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First description of archaeal communities in carbonate-rich seafloor and subseafloor sediments from the Southwestern Atlantic slope

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ABSTRACT

Deep-sea sediments comprise one of the largest habitats on Earth. The archaeal groups contribute to a large fraction of the deep-sea benthic biomass, playing a key role in biogeochemical cycles. However, their diversity in deep-sea benthic ecosystems remains poorly understood, mostly because only recently novel taxa have been proposed, thus remodeling the phylogenetic tree. Despite the difficulty in obtaining cultivated representatives, the metabolic capabilities of archaea have lately been described through metagenomic data, indicating that archaeal taxa are highly versatile. Here, we aimed to reveal the diversity of archaeal communities in surface (0 to 15 cm depth) and subsurface (200 cm depth) sediments from a carbonate-rich region in the Southwestern Atlantic upper slope. We performed 16S rRNA gene sequencing, and found that the archaeal composition in surface sediments was mainly dominated by ammonia-oxidizing archaea within Nitrososphaeria class. The distribution of Nitrososphaeria ASVs (amplicon sequence variants) indicates the presence of several species or ecotypes. Contrastingly, the subsurface sediment was dominated by uncultivated anaerobic and poorly known archaea, including representatives of all supergroups (Asgard, TACK, DPANN and Euryarchaeota). These archaea have been described as having potentially diverse metabolic capabilities, including autotrophic and heterotrophic pathways, such as acetogenesis, methylotrophy, and degradation of labile and recalcitrant organic compounds. This indicates an important role in the remineralization of organic matter in the SW (Southwest) Atlantic slope. They are likely enriched due to the transport and mixing of sediments by the IWBC (Intermediate Western Boundary Current) along the continental slope. However, further studies are needed to reveal the geochemical and oceanographic drivers of the archaeal distribution. This study provides the first description of the archaeal communities in carbonate-rich sediments in the SW Atlantic slope, and adds new biodiversity insights to this geological feature, which is considered a vulnerable marine ecosystem, thus helping for future conservation strategies.

Descriptors: Archaeal diversity, Surface and subsurface sediments, Deep sea, Southwestern Atlantic slope.

INTRODUCTION

Marine sediments cover 70% of the Earth's surface and harbor one of the largest microbial habitats on Earth. In deep-sea sediments, Bacteria and Archaea represent around 90% of the total benthic biomass and have a key role in nutrient cycles and remineralization of organic matter (Varliero et al., 2019). Firstly, microbial communities in deep-sea sediments were thought to be largely heterotrophic, being supported by sporadic influxes of nutrients from the overlying water column (Lauro and Bartlett, 2008). However, several recent studies have shown that these

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deep-sea benthic communities are dominated by metabolically diverse microbial groups, including chemoautotrophs, capable of surviving under high hydrostatic pressure and low temperatures (e.g., Corinaldesi, 2015; Oni et al., 2015; Hoshino et al., 2020). Within these microbial communities, archaeal groups contribute to a large fraction of the biomass, and their abundance is similar to or even higher than that of bacteria. Hoshino and Inagaki (2019) performed a recent study that estimated that archaeal cells constitute 37.3% of all cells found in marine sediments, with higher percentages in ocean-margin sediments than in open-ocean sediments.

Despite the high abundance of archaea in marine sediments, their diversity remains little known, since only recently, the development of shotgun metagenomics and bioinformatic tools allowed the proposal of novel archaeal taxa, thus remodeling the phylogenetic tree and improving the taxonomic database (Dombrowski et al., 2019; Baker et al., 2020). To date, at least four supergroups have been proposed within the archaeal domain: “TACK” (Thaumarchaeota, Aigarchaeota, Crenarchaeota, Korarchaeota), “Asgard” (Lokiarchaeota, Odinarchaeota, Thorarchaeota, and Heimdalarchaeota), “DPANN” (Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota, Nanohaloarchaeota, Pacearchaeota, Woesearchaeota, and Micrarchaeota) and Euryarchaeota (Thermoplasmata, among others) (Tahon et al., 2021).

The difficulty in obtaining cultivated representatives in the laboratory makes the description of archaeal metabolisms a tremendous challenge (Li, Yuting et al., 2019). By the reconstruction of archaeal genomes from metagenomic data and single-cell genomics studies, the metabolic potentials of archaea in deep-sea ecosystems have shown to be highly versatile, with members involved with the methane metabolism (methanogenesis and anaerobic methane oxidation) and nitrogen metabolism, mostly by oxidation of ammonia to nitrite performed by Nitrososphaeria members (Offre et al., 2013; Baker et al., 2020). Further, many archaeal taxa can fix carbon from inorganic sources, having a key role in chemosynthetic processes in the deep ocean in the last millions of years (Vuillemin et al., 2019).

One of the main factors influencing the distribution of archaea in the deep sea is the oxidation level of the sediments, where some taxa and ecotypes may be more adapted to oxic, anoxic or low-oxygen (oxic–anoxic) environments (Hoshino and Inagaki, 2019). Surface sediments (~up to 10 to 15 cm depth) are often oxygenated and thus select aerobic taxa, being dominated by ammonia oxidizer Nitrososphaeria members, while anoxic subsurface sediments are dominated by diverse anaerobic groups, such as those within Bathyarchaeia, Lokiarchaeia, and Euryarchaeota, most of them with not yet cultivated lineages (Baker et al., 2020; Hoshino et al., 2020). Generally, the abundance of Archaea in marine sediments decreases with increasing depth, but at lower rates than Bacteria (Hoshino et al., 2020).

Although microbial diversity in deep-sea sediments has been described in recent years, few studies have focused on the diversity and vertical distribution of archaeal communities and their ecotypes. Efforts to describe archaeal diversity have been performed, for example, in the subsurface sediments from the South China Sea (Yu et al., 2017; Li, Yuting et al., 2019), in the mid-ocean ridge flanks of the North Atlantic Ocean, in the Pacific abyssal plain and Mohns Ridge (Norwegian Sea) (Kerou et al., 2021), as well as in the Eastern Indian Ocean (Wang et al., 2017), and the active venting site Loki’s Castle (Arctic Mid-Ocean Ridge) (Spang et al., 2015). Despite the ecological and evolutionary importance of marine archaea, as demonstrated in these previous studies, their diversity in sediments from the deep South Atlantic Ocean remains poorly understood. This study reports the first description of the archaeome in surface and subsurface sediments from the Southwestern (SW) Atlantic slope.

