et al. Biodiversity estimates and ecological interpretations of meiofaunal communities are biased by the taxonomic approach. Commun Biol. 2018;1:112.

43. Giebner H, Langen K, Bourlat SJ, Kukowka S, Mayer C, Astrin JJ, et al. Comparing diversity levels in environmental samples: DNA sequence capture and metabarcoding approaches using 18S and COI genes. Mol Ecol Resour. 2020;20:1333–45.

44. Vanhove S, Lee HJ, Beghyn M, Gansbeke DV, Brockington S, Vincx M. The Metazoan Meiofauna in its biogeochemical environment: the case of an Antarctic coastal sediment. J Mar Biol Assoc UK. 1998;78:411–34.

45. Pasotti F, Saravia LA, De Troch M, Tarantelli MS, Sahade R, Vanreusel A. Benthic Trophic Interactions in an Antarctic Shallow Water Ecosystem Affected by Recent Glacier Retreat. PLoS ONE. 2015;10:e0141742.

46. Griffiths JR, Kadin M, Nascimento FJA, Tamelander T, Tornroos A, Bonaglia S, et al. The importance of benthic-pelagic coupling for marine ecosystem functioning in a changing world. Global Change Biology. 2017;23:2179–96.

47. Virta L, Gammal J, Järnström M, Bernard G, Soininen J, Norkko J, et al. The diversity of benthic diatoms affects ecosystem productivity in heterogeneous coastal environments. Ecology. 2019;100:e02765.

48. Malviya S, Scalco E, Audic S, Vincent F, Veluchamy A, Poulain J, et al. Insights into global diatom distribution and diversity in the world's ocean. Proc Natl Acad Sci USA. 2016;113:E1516–25.

49. Forster D, Dunthorn M, Mahe F, Dolan JR, Audic S, Bass D, et al. Benthic protists: the under-charted majority. Fems Microbiol Ecol. 2016;92:fiw120.

50. Fonseca VG, Carvalho GR, Nichols B, Quince C, Johnson HF, Neill SP, et al. Metagenetic analysis of patterns of distribution and diversity of marine meiobenthic eukaryotes. Glob Ecol Biogeogr. 2014;23:1293–302.

51. O'Malley MA. The nineteenth century roots of 'everything is everywhere'. Nat Rev Microbiol. 2007;5:647–51.

52. Pasotti F, Manini E, Giovannelli D, Wölfl A-C, Monien D, Verleyen E, et al. Antarctic shallow water benthos in an area of recent rapid glacier retreat. Mar Ecol. 2015;36:716–33.

53. Molari M, Janssen F, Vonnahme TR, Wenzhöfer F, Boetius A. The contribution of microbial communities in polymetallic nodules to the diversity of the deep-sea microbiome of the Peru Basin (4130–4198 m depth). Biogeosciences. 2020;17:3203–22.

54. Signori CN, Thomas F, Enrich-Prast A, Pollery RCG, Sievert SM. Microbial diversity and community structure across environmental gradients in Bransfield Strait, Western Antarctic Peninsula. Front Microbiol. 2014;5:647.

55. Ozturk RC, Feyzioglu AM, Altinok I. Prokaryotic community and diversity in coastal surface waters along the Western Antarctic Peninsula. Pol Sci. 2021;31:100764.

56. Ghiglione JF, Murray AE. Pronounced summer to winter differences and higher wintertime richness in coastal Antarctic marine bacterioplankton. Environ Microbiol. 2012;14:617–29.

57. Luria CM, Ducklow HW, Amaral-Zettler LA. Marine bacterial, archaeal and eukaryotic diversity and community structure on the continental shelf of the western Antarctic Peninsula. Aquat Microbial Ecol. 2014;73:107–21.

58. Cao S, He J, Zhang F, Lin L, Gao Y, Zhou Q. Diversity and community structure of bacterioplankton in surface waters off the northern tip of the Antarctic Peninsula. Pol Res. 2019;38:3491.

