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Dynamics of microbial stress responses driven by abiotic changes along a temporal gradient in Deception Island, Maritime Antarctica

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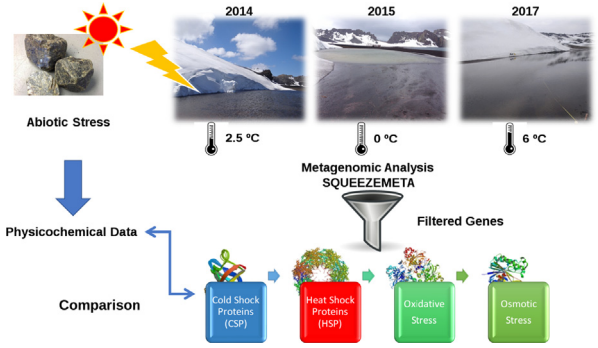
HIGHLIGHTS

- Temperature and solar radiation have influence on stress functions.
- Bacteria of WB have a rich repertoire of HSP, CSP, oxidative and osmotic genes.
- Abiotic factors influenced the microbial richness, but did not significantly alter the diversity.
- Warmer years have selected specific microbial groups, as Cyanobacteria.

GRAPHICAL ABSTRACT



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ABSTRACT

Whalers Bay (WB), Deception Island, is an environment that can drastically change its temperature within a few meters. The main forms of life inhabiting this environment are microorganisms, which, due to the high diversity and their adaptive potential, can survive and thrive under harsh stress conditions. However, the genetic potential and mechanisms to cope with fluctuating adverse conditions as well as what extent environmental variations shape the microbial community over the years it is still unknown in Antarctic environments. In this work, sediments collected in a transect in Whalers Bay, Deception Island, during the Austral Summers of 2014, 2015 and 2017 were analyzed using shotgun metagenomics. Sequence data were further processed with the SqueezeMeta tool for assembly, gene prediction, mapping, taxonomic and functional annotations. Results showed that stress-related functions had the influence of temperatures and solar radiation observed in the years of 2015 and 2017. The most differentiated functions were the ones related to oxidative stress, comparing 2014 vs 2015 and 2014 vs 2017. The genes coding for HSP20 and oxidoreductases (*nrd*H, *grx*A, *kor*C and *kor*D), as well as the genes *clp*E, *csp*L, and operons *mtr*AB and *vic*KR, were differentially enriched between the years, most of them found in gram-positive bacteria. The selective pressures of temperature and radiation may have favored the growth of gram-positive bacteria in 2017, with emphasis on *Arthrobacter* genus. Data gathered in this work showed that temperature and solar radiation could potentially be the primary driving forces shaping the repertoire of stress-response genes for the maintenance of microbial diversity in WB Antarctic sediments.

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1. Introduction

Extreme environments, according to the astrobiology encyclopedia, are habitats with stressful conditions, such as high and/or low values of temperatures, pH, nutrients, among others, beyond the ideal range for human development (Gargaud et al., 2015). Polar deserts, such as Antarctica, are considered extreme environments because of their low temperatures, high UV irradiation, low concentration of nutrients and low water availability (Bolter et al., 2002; Onofri et al., 2007, Duarte et al., 2019). In Maritime Antarctica, a volcanic island named Deception stands out as a stressful environment (Centurion et al., 2019), with emphasis on temperatures that can change dramatically in a few centimeters of distance (Bendia et al., 2018a, 2018b), serving as a model environment for astrobiology studies. In Deception, Whalers Bay area has attracted attention for having permanent ice and also geothermal activity (Bartolini et al., 2014; Centurion et al., 2019; Geyer et al., 2019). In Whalers Bay, persistent cold temperatures are often accompanied by freezing/defrosting cycles between winter and summer, extreme irradiance fluctuations (including ultraviolet radiation), and wide variations in nutrient supply and salinity. The microbiota of these habitats must, therefore, deal with extreme changes in temperature, freezing stress, desiccation, high solar radiation and high salinity. Given its volcanic nature, Deception island has high levels of heavy metals that could lead to electrostatic destabilization through the exchange of charges between molecules and oxidation, leading to the formation of reactive oxygen species (ROS). In cold environments, a greater solubility of gases makes microorganisms more affected by ROS, being constantly sensitized by oxidative stress (Chattopadhyay, 2006). In a comparative study between Arctic and Antarctic stress genes, a greater amount of oxidative stress genes was observed in Antarctica (Varin et al., 2011).

Stress response functions such as heat (HSPs) and cold (CSPs) shock proteins are shaped by natural selection and may play an important role in contributing to microbial survival under rapidly changing conditions (Chattopadhyay, 2006). HSPs represent a family of proteins known as chaperones. In addition to being involved in thermal variation response, as the name refers, they have essential importance under exposure to a wide variety of environmental stresses (Maleki et al., 2016). They act in protein folding/unfolding and degradation processes to maintain cellular homeostasis. CSPs act as RNA chaperones on the secondary structure of destabilized messenger RNAs, assisting the translation of new proteins (Rabus and et al., 2004; Chaikam and Karlson, 2010). In addition to regulating the adaptive response to cold stress, CSPs may also help to regulate oxidative and osmotic stress functions. To survive under oxidative stress, many microorganisms produce high concentrations of antioxidant enzymes such as superoxide dismutase, catalase and peroxidase to help in the detoxification of ROS. Likewise, microorganisms are able to capture inorganic ions from the environment to balance extracellular ion concentrations, as well as producing organic osmolytes such as betaine and proline that act as cryoprotectant agents (Chattopadhyay, 2002; Oren, 2008). Therefore, based on the diverse genetic and functional adaptations to cope with abiotic conditions imposed by harsh habitats, from a microbial ecology point of view, the "extreme" term may not match the adaptive reality of so-called extremophiles (Maccario et al., 2015). Nonetheless, information on novel genetic mechanisms used by such extreme microorganisms to cope with different combinations of stressful conditions in Antarctic environments are still scarce. Changes in abiotic factors over the years, such as temperature, radiation and heavy metal concentration, may shape the stress response profiles of microbiomes inhabiting the deicing area of Deception - Whalers Bay, in addition to favoring the establishment of new microbial groups. Based on that, this work aimed to investigate the dynamics of the genetic arsenal of microbial communities from sediments of Whalers Bay, Deception Island, along spatial and temporal gradients by using shotgun metagenomics analysis, seeking to understand what environmental changes can bring to the microbial adaptive response.