The Santos marginal basin (SW Atlantic) harbors diverse geological features, including pockmark fields (Ramos et al., 2020), carbonate-rich sediments, and carbonate ridges, such as the recently described Alpha Crucis Carbonate Ridge (ACCR) (Maly et al., 2019). These environments are considered vulnerable marine ecosystems that urge a better understanding for future conservation strategies. This study aimed to reveal the diversity of archaeal communities in surface (0 to 15 cm depth) and subsurface (200 cm depth) sediments of a carbonate-rich region in the deep SW Atlantic Ocean. Using high-throughput Illumina sequencing targeting the 16S rRNA gene, we found a high number of Nitrososphaeria ASVs (amplicon sequence variants) in surface sediments. Their vertical distribution probably indicates species or ecotypes which are ubiquitous or colonizers of upper or lower sediment strata. Contrastingly, the subsurface sediment lacks members of Nitrososphaeria, being dominated by different anaerobic and uncultivated taxa, including representatives of all archaeal supergroups (Asgard, TACK, DPANN, and Euryarchaeota). These archaea are known to potentially participate in marine nitrogen and carbon cycles, including carbon fixation, methane metabolism, and degrading label and recalcitrant organic compounds by heterotrophic pathways, likely playing an important role in the remineralization of the organic matter in the deep sea.

METHODS

Study area and sampling strategy

The study area is located between the 700 and 1000 m isobaths in the central portion of the Santos Basin, southeastern Brazilian margin (Figure 1A). The area is influenced and shaped by the bottom currents system of Brazil Current (BC) and the Intermediate Western Boundary Current (IWBC) with opposite flow (Soutelino et al., 2013; Biló et al.,2014; Maly et al., 2019) (Figure 1B). The area is adjacent to the large carbonate mound province The Alpha Crucis Carbonate Ridge (AACR) (Maly et al., 2019) and presents a seafloor with carbonate composition. A study in the area shows that the region has a high concentration of nutrients through the metal ratios of the corals that compose the bottom (Trevizani et al., 2022). This study is part of a multidisciplinary project called "BIOIL" financed by Shell Brazil. The description of the cruise onboard RV Alpha Crucis is detailed in Sumida et al. (2022).

Surface sediment samples (0 to 15 cm) from stations P681, P683 and P684 (Figure 1C) were collected using an Ocean Instruments® box corer (50 cm x 50 cm x 50 cm). The overlying water was drained before the box corer opening, and then corers (7 x 20 cm) were collected, extruded, and subsampled in the following strata: 0-5, 5-10, and 10-15 cm. Subsurface sediments were collected at station P684 using a gravity-corer sampler (3" diameter, 3 m long), and the 200th-cm deep layer was selected for microbiological analysis. Surface (n=18) and subsurface (n=1) sediment samples were stored in Whirl-pack bags at -80°C until arrival at the Oceanographic Institute, University of Sao Paulo, Brazil, in November 2019. Details of each sample, including strata, coordinates and local depth, are described in Supplementary Table 1.

Characterization of water masses

The characterization of water masses in the study area was performed by measuring temperature and salinity *in situ* using a CTD-Rosette onboard the R/V Alpha Crucis system. Further, nutrient analyses of three water depths from stations P681, P683, and P684 were performed, including nitrate and nitrite (SEAL Analytical AutoAnalyzer II), phosphate, silicate, and ammonium (Thermo Scientific Spectrophotometer Evolution 200) measurements. Nutrient analyses were performed in the "Laboratório de Nutrientes, Micronutrientes e Traços no Mar" at the Oceanographic Institute of the University of São Paulo (São Paulo, Brazil).

Physical-chemical analyses of sediments

Sediments were analyzed according to their grain size, calcium carbonate ($CaCO_3$) content, total organic carbon (TOC), total nitrogen, and $\delta^{13}C/^{12}C$. The strata from 0 cm to approximately 28 cm (depending on each sample) were analyzed. Grain size analysis was performed according to Ramos et al. (2020). For calcium carbonate content, we performed a gravimetric analysis by the weight difference before and after the acidification of each sample with 2M HCl. Total organic carbon (TOC), total nitrogen, and $\delta^{13}C$ (reported in ‰ PDB) analyses were performed with a Costec elemental analyzer (EA) coupled to an isotopic ratio mass spectrometer (IRMS) (Thermo Scientific Delta Advantage) after the complete elimination of calcium carbonate of the samples with 2M HCl.