59. Walsh EA, Kirkpatrick JB, Rutherford SD, Smith DC, Sogin M, D'Hondt S. Bacterial diversity and community composition from seasurface to subseafloor. ISME J. 2016;10:979–89.

60. Kiko R, Werner I, Wittmann A. Osmotic and ionic regulation in response to salinity variations and cold resistance in the Arctic under-ice amphipod Apherusa glacialis. Pol Biol. 2009;32:393–8.

61. Zeppilli D, Leduc D, Fontanier C, Fontaneto D, Fuchs S, Gooday AJ, et al. Characteristics of meiofauna in extreme marine ecosystems: a review. Mar Biodivers. 2018;48:35–71.

62. Arnosti C, Joergensen BB, Sagemann J, Thamdrup B. Temperature dependence of microbial degradation of organic matter in marine sediments: polysaccharide hydrolysis, oxygen consumption, and sulfate reduction. Mar Ecol Prog Ser. 1998;165:59–70.

63. Fabiano M, Danovaro R. Enzymatic activity, bacterial distribution, and organic matter composition in sediments of the ross sea (Antarctica). Appl Environ Microbiol. 1998;64:3838–45.

64. Kujawinski EB, Longnecker K, Barott KL, Weber RJM, Kido Soule, MC. Microbial community structure affects marine dissolved organic matter composition. Front Mar Sci. 2016;3:45.

65. Barrett JE, Virginia RA, Hopkins DW, Aislabie J, Bargagli R, Bockheim JG, et al. Terrestrial ecosystem processes of Victoria Land, Antarctica. Soil Biol Biochem. 2006;38:3019–34.

66. Ganzert L, Lipski A, Hubberten H-W, Wagner D. The impact of different soil parameters on the community structure of dominant bacteria from nine different soils located on Livingston Island, South Shetland Archipelago, Antarctica. Fems Microbiol Ecol. 2011;76:476–91.

67. Rusch A, Huettel M, Reimers CE, Taghon GL, Fuller CM. Activity and distribution of bacterial populations in Middle Atlantic Bight shelf sands. Fems Microbiol Ecol. 2003;44:89–100.

68. Hemkemeyer M, Dohrmann AB, Christensen BT, Tebbe CC. Bacterial preferences for specific soil particle size fractions revealed by community analyses. Front Microbiol. 2018;9:149.

69. Giere O. Meiobenthology: the microscopic motile fauna of aquatic sediments. 2nd ed: Springer-Verlag Berlin Heidelberg; 2009. 527 p.

70. Fonseca VG, Carvalho GR, Sung W, Johnson HF, Power DM, Neill SP, et al. Second-generation environmental sequencing unmasks marine metazoan biodiversity. Nat Commun. 2010;1:98.

71. Pitcher RC, Lawton P, Ellis N, Smith SJ, Incze LS, Wei C-L, et al. Exploring the role of environmental variables in shaping patterns of seabed biodiversity composition in regional-scale ecosystems. J Appl Ecol. 2012;49:670–9.

72. Rose A, Ingels J, Raes M, Vanreusel A, Arbizu PM. Long-term iceshelf-covered meiobenthic communities of the Antarctic continental shelf resemble those of the deep sea. Heidelberg: Springer; 2014. 743–62 p.

73. Gonçalves-Araujo R, de Souza MS, Tavano VM, Garcia CAE. Influence of oceanographic features on spatial and interannual variability of phytoplankton in the Bransfield Strait, Antarctica. J Mar Syst. 2015;142:1–15.

74. Learman DR, Henson MW, Thrash JC, Temperton B, Brannock PM, Santos SR, et al. Biogeochemical and microbial variation across 5500 km of Antarctic surface sediment implicates organic matter as a driver of benthic community structure. Front Microbiol. 2016;7:284.