2. Material and methods

2.1. Sampling

Sampling was carried out from sediments with a reddish biofilm formed on the surface of melting water, derived from a close glacier, in Whalers Bay (WB), Deception Island, South Shetlands archipelago (Maritime Antarctica; - S 62° 97′ 934″ and W 060° 55′ 532″), in December of 2014, 2015 and 2017 (austral summer). Sediment samples were collected in duplicate (2014) and triplicate (2015 and 2017), from the surface of a transect along the extension of the melting water, comprising: site 1: WB1, subglacial sediments; site 2: WB2, 50 cm far from the glacier; site 3: WB3, central area; site 4: WB4, transect end. Samples were collected using sterilized bags (for chemical analysis) and Falcon tubes (for DNA extraction), in order to prevent further contamination with microorganisms that might alter concentrations of heavy metals and other soil/sediment components during the long trip back to Brazil, and stored at −20 °C for transportation to the laboratory of the Microbial Resources Division (CPQBA/UNICAMP). Sediments samples from each summer were immediately processed for DNA extraction and sequencing after arrival at the laboratory. At the sampling site, sediment and environmental temperature data were collected (Fig. 1).

2.2. Physicochemical analysis of WB sediments

Physicochemical analyses of samples corresponding to each site of the WB sediments were carried out at the Soil Science Department of the Luiz de Queiróz School of Agriculture (ESALQ - USP, Piracicaba, SP). The concentrations of the elements Fe, Cu, Mn, Zn, Pb, Cd, Cr, Ni, and Co were determined by flame atomic absorption spectroscopy. The concentrations of total N and inorganic N ($N\text{-}NH^{+4}$ and $N\text{-}NO^{-3}$) were evaluated by the Kjeldahnl test and steam distillation, respectively. The levels of organic carbon were evaluated by titration of excess dichromate ions with Fe^{+2} ions and colorimetric method based on reading of green color of Cr (III) ion reduced by organic carbon (Quaggio and Raij, 1979) and detection limit of 0.00 mg/dm^3. The concentration of organic matter was obtained from the results of organic carbon by conversion using Van Bemmelen factor. Temperature and solar radiation data from the austral summers (from November to February) of the years 2014 to 2017 were obtained from the Spanish Meteorological Station, Gabriel de Castilla, located on the Deception Island, through the website https://antartida.aemet.es/.

2.3. Metagenomic analysis

2.3.1. DNA extraction and sequencing

Metagenomic DNA of the samples collected from the sediments of Whalers Bay along the three summers was extracted with the DNAsy PowerSoil Kit (Qiagen, Inc., Hilden, Germany), according to the manufacturer's instructions. Total DNA obtained from 2014 (duplicate) and 2015 (triplicate) samples was sent for large-scale sequencing using the Illumina HiSeq platform (2 × 150 bp, paired end) at the MR DNA company (Texas, United States). Samples of the year 2017 were sent to the company GenOne Soluções em Biotecnologia (Rio de Janeiro, RJ, Brazil) for sequencing at the Illumina NovaSeq 6000 platform (2 × 150, paired-end). Sequences obtained in this study were submitted to the European Nucleotide Archive (http://www.ebi.ac.uk) under the project accession number PRJEB29861 for 2014 samples and PRJEB38669 for 2015 and 2017 samples.

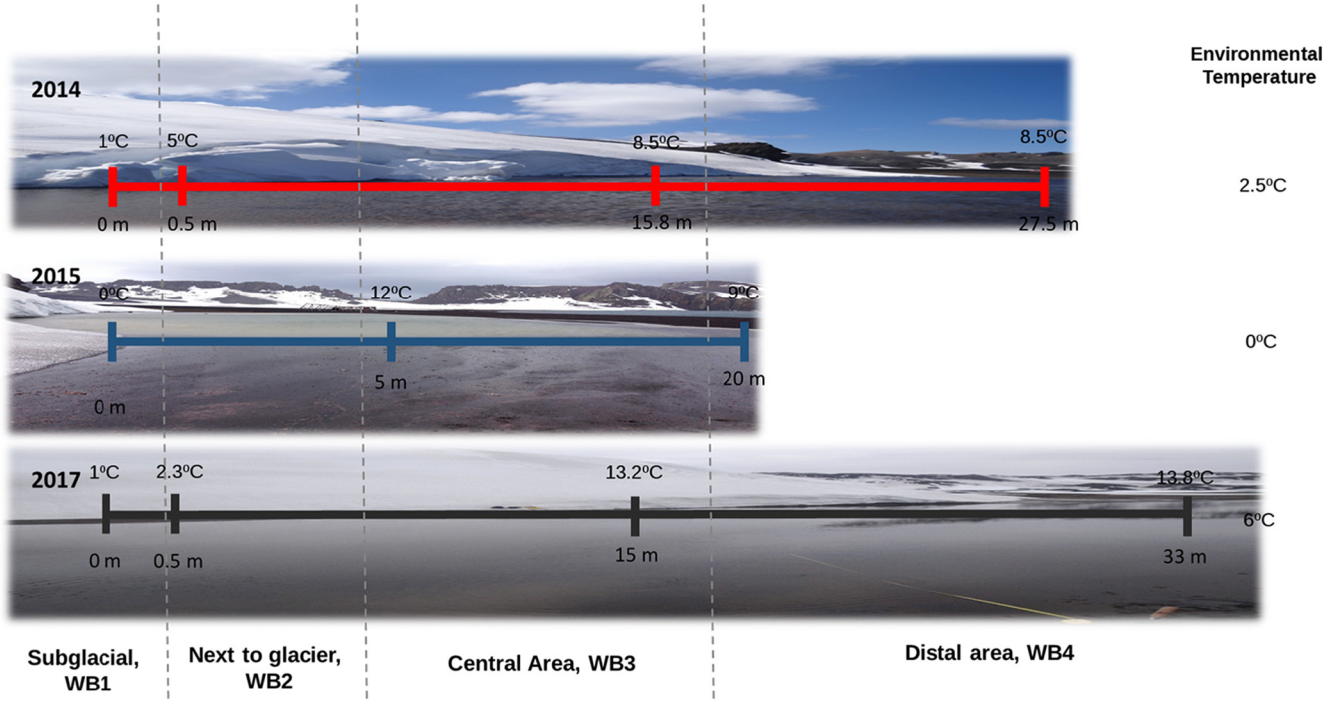

Fig. 1. Images of the sampling area showing the distances and temperatures of sediment and environmental of each site (WB1, WB2, WB3 and WB4) along the transect and the environmental temperature in each year.