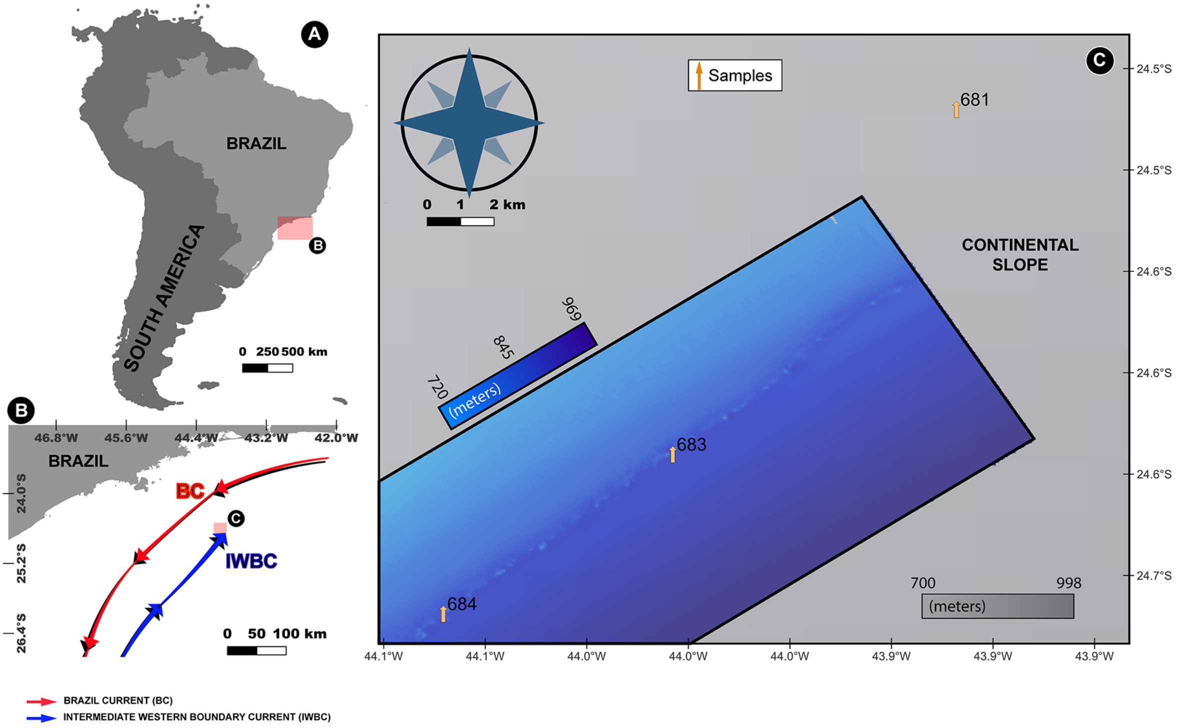

Figure 1. Sampling map describing the location of the sites P681, P683, and P684. A: general location according to the Brazilian Southeast coast; B: detail of the local influence of the oceanographic currents (IWBC and BC); C: location of sampling sites according to bathymetry (in blue shades). The station P681 is located in a region which does not has bathymetry measurements.

DNA EXTRACTION AND 16S RRNA GENE SEQUENCING

DNA extraction of sediments (0.3 g per sample) was performed using the DNEasy PowerSoil Kit (Qiagen, Hilden, Germany), following manufacturing protocols. Total DNA was then quantified using Qubit dsDNA HS Assay (Thermo-Fisher Scientific, Waltham, U.S.A.) and Qubit Fluorometer 1.0 (Thermo-Fisher Scientific, Waltham, U.S.A.). The sequencing of the 16S rRNA gene was carried out through Illumina Miseq paired-end system 2 x 300 bp, with the primers 515F-926R (Parada et al., 2016), targeting the V3–V4 regions of the gene. Sequencing was performed by ZymoBIOMICS® Service (Irvine, U.S.A.).

BIOINFORMATIC ANALYSES

After sequencing, raw reads were imported into QIIME 2 (v.2020.2, https://docs.qiime2.org/) (Bolyen et al., 2019) using the q2-tools-import script. DADA2 software was used to obtain a set of observed sequence variants (SVs) (Callahan et al., 2017). Based on the quality scores, the forward reads were truncated at position 270 and the reverse reads at 200, using the q2-dada2-denoise script. Primer sequences were removed using the same script. Taxonomy was assigned through feature-classifier classify-sklearn and SILVA database v138. The phylogenetic tree was built by FastTree (Price et al., 2009) and the MAFFT aligner (Katoh et al., 2002).

QIIME 2 outputs were imported in R (version 4.2.0) using the package qiime2R (https://github.com/jbisanz/qiime2R). Archaeal taxonomic groups were filtered using the phyloseq package (McMurdie and Holmes, 2012), and then alpha and beta diversity metrics at a rarefied sampling depth of 16,443 sequences were calculated in phyloseq. Beta diversity was measured by weighted Unifrac distance and visualized via PcoA (Principal Coordinate Analysis). Differences in the archaeal community structure among sediment strata were tested by performing a permutational multivariate analysis of variance (PERMANOVA) on the community matrix (Anderson, 2001). Sequencing data were deposited in the National Center for Biotechnology Information Sequence Read Archives (SRA) under BioProject ID PRJNA843713.

RESULTS

CHARACTERIZATION OF WATER MASSES IN THE STUDY AREA

Seawater temperature ranged from 4.6°C to 24.5 °C, and salinity, from 34.3 to 37.2 psu, both decreasing with depth. Phosphate and nitrate presented their minimum concentrations (0.05 µmol L^{-1}, 0.36 µmol L^{-1}, respectively) at the surface of station P681 and their maximum concentrations (1.92 µmol L^{-1}, 27.51 µmol L^{-1}, respectively) at the most profound depth of station P684. Silicate concentrations ranged from 0.69 µmol L^{-1} at the surface of station P681 to 20.25 µmol L^{-1} at the greatest depth of station P684. Nitrite concentrations varied from 0.01 µmol L^{-1} at the surface of station P683 to 0.04 µmol L^{-1} at the intermediate depth of station P684. Ammonium concentrations ranged from 0.05 µmol L^{-1} at the surface of station P684 to 0.28 µmol L^{-1} at the intermediate depth of station P684. The results of the physical-chemical characteristics of seawater are detailed in Supplementary Table 2.

PHYSICAL-CHEMICAL CHARACTERIZATION OF SEDIMENTS

The grain size analysis of the sediments (0-28 cm, varying according to each sampling site) resulted in prevalent mud sediments among all analyzed samples (Supplementary Table 3). The chemical analyses resulted in a $CaCO_3$ content varying between 13.10% at P681 (0-2 cm) to 7.37% at P683 (24-26 cm). Generally, TOC was lower in the deepest strata, varying between 0.73% at P683 (0-2 cm) to 0.15% at P684 (8-10 cm), as well as total nitrogen, which varied from 0.09% at P684 (2-4 cm) to 0.01% at P681 (16-18 cm) and P683 (8-10 cm and 24-26 cm). Some samples were below the detection level for TOC and total nitrogen. $\delta^{13}C/^{12}C$ varied from -22.15‰ at P681 (12-14 cm) to -20.68‰ at P81 (4-6 cm).