75. Ghiglione JF, Galand PE, Pommier T, Pedros-Alio C, Maas EW, Bakker K, et al. Pole-to-pole biogeography of surface and deep marine bacterial communities. Proc Natl Acad Sci USA. 2012;109:17633–8.

76. Rosli N, Leduc D, Rowden A, Probert P. Review of recent trends in ecological studies of deep-sea meiofauna, with focus on patterns and processes at small to regional spatial scales. Mar Biodivers. 2017;48:13–34.

77. Ruff SE, Probandt D, Zinkann A-C, Iversen M, Klaas C, Schwabe L, et al. Indications for algae-degrading benthic microbial communities in deep-sea sediments along the Antarctic Polar Front. Deep Sea Res Part II: Top Stud Oceanogr. 2014;108:6–16.

78. El-Serehy HA, Al-Rasheid KA, Al-Misned FA, Al-Talasat AA, Gewik MM. Microbial-meiofaunal interrelationships in coastal sediments of the Red Sea. Saudi J Biol Sci. 2016;23:327–34.

79. Danovaro R, Company JB, Corinaldesi C, D'Onghia G, Galil B, Gambi C, et al. Deep-sea biodiversity in the Mediterranean Sea: the known, the unknown, and the unknowable. PLoS ONE. 2010;5:e11832.

80. Mussmann M, Pjevac P, Kruger K, Dyksma S. Genomic repertoire of the Woeseiaceae/JTB255, cosmopolitan and abundant core members of microbial communities in marine sediments. ISME J. 2017;11:1276–81.

81. Hinger I, Pelikan C, Mußmann M. Role of the ubiquitous bacterial family Woeseiaceae for N2O production in marine sediments. Geophys Res Abstracts. 2019;21:17441.

82. Hoffmann K, Bienhold C, Buttigieg PL, Knittel K, Laso-Pérez R, Rapp JZ, et al. Diversity and metabolism of Woeseiales bacteria, global members of marine sediment communities. ISME J. 2020;14:1042–56.

83. Mare MF. A study of a marine benthic community with special reference to the microorganisms. J Mar Biol Assoc UK. 1942;25:517–54.

84. Bott TL, Borchardt MA. Grazing of protozoa, bacteria, and diatoms by Meiofauna in lotic epibenthic communities. J North Am Bentholog Soc. 1999;18:499–513.

85. Griffiths HJ. Antarctic marine biodiversity-what do we know about the distribution of life in the Southern Ocean? PLoS ONE. 2010;5:e11683.

86. Convey P, Chown SL, Clarke A, Barnes DKA, Bokhorst S, Cummings V, et al. The spatial structure of Antarctic biodiversity. Ecol Monogr. 2014;84:203–44.

87. Li L, Ma ZS. Species sorting and neutral theory analyses reveal archaeal and bacterial communities are assembled differently in hot springs. Front Bioeng Biotechnol. 2020;8:464.

88. Lee JE, Buckley HL, Etienne RS, Lear G. Both species sorting and neutral processes drive assembly of bacterial communities in aquatic microcosms. Fems Microbiol Ecol. 2013;86:288–302.
89. Gansfort B, Fontaneto D, Zhai M. Meiofauna as a model to test paradigms of ecological metacommunity theory. Hydrobiologia. 2020;847:2645–63.
90. Convey P, Peck LS. Antarctic environmental change and biological responses. Sci Adv. 2019;5:eaaz0888.

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AUTHOR CONTRIBUTIONS

MSC conceived and supervised the project. VGF and MSC wrote the first draft of the manuscript. VGF, HG and AK performed the bioinformatic analysis. BJV collected the samples and LSP supervised sample collection. TD performed the sedimentological analyses. VGF performed the ecological analysis and interpreted the data. MSC and DMP assisted with the biological interpretations and the production of the manuscript. All authors contributed to the final draft and gave approval for publication.

COMPETING INTERESTS

The authors declare no competing interests.

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