2.3.2. Bioinformatics analyses

Functional and taxonomic diversity of the microbiota present in WB sediment samples from Deception Island was analyzed and compared using bioinformatics tools. First, the quality of the raw sequence data was verified using the fastqc tool (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were then filtered by the Trimmomatic v 0.36 tool (Bolger et al., 2014) to remove adapters and low-quality reads (phred score ≤ 30). For each sampling year, the data were run using the SqueezeMeta v 1.0.0 tool (Tamames and Puente-Sánchez, 2019). For this, the "co-assembly" option was used to assemble the contigs with the MegaHit tool (Li et al., 2015), using the k-mers 21, 29, 39, 59, 79, 99, 119, within the SqueezeMeta. Taxonomic and functional annotation were performed using an 80% identity cutoff and an e-value of $<1e^{-3}$.

The SqueezeMeta pipeline uses the tools Bowtie2 (Langmead and Salzberg, 2012) and BedTools (Quinlan and Hall, 2010) for mapping reads in contigs and counting reads for statistical purposes, respectively. The Prodigal tool (Hyatt et al., 2010) was used for the prediction of ORFs (Open Reading Frames). The Diamond tool (Buchfink et al., 2015) was used for searching and aligning ORFs against GenBank nr database for taxonomic assignment, while the eggNOG database (Huerta-Cepas et al., 2019) and the latest publicly available version of KEGG database (Kanehisa and Goto, 2000) were used for functional annotation. SqueezeMeta also classifies genes against the PFAM database (Finn et al., 2014), using the HMMER3 tool (Finn et al., 2011). With this methodology, we can annotate the sequences in three different databases, comprising one of orthology (KEGG – Kyoto Encyclopedia of Genes and Genomes) - KO groups, one of proteins encoded in complete genomes (COG) and one of protein domains (PFAM), enabling an integration of information and facilitating annotations of hypothetical proteins. Because it is only possible to classify CSPs in KEGG, COG and PFAM in the CSP-A protein family, an in-house database of CSPs was created from the UniProt database. This was inserted in SqueezeMeta as an external database and run together with the other databases (KEGG, COG and PFAM). Once the tables with taxonomic and functional data for each sampling year were obtained, the genes related to cold shock proteins (CSPs), heat shock proteins (HSPs), oxidative and osmotic stresses were manually filtered using information from the literature and UniProt and InterPro (Protein sequence analysis & classification) and the classifications of the databases (KEGG, COG and PFAM).

2.4. Statistical analysis

The tables with taxonomic and functional annotations for specific genes of each sampling year were exported to the R Statistical Environment platform. First, samples were normalized by the DeSeq2 package (Differential gene expression analysis based on the negative binomial distribution; Love et al., 2014). Then, replicates of each site for each year were evaluated using the pvclust package (Suzuki and Shimodaira, 2006), to assess the correlation between samples. For this, average method, distance by correlation, bootstrap of 1000 replicates and AU (approximately unbiased) values above 90% were used. After that, a PCA (Principal Component Analysis) was performed to assess the logarithmic relationship of the replicates and their dissimilarity. For the analysis of statistical differences, the DeSeq2 package with a p value <0.05 was used. Volcano plots were generated to allow visualization of functional differences of metagenomes between the sampling years. Taxonomic information inferred from the functions that showed statistical differences between the sampling years was extracted; the read count transformed into relative abundance and represented as alluvial diagrams to show changes in the taxonomic level of family and genus over the years. Diversity analyses of the sampling years were also performed using the Phyloseq package (McMurdie and Holmes, 2013), evaluating Simpson's and Shannon's diversity indices, in addition to functional evaluation of stress genes through rarefaction curves. The values of Shannon and Simpson indices were compared using the t-test ($p < 0.05$).

The solar radiation and temperature data from Gabriel de Castilla weather station for each sampling year of austral summer were evaluated in scatter plots generated in the ggplot2 package. The correlation between stress genes and environmental data was performed in R Statistical environment, using VEGAN package v.2.5–5 (Oksanen et al., 2018), by Non-metric Multidimensional Scaling (NMDS) based on

Chi-square distance with environmental data significantly correlated to the ordination (envfit, $p < 0.05$).

3. Results and discussion

3.1. Physicochemical characterization of WB sediments

The levels of solar radiation and temperature in the summers of each sampling year were constant, showing a median value for solar radiation and a slight increase of 1 °C for the year 2017 (Fig. 2). Interestingly, it was observed a higher environmental temperature, and consequently greater deicing in samples collected in 2017 when compared to other years. PCA analysis based on physicochemical data also showed a strong correlation of solar radiation and environmental temperature parameters with samples collected in the year 2017 (Fig. 3). Solar radiation values ranged from 0 to 350 W/m^2, depending on the time of day, with an average of 100 W/m^2. In the austral summer, the days are longer due to the terrestrial position of Antarctica, receiving higher doses of solar radiation between 12:00 to 22:00 h. The average values observed in this work coincided with those showed by Obryk et al. (2018) on McMurdo Island, Dry Valleys site, for the years 1990 to 2015. However, higher individual values were observed in Deception Island.

Physicochemical data (Table S1 sites; Table S2 years) showed that the concentrations of all heavy metals decreased during 2017, except for Cu concentration, which remained similar to the year 2015. This is confirmed by the PCA (Fig. 3), where heavy metal concentrations with significant differences (envfit $p < 0.05$) were most associated to the years of 2014 and 2015. Therefore, a variation in metal concentration might be occurring due to geothermal events, rising tide, and deicing of the glacier. Depending on the freezing state of the site, a greater concentration of metals can be observed. This hypothesis is reinforced by the sampling of year 2015, when the place was more frozen than the usual for the month of December, and by higher environmental temperature of 2017 (Fig. 3). The highest concentrations of nitrate (NO^{-3}) and ammonia (NH^{+4}) found in samples from the years 2014 and 2015 may be related to greater nitrogen fixation in sediments. In fact, the presence of reactive oxygen species (ROS) caused by solar radiation can inhibit the microbial enzyme nitrogenase, responsible for nitrogen fixation (Berman-Frank et al., 2003). As a result, the microbial community of sediments from the year 2017, more exposed to solar radiation, may suffer a greater inhibition of biological nitrogen fixation due to the higher concentrations of ROS.