ALPHA AND BETA-DIVERSITY

We obtained 571,650 reads and 32,912 ASVs distributed among 19 samples. A mean of 852.36 ASVs (SD ± 201.14) was detected in sediment samples. For total communities, richness values measured by Chao1 varied between 1061.37 to 595.80 among surface sediments and was 192.50 for the subsurface sediment sample. Alpha diversity using the Shannon index varied from 6.16 to 5.51 for surface sediments and was 4.88 for the subsurface sediment. By considering only the surface microbial communities, the ANOVA result was significant by strata for Chao1 (p=0.00778) but was no significant for Shannon (p=0.163).

When considering only archaeal communities, surface sediments exhibited a number of ASVs varying from 99 to 148, Chao1 from 99.33 to 151.47, and Shannon from 3.60 to 4.07. Subsurface sediment values for archaeal diversity were 32 for the number of ASVs, 32.00 for Chao1, and 3.29 for Shannon. The values of alpha diversity indices are described in Supplementary Table 4. The ANOVA result considering the surface archaeal communities exhibited the same pattern for the total communities, where only Chao1 was significant (p= 0.0436) according to the strata.

The weighted Unifrac distance observed in PcoA (Figure 2) showed a clear pattern of sample distribution according to sediment layers by considering both the total community and the archaeal community. Sediment samples of 0-5 cm harbored similar communities in all stations, and a transition of community similarity from 5-10 cm to 10-15 cm strata was clearly observed. The subsuperficial sediment sample (200 cm stratum) was distantly distributed from all surface strata (0-15 cm). Based on PERMANOVA, the total community structure was significantly influenced by depth (R^2=0.52, p=0.001), as was the archaeal community structure (R^2=0.65, p=0.001).

TOTAL COMMUNITY COMPOSITION

The most abundant phyla in surface sediments were Crenarchaeota, Proteobacteria, Acidobacteriota, Planctomycetota, Methylomirabilota, NB1-j, Bacteroidota, Nitrospirota, Gemmatimonadota, and Chloroflexi (Figure 3). For subsurface

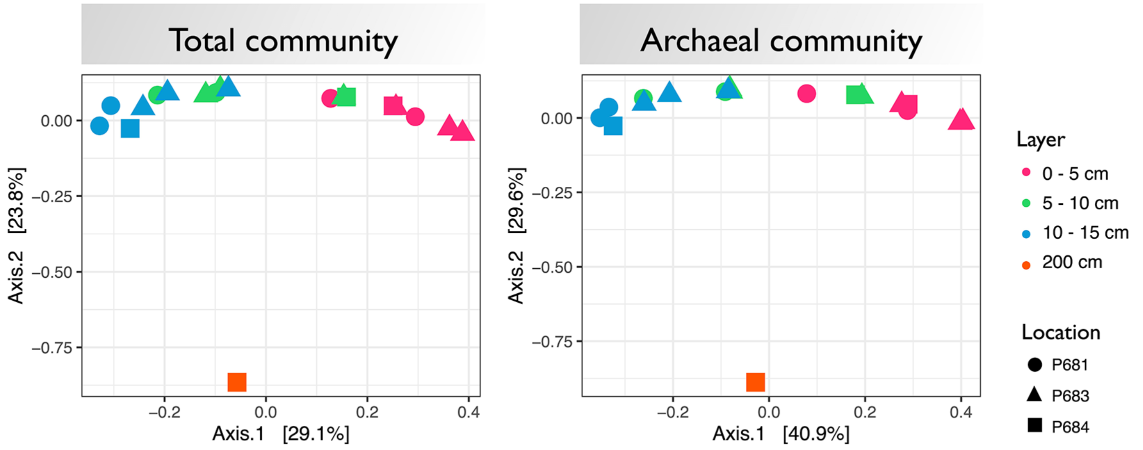

Figure 2. PcoA (Principal Coordinate Analysis) ordination based on weighted UNIFRAC distances for the entire and archaeal communities. Colors are classified according to the sediment strata (layer). Total community PC1: 29.1%, PC2: 23.8%; Archaeal community PC1 40.9%, PC2 29.6%.

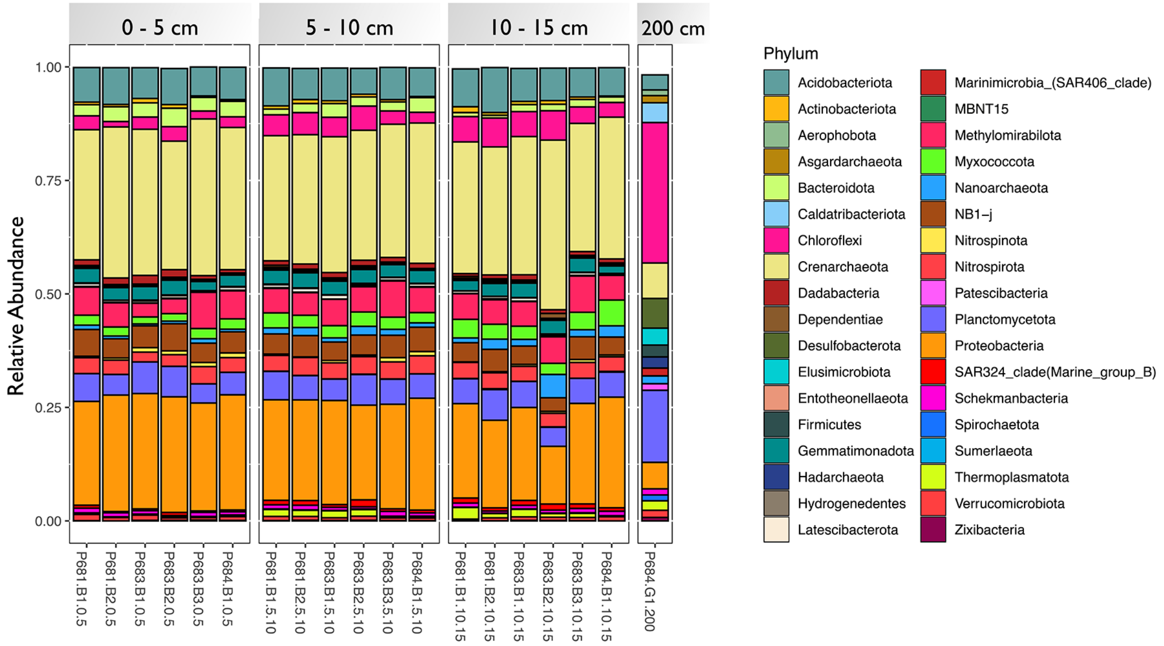

Figure 3. Microbial community composition in surface and subsurface sediment samples from the Southwestern Atlantic Ocean. The figure shows the relative abundance of bacterial and archaeal taxonomic groups at the phylum level. Samples are grouped according to sediment strata. Only phyla with more than 0.1% of abundance are represented. Sequences were taxonomically classified using the Silva database v. 138.