3.2. Metagenomic analysis of the WB microbiota

To eliminate the redundancy of the size effect of libraries on functional annotation, a rarefaction analysis was carried out according to the guidelines of the phyloseq package (Fig. S1) and McMurdie and Holmes (2014). The curve plateau was reached with 25,000 reads in all analyzed metagenomes, indicating that the sequence depth of all libraries was enough to access most of functional attributes of the microbiome. In addition, a clustering analysis using the pvclust package and PCA graph was performed among replicates to evaluate their similarities. Replicates of all sites showed similarity of AU 90% (Fig. S2). Only replicate WB11_2015 (year 2015, site WB1) showed AU of 72% and was excluded from further analyses. PCA graph based on the functional profile showed that replicates belonging to the same year and sampling site clustered close to each other, proving their reproducibility (Fig. S3). The years 2015 and 2017 differed mainly due to the sites WB1 and WB2 of 2017, with sites WB3 and WB4 being grouped closer to the sites of 2015. This distribution characteristic is probably linked to the atypical sampling of the year 2015. It can be observed that the sampling transect in the year 2015 was smaller than in the years 2014 and 2017 (Fig. 1), because the sediment was more frozen than the usual in December. Therefore, site WB1 from the year 2015 would be physically closer to the site WB3 from the year 2017. Sites of the year 2014 clustered separately from the two other years. These differences observed in the PCA and clustering analysis are also evidenced by the statistical analysis of DeSeq2, where the main significant differences in the stress gene profiles were found in the year 2014 versus the years 2015 and 2017. Information on the libraries and analyses can be found in Table S2.

3.2.1. Diversity and taxonomy of stress response genes

The libraries normalized by DeSeq2 were compared and their significant differences ($p < 0.05$) were analyzed two by two considering the 15 most abundant taxa. The main differences were related to the phylum Euryarchaeota and the family Caulobacteraceae, more abundant in 2017, and to the class Cyanobacteria, more abundant in 2015 and 2017 (Fig. 4). Only a few differences were observed at the genus taxonomic level, including the higher abundance of *Psychrobacter* in 2014 in comparison to 2015 and of *Arthrobacter* in 2017, in comparison to 2014. Members of the phylum Euryarchaeota, belonging to the marine group II (MG II), are the most abundant planktonic archaeal group in ocean surface waters due to their photoheterotrophic capability (Zhang et al., 2015). Interestingly, knowledge of the lifestyle and ecological role of this group has been limited by a lack of cultured representatives and comparative phylogenomic analysis (Rinke et al., 2019). The Caulobacteraceae family comprise bacteria with oligotrophic lifestyle, harboring several mechanisms to deal with low nutrient concentrations. These bacteria also show resistance to oxidative stress by expression of the *rpo*E gene (Abraham et al., 2014). Cyanobacteria are diazotrophic and photoautotrophic microorganisms, recognized for their important role in biogeochemical cycles and for carbon or nitrogen fixation in extreme environments, such as Antarctica (Cary et al., 2010). Their greater abundance in 2015 and 2017 may be related to higher temperatures at sites WB3 and WB4 (from 9 to 13.8 °C) and higher

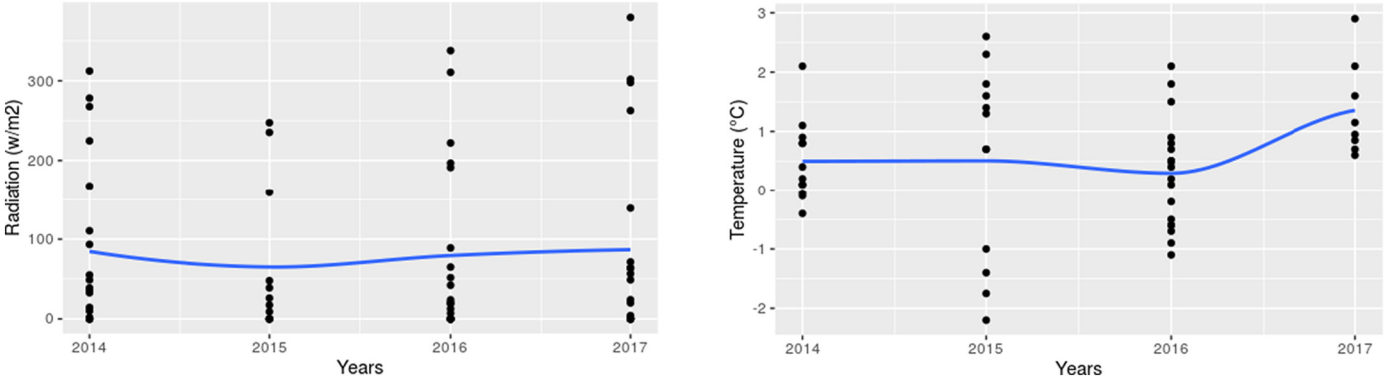

Fig. 2. Scatter plots showing the monthly average values of the solar radiation (left) and temperature (right) obtained from the austral summer in each sampling year.

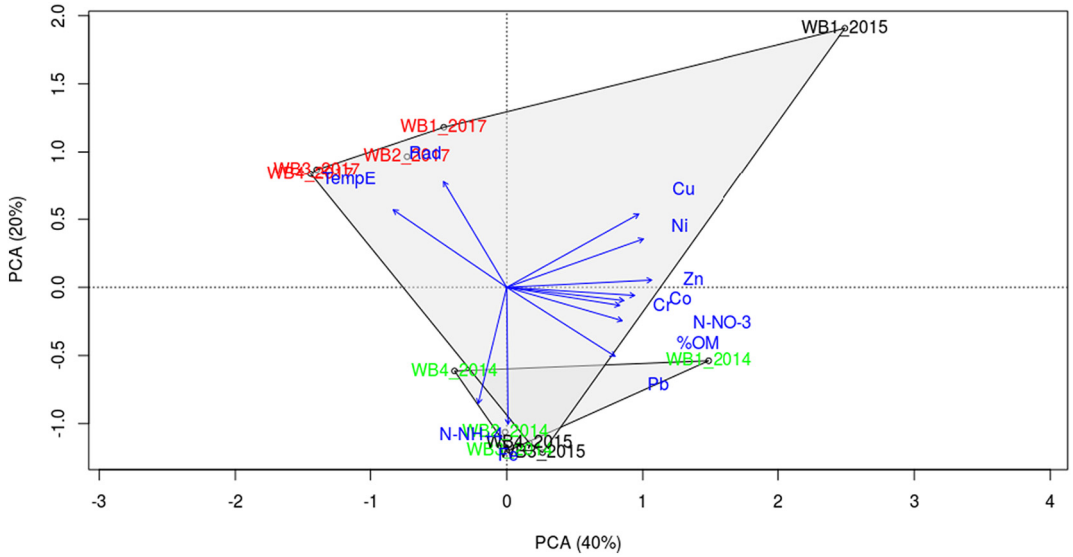

Fig. 3. Principal component analysis (PCA) based on physicochemical data from sampling sites of each year. Each arrow is significantly correlated to the ordination (envfit, $p < 0.05$) and represents the direction and strength of the environmental parameter. TempE = environmental temperature, measured at the time of sampling (°C); Rad = solar radiation, data from the Spanish station (W/m^2). Only solar radiation data from the time with the highest incidence of sunlight (12:00–22:00) was used. Sites: WB1: subglacial; WB2: next to glacier; WB3: central area; WB4: distal area.

incidence of solar radiation. This relationship can also be applied to the phylum Euryarchaeota, the family Caulobacteraceae and the genus *Arthrobacter*, all of them more abundant in 2017. The genus *Arthrobacter* has already been described in the literature as a producer of antioxidant substances, being more resistant to oxidative stress (Silva et al., 2018).

Acidovorax, *Hydrogenophaga*, *Polaromonas*, *Rhodoferax* and *Variovorax* were the most abundant genera found in all sampling years of WB Deception. These genera encompass free-living diazotrophic bacteria widespread in diverse soils and lakes, able to fix nitrogen in nitrate (NO-3) and ammonia (NH + 4) (Roesch et al.,

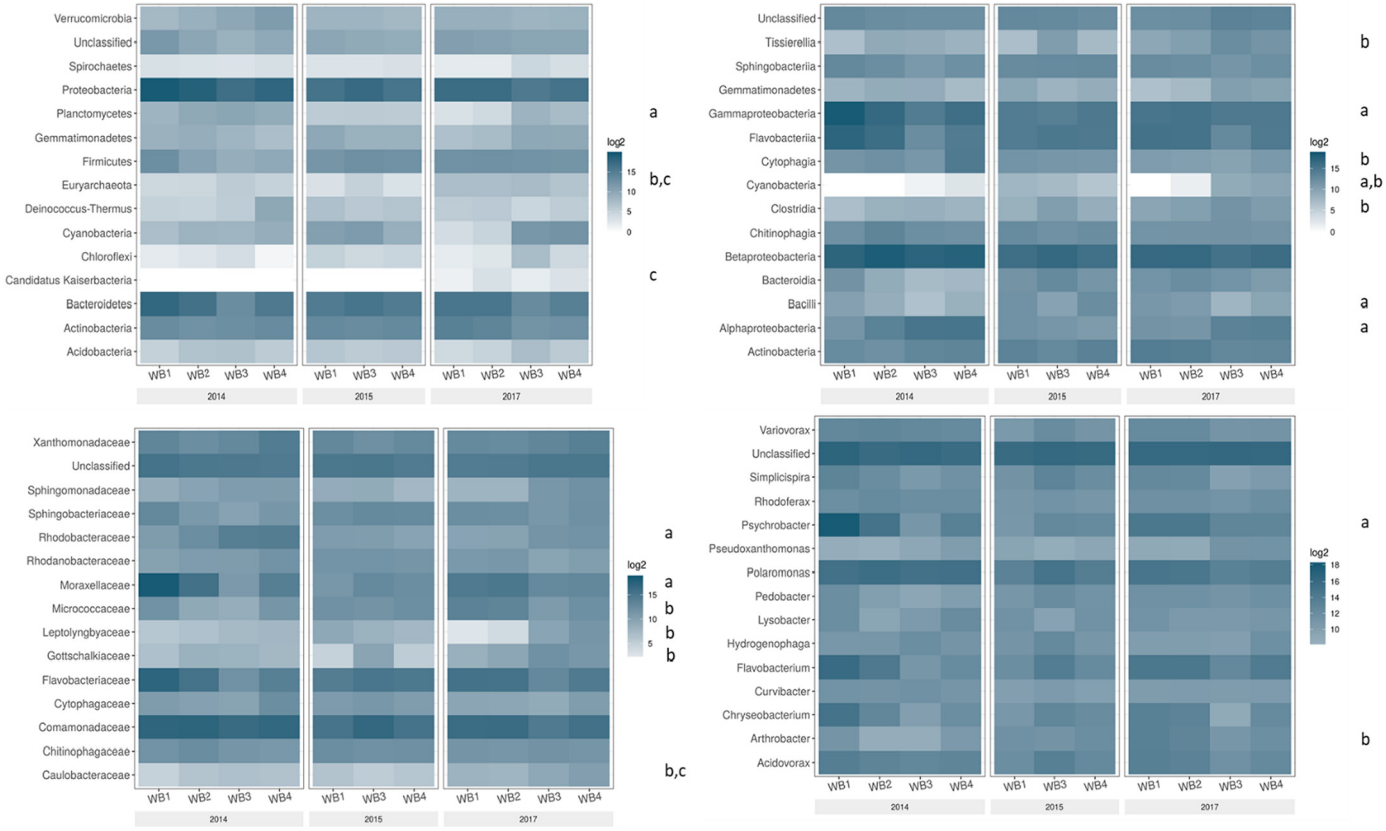

Fig. 4. Heatmap showing the microbial taxonomic composition of WB sites over the years. Phylum level (top left); Class level (top right); Family level (bottom left); and Genus level (bottom right). Values were normalized by DeSeq2. Only the 15 most abundant taxonomies are shown for each level. Letters a, b and c show the taxa with significant differences ($p < 0.05$) between the years, where: a, 2014 vs 2015; b, 2014 vs 2017; c, 2015 vs 2017. Sites: WB1: subglacial; WB2: next to glacier; WB3: central area; WB4: distal area.

2010). They have also been reported carrying antibiotic, heavy metal and biocide resistance genes in WB Deception Island sediments (Centurion et al., 2019).