sediment, the most abundant phyla were Chloroflexi, Planctomycetota, Crenarchaeota, Desulfobacterota, Proteobacteria, Caldatribacteriota, Elusimicrobiota, Acidobacteriota, Firmicutes, Hadarchaeota, Thermoplasmatota, Nanoarchaeota, Marinimicrobia (SAR406_clade), Verrucomicrobiota and Asgardarchaeota. The relative abundances and standard deviations for each phylum per sediment stratum (layer) are detailed in Supplementary Table 5.

ARCHAEAL COMMUNITY: TAXONOMIC COMPOSITION AND PHYLOGENY

Looking at phylum levels within archaeal communities, the 0-5 cm deep sediments showed Crenarchaeota and Nanoarchaeota as the most abundant. These phyla were also abundant in sediments from 5 to 15 cm, with the addition of Thermoplasmatota (Figure 4 and Supplementary Figure 1). All ASVs related to Crenarchaeota in 0 to 15 cm depth sediments were affiliated with Nitrososphaeria class. Contrastingly, the most abundant phyla in subsurface sediments (200 cm) were Crenarchaeota assigned within Bathyarchaeia class, Asgardarchaeota, Hadarchaeota, Nanoarchaeota, and Thermoplasmatota. The relative abundances and standard deviations for each archaeal phyla per sediment layer are detailed in Supplementary Table 6.

Phylogenetic relationships among archaeal taxa were analyzed through 16S rRNA gene and by grouping ASVs at order level, selecting those with >0.001% relative abundance. The selected orders belonged to 8 archaeal phyla and showed different pattern distributions according to the sediment strata (Figure 5A). Briefly, the phylum with more branches in the phylogenetic tree was Crenarchaoeota (Bathyarchaeia, Marine Benthic Group A, Nitrosopumilales, Nitrososphaerales, and uncultured order), followed by Thermoplasmatota (Methanomassiliicoccales, SG8-5, Marine Benthic Group D and DHVEG-1, and uncultured order), Nanoarchaeota (Woesearchaeales) and Aenigmarchaeota (Aenigmarchaeales and DSEG). Other phyla were represented by only one branch, such as Hadarchaeota, Asgardarchaeota, and Micrarchaeota.

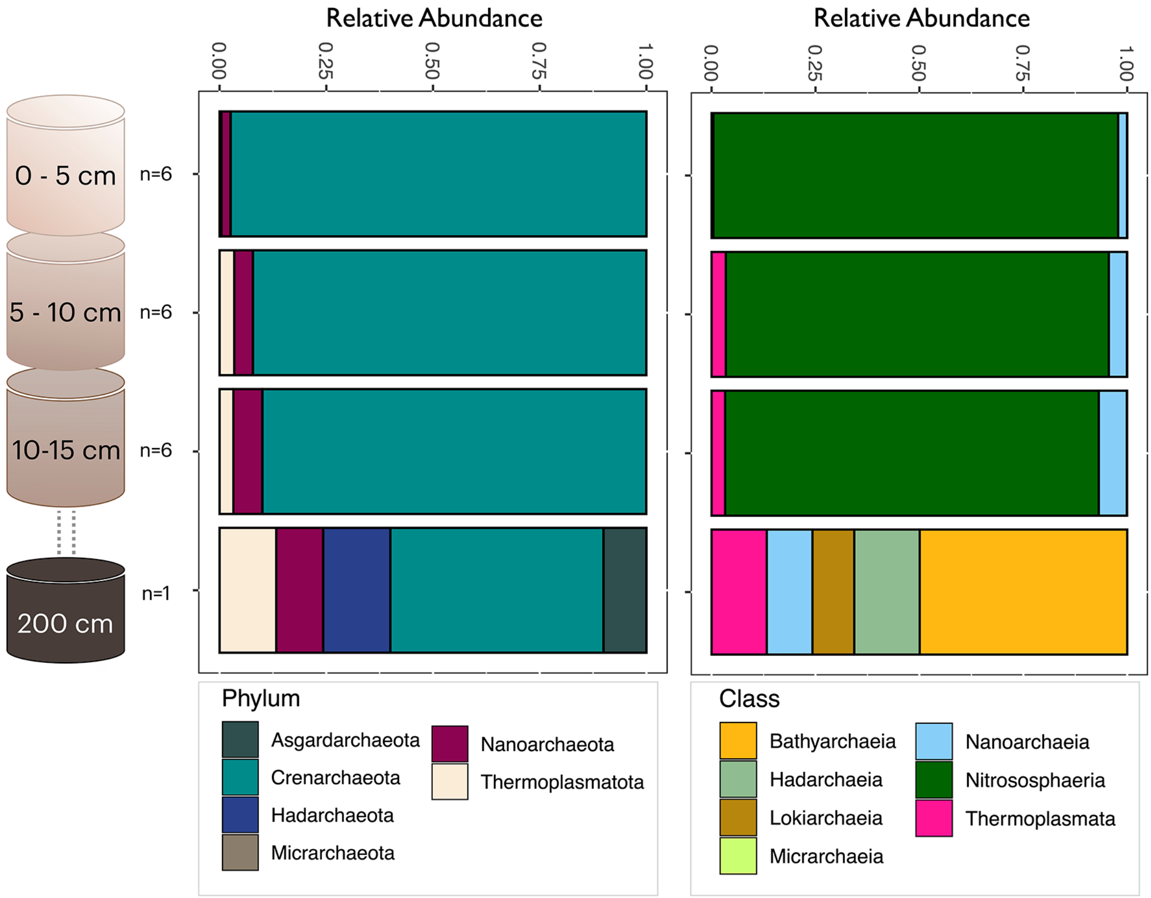

Figure 4. Archaeal community composition in surface and subsurface sediment samples from the Southwestern Atlantic Ocean. The figure shows the relative abundance of archaeal taxonomic groups at phylum and class levels. Samples are grouped according to sediment strata. Sequences were taxonomically classified using the Silva database v. 138.