To investigate the impact on the microbial diversity of WB sediments over the years, alpha diversity analyses were carried out using the Shannon index and its Simpson complement (diversity indices) (Table S4). Results revealed that the years of 2015 and 2017 had a greater microbial richness in comparison to the year of 2014. However, values of the Simpson diversity index observed were very close (0.81 to 0.84) and did not show significant difference. In addition, a higher number of low-abundant groups was observed in the years of 2015 and 2017, with a greater OTU richness. However, only slight changes in the microbial diversity was observed over the years, with the same most abundant genera prevailing among the years, as seen in Fig. 4.

3.2.2. Composition and dynamics of stress response genes in WB metagenomes over the years

In general, 12 CSPs, 26 HSPs, 85 oxidative stress genes and 55 osmotic stress genes were identified in the metagenomes of WB sediments. Two CSP genes for survival in low temperature environments stood out (Fig. S4): *csp*A, the main group of CSP; and *dea*D (or *csd*A), coding for DEAD-box RNA helicase, essential in the regulation of gene expression during microbial growth at low temperatures due to their helicase activity (Rocak and Linder, 2004). The metagenomes analyzed harbored genes belonging to all HSP classifications (sHsps, Hsp40, Hsp60, Hsp70, Hsp90 and Hsp100), with greater abundance of *groe*L (Hsp60), *dna*K (Hsp70), *htp*G (Hsp90) and *clp*B (Hsp100) genes (Fig. S5). HSPs are a well-conserved gene family and widely distributed among members of Bacteria and Archaea domains from different environments (Noronha et al., 2017), being used in phylogenetic studies (Hu et al., 2018). Therefore, their presence may not necessarily be associated with an increase in stress levels due to temperature or other factors in a functional metagenomic analysis. However, we can see a shift from gram-negative to gram-positive bacteria depending on the abundance of HSP genes (Derré et al., 1999; Van De Guchte et al., 2002; Miethke et al., 2006; Ventura et al., 2006).

Most of the functions related to osmotic stress found in the metagenome of WB sediments from different samples belonged to the OmpR family (26 of 55). This family includes proteins responsible for regulating external proteins, playing a central role in the bacterial response to acid and osmotic stress (Chakraborty et al., 2015, 2017). In this study, *tct*E, *kdp*D, *env*Z and *pho*R were the most abundant genes, all linked to the OmpR family of the histidine kinase enzyme, known for its hyperosmotic stress detection sensor and the Hik domain, which signals the osmolarity regulation pathway (Paithoonrangsarid et al., 2004) (Fig. S6). Other abundant genes observed in the WB metagenomes are active in the transport of molecules, such as *ku*P, a system responsible for the transport of potassium under osmotic stress at low pH. The *bet*T/*bet*S system is involved in osmoprotection under both hyperosmotic and hyposmotic stress through the transport of osmoprotective compounds, such as choline, glycine, proline and betaine solutes (Chen and Beattie, 2008). pH data showed a variation ranging from 5 to 7, which may explain the functions that act on acidic pH and osmotic stress (Table S1).

Regarding the oxidative stress gene profile, the enzymes oxidoreductase monooxygenase trimethylamine [EC: 1.14.13.148] (*tmm*), indolepyruvate ferredoxin oxidoreductase [EC: 1.2.7.8] (*ior*A), 2,3-dihydroxybenzoate 3,4 -dioxygenase [EC: 1.13.11.14] (*dhb*A), catalase [EC: 1.11.1.6] (*kat*E, CAT, *cat*B, *srp*A), aconitate hydratase [EC: 4.2.1.3] (*acn*A) and the *suf*B gene of the SufBCD complex were more abundant in all years (Fig. S7). Oxidoreductases catalyze the exchange of electrons or redox equivalents between a donor (reductant) and an acceptor (oxidant) molecule. Oxidoreductases act not only eliminating free radicals, but in processes of energy production and cellular respiration. The SufBCD complex acts to repair unstable iron and sulfur molecules in oxidative stresses (Outten et al., 2003). It is worth to note that WB sampling sites have a high concentration of iron (Table S1) and possible geothermal activity (sulfur elimination) (Bartolini et al., 2014; Centurion et al., 2019; Geyer et al., 2019), which could explain the high abundance of *suf*B gene in the microbial community from WB sediments. The enzyme aconitate hydratase - AcnA (gene *acn*A) belongs to a family of lyases, responsible for the production of water, carbon and ammonia molecules. Varguese et al. (2003) showed that AcnA is activated in the presence of superoxide radicals, acting in the neutralization of these molecules.

The only HSP genes with significant differences between the sampling years were the sHSP HSP20 and *ibp*A (2014 versus 2015 and 2014 versus 2017) and Hsp100 *clp*E (2015 versus 2014 and 2015 versus 2017) (Fig. 5). HSP20 and *clp*E were more abundant in 2015, a year that showed a temperature variation from −2 to +2.6 in the austral summer according to the Spanish weather station. Furthermore, this sampling area was more frozen than the usual in December. In addition, studies have shown that, depending on the salt concentration, HSP20 and *clp*E are expressed in psychrophilic bacteria at low temperature (4 °C) (Zheng et al., 2007; Kurihara and Esaki, 2008). The NMDS graph indicated that the environmental temperature was the main factor driving the stress gene profile of the microbial community from year 2015 (Fig. 6), which may explain the greater abundance of HSPs. In addition, sites