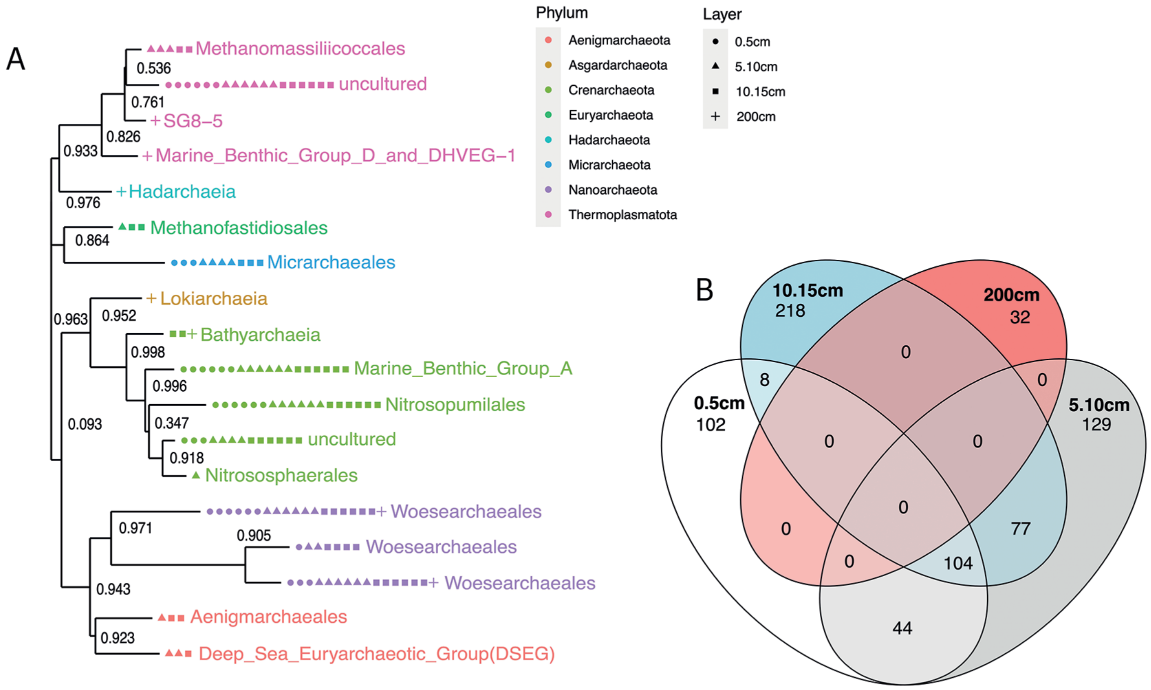

Figure 5. (A) Phylogenetic tree of the archaeal taxa through 16S rRNA gene, grouped at the order level (>0.001%), and the contribution of samples by sediment stratum (layer). Orders are colored according to their respective phyla. Taxonomic groups which were not classified at the order level are represented at the class level. The numbers on the nodes are bootstrap values. (B) Venn diagram showing the number of shared archaeal ASVs along the sediment strata (layers).

No archaeal ASV was shared between surface strata and subsurface stratum (Figure 5B). The 0-5 cm samples shared 148 ASVs with 5-10 cm stratum and 112 ASVs with 10-15 cm stratum. Samples from the 5-10 cm and 10-15 cm layers shared 181 ASVs.

To deeply explore archaeal taxa, we selected the 120 most abundant ASVs to understand their distribution among the surface and subsurface sediments. We observed that most of the highly abundant ASVs were classified within Nitrosopumilaceae (Figure 6), which exhibited different patterns of distribution, with some ASVs preferably present in the upper strata (0-10 cm) (e.g., ASV1615, ASV1700, and ASV1948), others more prevalent in the 10-15 cm stratum (e.g., ASV1480, ASV1621, and ASV1720), and ASVs highly distributed among all surface sediments (0 to 15 cm) (e.g., ASV1542, ASV1547 and ASV1701). Woesearchaeles (Nanoarchaeota phylum) showed ASVs prevalent among all surface sediments (0 to 15 cm), and others present only in 5 to 15 cm strata (ASV919) or only in the subsurface sediment (200cm) (ASV806 and ASV1081). Marine Benthic Group A (Nitrososphaeria class) ASV was found in all samples from 0 to 15 cm, whereas SCGC-AA011-d5 (Nanoarchaeota phylum) ASVs were present from 5 to 15 cm strata. Subsurface sediment did not exhibit ASVs belonging to Nitrosopumilaceae, SCGC-AA011-d5, and Marine Benthic Group A. The prevalent ASVs in the subsurface were related to Bathyarchaeia, Hadarchaeales, Lokiarchaeia, Marine Benthic Group D (Thermoplasmatota phylum), Woesearchaeles, and SG8-5 (Euryarchaeota).

DISCUSSION

ARCHAEAL COMMUNITY IN SURFACE SEDIMENTS

This study provided the first survey describing archaeal communities in surface and subsurface sediments from the SW Atlantic Ocean margin. The values of richness showed that both total and archaeal communities varied significantly according