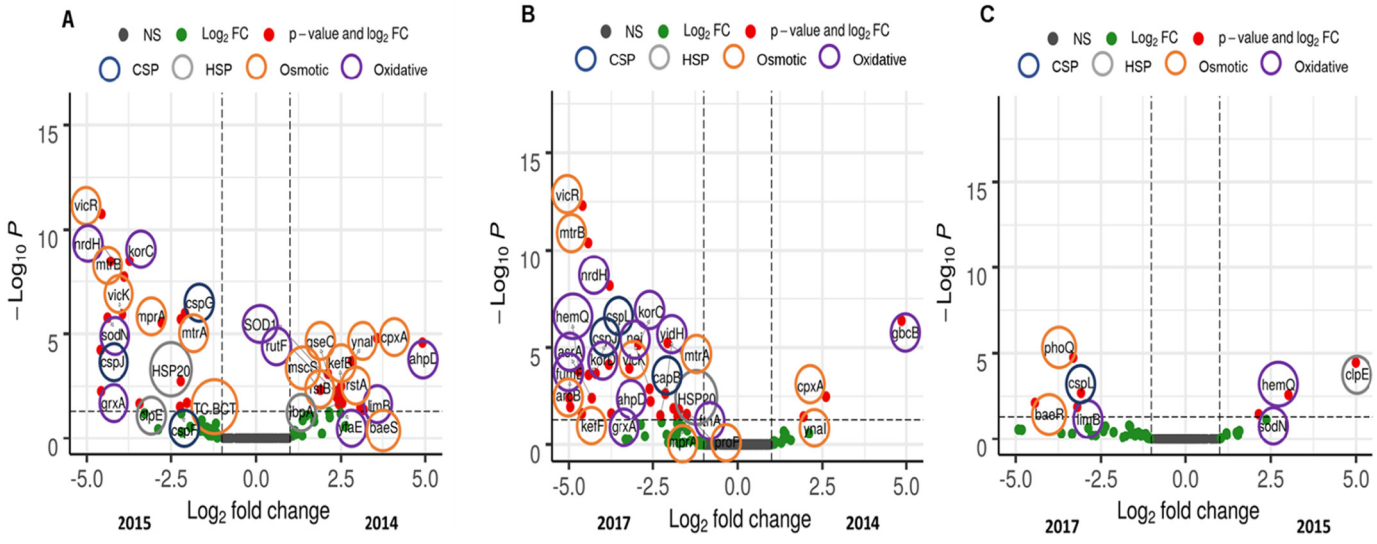

Fig. 5. Volcano plot showing the significant differences in stress-response genes between the years (p-value <0.05 and log2 FC), where A: 2014 vs 2015, B: 2014 vs 2017, C: 2015 vs 2017.

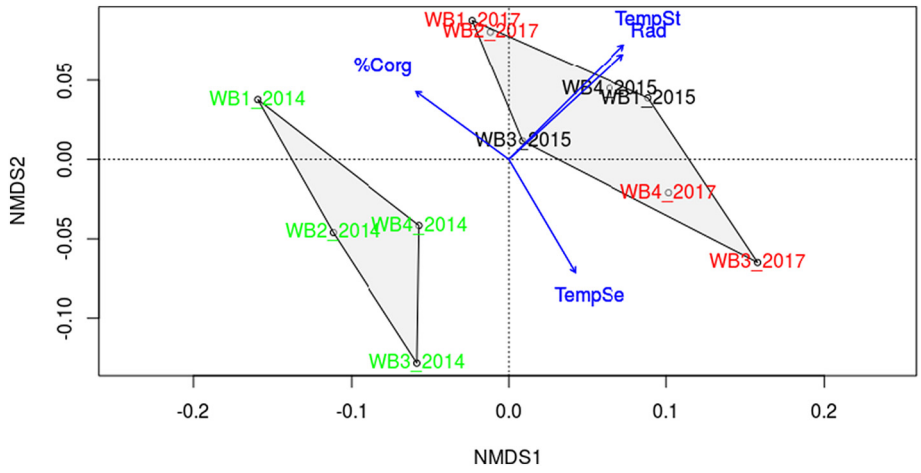

Fig. 6. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance (stress value of 0.05) showing the correlation between the environmental parameters and stress-response genes. Each arrow is significantly correlated to the ordination (envfit, $p < 0.05$) and represents the direction and strength of the environmental parameter. TemSt: Spain station Temperature measure (°C); TempSe: Sediment Temperature (°C); Rad: Spain station solar radiation measure (W/m^2); %Corg: Percentage of organic carbon. Sites: WB1: subglacial; WB2: next to glacier; WB3: central area; WB4: distal area.

WB3 and WB4 showed temperatures of 12 and 9 °C, respectively. The temperature variations in the sampled area may have selected microorganisms with a greater genetic potential for thermal shock response. HSP20 helps denatured proteins not to fold in the wrong way, preventing their irreversible aggregation by linking the N-terminal and C-terminal portions of the proteins and taking them to be correctly folded by the Hsp60 (*groe*L and *groe*S), one of the most abundant HSP mechanisms in WB. ClpE is a protein of the Hsp100 class, induced by heat shock and the substance puromycin, an antibiotic that inhibits protein synthesis (Derré et al., 1999). This protein acts mainly in the degradation of malformed proteins and is produced by sporulating gram-positive bacteria, such as *Bacillus*, *Clostridium* and *Streptomyces*. In this work, *clp*E gene was found in members of the Bacillaceae family, without classification at genus level (Fig. S10). The higher abundance of HSP20 and *clp*E genes in the WB microbiome from the year 2015 suggests a greater functional capability to deal with the malformation and denaturation of proteins. Also, the results indicate a higher abundance of sporulating organisms, a resistance factor found in gram-positive microbes.

Genes *csp*F, *csp*G and *csp*J, belonging to the CSPs family, were more abundant in sediment samples from 2015 compared to 2014. In contrast, *csp*L was significantly enriched in 2017 in comparison with the years 2014 and 2015. There are not many reports on these CSPs in the literature, however, it is known that they show up to 90% similarity with the protein coded by *csp*A (Keto-Timonen et al., 2016). A study with *Lactobacillus plantarum* reported that *csp*J codifies an important CSP compared to other CSPs (coded by *csp*C and *csp*P), being expressed when temperature drops suddenly, and remaining until the logarithmic phase (Derzelle et al., 2003). As already mentioned, the pronounced temperature variation observed in the years 2015 and 2017 could explain the greater abundance of bacteria harboring mechanisms able to cope with temperature variations. In this study, the most abundant taxa affiliated to *csp*F, *csp*G, *csp*L and *csp*J genes could not be classified at family or genus level (Figs. S9 and S10), suggesting a vast group of microbes still unknown that could be further explored and isolated by culturing techniques.