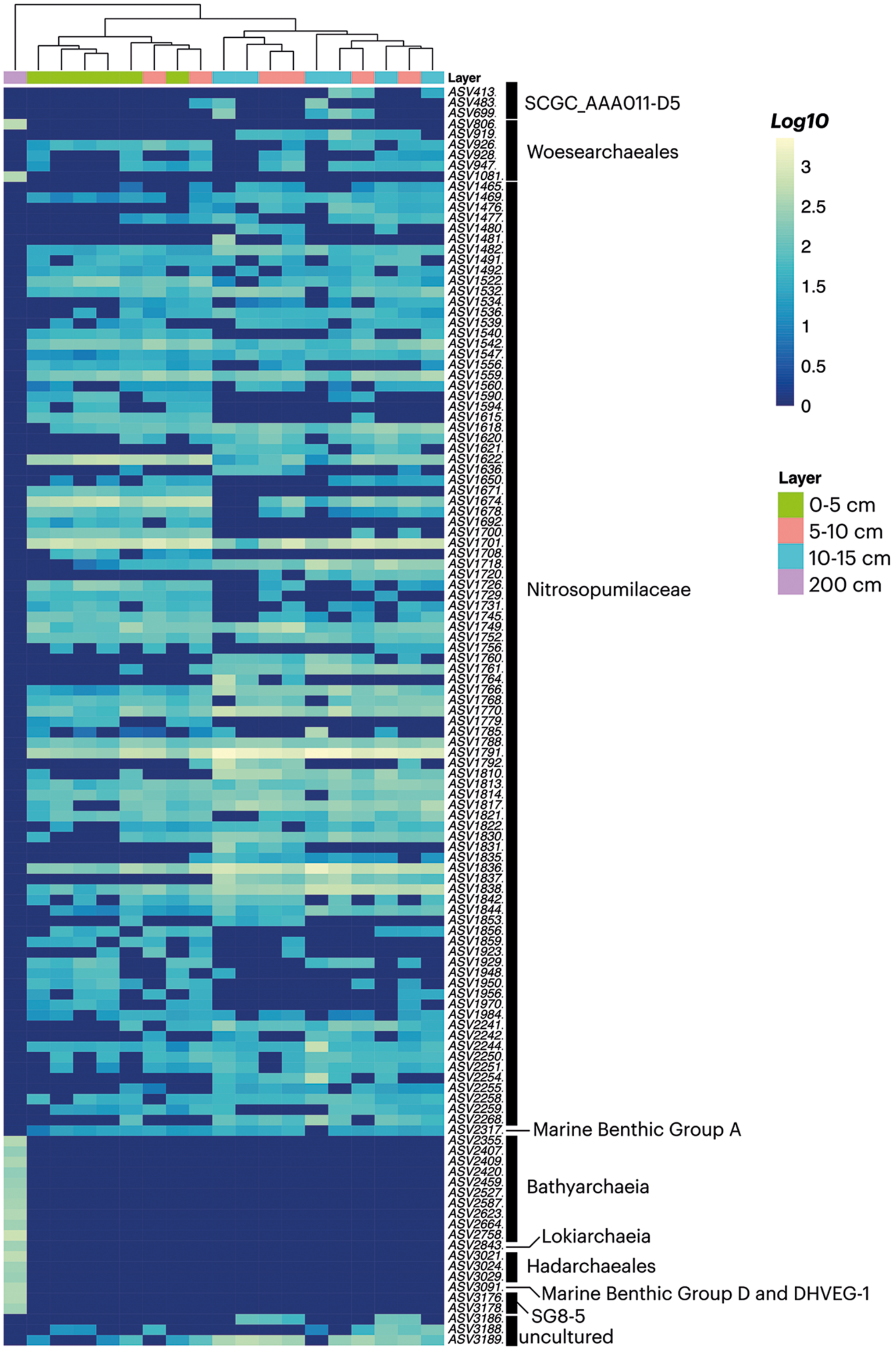

Figure 6. Heatmap showing the log 10 transformed relative abundance of the 120 most abundant OTUs. Samples are clustered and colored according to sediment strata (layer). Taxonomic names are related to the deepest classification according to Silva database v. 138.

to sediment depth, what was not true for the alpha diversity index measured by Shannon. In general, the most superficial layer (0-5 cm) was less rich than 5-10 cm and 10-15 cm layers. Regarding the archaeal community composition, we found that it was significantly correlated with sediment depth, with ASVs assigned within Nitrososphaeria being dominant in surface sediments (0 to 15 cm) and absent in subsurface sediments. Nitrososphaeria encompasses ammonia oxidizing chemoautotrophic members and is thought to be the most numerous pelagic living organisms in the dark ocean (Zhong et al., 2020; Lin et al., 2021). Recent studies have also been detected Nitrosphaeria members (formerly classified within the Thaumarchaeota phylum) in deep-sea surface sediments, such as in the western flank of the Mid-Atlantic Ridge (Zhao et al., 2019, 2020) and hadopelagic sediments from Ogasawara Trench (Nunoura et al., 2013). As in our results, these studies identified Nitrososphaeria decreasing with sediment depths. Nitrososphaeria members have an important role in transforming ammonia in overlying sediments from the sediment-water interface, producing nitrate and thus creating a downward flux into the underlying oceanic crust (Zhao et al., 2019).

Our results showed that several Nitrososphaeria ASVs presented different pattern distributions along the surface sediment strata, with some being prevalent in upper (0-10 cm) or lower layers (10-15 cm) and others ubiquitously distributed among surface layers (0-15 cm). This pattern suggests a distinct ecophysiological potential to colonize sedimentary environments. Previous studies have shown that different Nitrososphaeria clades likely harbor single or multiple phylotypes and ecotypes, each adapted to specific environmental conditions, such as oxygen concentration, water or sediment depth, nutrient availability, and pressure (Zhao et al., 2020; Kerou et al., 2021). Some adaptation mechanisms within Nitrososphaeria clades are thought to be obtained through horizontal gene transfer (HGT), as the genes encoding the putative di-myo-inositol-phosphate (DIP) synthases, which might contribute to the success of hadal clades under extreme conditions (Zhao et al., 2020).