For osmotic stress, in addition to the OmpR family (*qse*C, *cpx*A and *rst*B sensor genes and *bae*S and *rst*A regulator genes), the microbiome of WB sediments from 2014 showed higher abundance of ion channel functions (*yna*I and *msc*S) and potassium efflux pump (*kef*B) in comparison to the year of 2015, which exhibited only members of the OmpR family and the TC.BCT betaine and carnitine transporter (BCCT family). *cpx*A and *yna*I were the most abundant genes in the WB sediment microbiome of 2014 compared to 2015 and 2017. They were affiliated with *Psychrobacter*, the most abundant genus, especially in 2014 (Fig. 4), which harbors the majority of osmotic stress genes (Fig. S8). Genes of the family OmpR, *mtr*A, *mtr*B, *vic*K and *vic*R were enriched in 2015 and 2017 when compared to the year of 2014. The operons *mtr*AB and *vic*KR have similar functions and are more conserved in gram-positive bacteria with low GC content, regulating several responses to extracellular stresses (Takada and Yoshokawa, 2018). In this work, these genes were shown to be more related to the Micrococcaceae and Carnobactereaceae families (Fig. S9 and S10), both gram-positive bacteria. Other genes, like *mpr*A and *pro*P, more abundant in 2017 compared to 2014, were also demonstrated to be mainly related to gram-positive bacteria (Fig. S10). These results suggest that there was a shift in the diversity of gram-positive bacteria along the years. In addition, *arc*B and *kef*F genes, which control cellular respiration at the aerobic level, were more abundant in 2017 compared to 2014. Therefore, *arc*B and *kef*F are not only linked to osmotic stress, but also to the regulation of ROS (Loui et al., 2009; Lyngberg et al., 2011). The year of 2017 showed the highest value of solar radiation (Figs. 2 and 3) and prevalence of oxidative stress functions.

For oxidative stress, 2017 showed more abundant functions compared to 2014, as previously mentioned. The WB sediments from the years 2014 and 2015 had higher concentrations of heavy metals and lower temperatures compared to 2017, which showed higher solar radiation and temperature values (Fig. 3). Heavy metals and solar radiation are the main abiotic factors that lead to the production of ROS in microorganisms. However, only solar radiation had an influence on stress functions (Fig. 6). All genes that are more abundant in 2015 (*nrd*H, *grx*A, *kor*C and *kor*D) are more abundant in the year 2017 and are related to oxidoreductase enzymes that neutralize free radicals. Taxonomic assignment showed that *nrd*H was affiliated with *Arthrobacter* genus of the Micrococcaceae family, the main difference between taxonomic abundances of the year 2014 versus 2017 (Fig. 4). The other genes were affiliated with several families (Figs. S9 and S10). The years of 2015 and 2017 showed a greater richness than 2014, which could explain the greater abundance of these genes. Comparing 2015 and 2017, only two genes were more abundant in 2015 (*hem*Q and *sod*N) and one in 2017 (*lim*B). The enzyme hydrogen peroxide-dependent heme synthase [EC: 1.3.98.5] (*hem*Q) catalyzes the degradation of hydrogen peroxides (H_2O_2), *sod*N in turn is a nickel superoxide dismutase [EC: 1.15.1.1], which catalyzes the dismutation (or partitioning) of the cytotoxic superoxide (O_2^-) radical into ordinary molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) (Barondeau et al., 2004). The higher abundance of these two genes (*hem*Q and *sod*N) in 2015 compared to 2017 might be due to the use of iron and nickel in their reactions (Barondeau et al., 2004; Dayley et al., 2010), found only in gram-

positive bacteria. Sediment samples of 2014 and 2015 showed higher concentrations of heavy metals compared to 2017 (Fig. 3). However, in the NMDS graph (Fig. 6), heavy metals did not show influence on the functions.

Taxonomic affiliation indicated that the *Psychrobacter* genus was the most abundant group in 2014, harboring most of the genes for resistance to osmotic stress (Fig. S8). Although *Psychrobacter* members still predominated in WB sediments in 2015, an increase in the abundance of *Arthrobacter* members was observed (Fig. S9). In 2017, there was a shift in the microbial community profile and *Arthrobacter* became the most abundant genus. Interestingly, nitrogen-fixing microorganisms belonging to the family Comamonadaceae (*Pseudorhodoferax*, *Acidovorax* and *Polaromonas*) were also abundant. In 2015 and 2017, temperatures of sites WB3 and WB4 were similar and higher than those in 2014 (Fig. 1). There was also a higher incidence of solar radiation in 2015 and 2017 (Figs. 3 and 6). *Arthrobacter* spp. are pigmented bacteria, already reported in Antarctica environment (Silva et al., 2018), that are presumably more resistant against solar radiation than *Psychrobacter* members (Rainey et al., 2005; Kumar et al., 2016; Mark Li et al., 2019). In addition, *Psychrobacter* genus showed greater abundance at lower temperatures and close to the ice (Fig. 4 - 2014 WB1, WB2), and a decrease in abundance in the years 2015 and 2017. With higher temperatures, the environment tends to become more friendly for the development of mesophilic microorganisms (> 10 °C), resulting in greater microbial richness in Antarctic sediments. It was also observed an increase of members belonging to *Cyanobacteria* phylum (Fig. 4), that may be involved in the carbon and nitrogen fixation in Antarctic sediments, contributing for the development of other microbial groups (Cary et al., 2010).

4. Conclusions

In this work, results showed that abiotic factors differentially influenced the microbial abundance at WB sites, but did not significantly alter taxonomic composition of the microbiome. As a result, microbial communities carrying stress-related genes, such as HSP20, *clp*E, *csp*L, *mtr*AB and *vic*KR, and oxidoreductases (encoded by *nrd*H, *grx*A, *kor*C and *kor*D genes) showed different profiles in response to environmental variations over the years. Most of these functions were associated to gram-positive bacteria, suggesting that environmental drivers shape a microbial community able to cope with high solar radiation and temperature variations. The temperature also favored the enrichment of Cyanobacteria members, which may support a functional community in such extreme systems by autotrophic carbon and/or nitrogen fixation. With that, we conclude that temperature and solar radiation could potentially be the primary driving forces shaping the genetic repertoire of stress-response genes for the maintenance of the microbial diversity in the WB Antarctic sediments.

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CRediT authorship contribution statement

V.B. Centurion: Conceptualization, Methodology, Software, Validation, Formal analysis, Writing - original draft. **G.V. Lacerda-Júnior:** Methodology, Investigation, Writing - review & editing. **A.W.F. Duarte:** Methodology, Investigation, Resources. **T.R. Silva:** Investigation, Resources. **L.J. Silva:** Investigation, Resources. **L.H. Rosa:** Investigation, Resources, Supervision. **V.M. Oliveira:** Supervision, Project administration, Methodology, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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