While absent in subsurface, the sediment strata between 5 to 15 cm have also presented Methanomassiliicoccales members (Thermoplasmatota), which are host-associated or free-living methanogens (Borrel et al., 2020). Methanogenic archaea can use substrates produced by fermentation (H2, CO_2, acetate, or methylated compounds) or geological processes (H_2, CO_2, CO) to produce methane (Cozannet et al., 2021). The order Methanomassiliicoccales has been reported to perform methyl-dependent hydrogenotrophic methanogenesis, reducing methyl compounds with H_2 as electron donor (Kröninger et al., 2016). Contrasting with acetoclastic and hydrogenotrophic methanogens, inhibited thermodynamically by sulfate reduction, methylotrophic methanogens can compete with sulfate reducers for methylated substrates and colonize upper marine sediment layers (Zhuang et al., 2018). Indeed, the presence of Methanomassiliicoccales ASVs in surface strata (5-15 cm) might indicate their adaptation in colonizing the sulfate reduction zone. The methylotrophic methanogenesis in the surface sediment could be a source of methane to the water column, thus influencing the benthic methane budgets in the ocean (Chronopoulou et al., 2017). Since marine sediments are the most significant global methane reservoir, marine methanogenic and methanotrophic microorganisms have an important role in balancing the global methane emission and sink (Milkov, 2004).

ARCHAEAL COMMUNITY IN SUBSURFACE SEDIMENTS

The subsurface archaeal communities showed to be less rich and diverse when compared to surface communities, as previous studies have demonstrated (e.g., Vuillemin et al., 2019; Varliero et al., 2019; Ramírez et al., 2020). Subsurface taxa were absent in the surface strata and comprised the uncultivated classes Bathyarchaeia, Hadarchaeia, Lokiarchaeia, Micrarchaeia, Nanoarchaeia, and Thermoplasmata (SG8-5 and Marine Benthic Group D and DVHEG orders). Bathyarchaeia is an uncultured class that belongs to the Crenarchaeota phylum (TACK supergroup) and was the most abundant archaea in our subsurface sediment sample. This class encompasses phylogenetically diverse and widely distributed lineages, often in high abundance in anoxic marine sediments, from shallow regions (Zou et al., 2020; Romano et al., 2021) to the deep sea (He et al., 2016; Ramírez et al., 2020). Bathyarchaeia might be involved with CO_2 fixation, and recent findings showed that some lineages contain genes related to the methanogenesis pathway, being the first evidence of the presence of *mcr* genes outside the Euryarchaeota (Evans et al., 2015). However, the role of Bathyarchaeia in the methane cycle remains unclear. More recent findings have shown that Mcr proteins might be involved with butane oxidation or other short-chain hydrocarbons oxidation (Baker et al., 2020). Bathyarchaeia lineages are thought to have a critical role in degrading recalcitrant organic matter (Yu et al., 2017), and some lineages have shown to be active in subsurface marine sediments, such as those potentially performing acetogenesis, which indicates that this archaeal group has adopted a versatile life strategy to thrive under energy-limiting conditions (He et al., 2016; Baker et al., 2020). Further, recycling fermentation products (CO_2, hydrogen) through acetogenesis may provide an energetic advantage over classical fermenters in the anoxic sediments (Ramírez et al., 2020). The abundance of Bathyarchaeia in our SW Atlantic subsurface sediments may suggest an important role in the remineralization of the subseafloor organic matter, thus needing further investigation to elucidate the niche differentiation patterns and ecological functions of the extant subgroups.

As in our results, previous studies have shown that Bathyarchaeia in subsurface marine sediments often co-occurs with other archaeal taxa, such as those within Hadarchaeia and Lokiarchaeia (e.g., (Li, Yingjie et al., 2019; Ramírez et al., 2020; Kong et al., 2021). Lokiarchaeia belongs to the recently discovered Asgard superphylum, closely linked to the emergence of eukaryotes (Spang et al., 2015; Zaremba-Niedzwiedzka et al., 2017). Lokiarchaeotal subgroups vary in their metabolic capabilities. Some are thought to be homoacetogens. Others can syntrophically grow with methanogens or sulfate-reducing bacteria while degrading amino acids (Imachi et al., 2020) or even can use inorganic carbon through the tetrahydromethanopterin-dependent Wood-Ljungdahl pathway (Orsi et al., 2020). These diverse metabolic potentials suggest the high ecological plasticity of Lokiarchaeia members, with distinct subgroups specialized in degrading different classes of organic compounds (Yin et al., 2021). Due to these variations of metabolic potentials, further studies are needed to elucidate which subgroups are inhabiting the Southwestern Atlantic subsuperficial seafloor and their phylogenetic and evolutionary relationships with groups from other regions.

Another archaeal group detected only in the SW Atlantic subsurface was Hadarchaeia (formerly Hadesarchaea), originally described as the South-African Gold Mine Miscellaneous Euryarchaeal Group (SAGMEG), being found in a broad range of anoxic subsurface environments. Hadarchaeia is phylogenetically related to Euryarchaeota and encompasses versatile anaerobic heterotrophic lineages, potentially coupling CO and H_2 oxidation with nitrite reduction to ammonia (Baker et al., 2016). They potentially perform carbon fixation due to central carbon metabolic genes found in methanogens and are being described as prominent members of the deep dark subsurface biosphere (Baker et al., 2016, 2020).

The order Woesearchaeales (Nanoarchaeota phylum from the monophyletic DPANN superphylum) was the unique archaeal group present in both surface and subsurface sediments, although the ASVs belonging to this group were distinct among the sediment strata. Nanoarchaeota members were initially thought to be restricted to extreme acidic and hydrothermal environments. However, we now know that they are ubiquitously found in nature (Liu, Li, et al., 2018). Based on metabolic predictions, Woesearchaeales members might be able to associate carbon and hydrogen metabolism with symbiotic or fermentation-based lifestyles under anoxic conditions (Castelle et al., 2015). However, their distribution, biodiversity, and metabolism group remain largely unknown. Liu et al. (2021) suggested that Woesearchaeales members often occur together with methanogenic archaea, indicating a syntrophic relationship, where Woesearchaeales might produce H_2/CO_2 and acetate, which can be used by hydrogenotrophic and acetotrophic methanogens, respectively. In turn, Woesearchaeales may receive amino acids and other compounds to compensate for their metabolic deficiencies due to their reduced genomes typical of the groups within DPANN (Liu et al., 2021). Despite Woesearchaeales being previously detected in distinct ecosystems (e.g., Castelle et al., 2015; Liu, Pan, et al., 2018), they might not be ubiquitously distributed among the marine sediments (Hoshino et al., 2020; Ramírez et al., 2020). Thus, revealing their environmental drivers is crucial to understanding their distribution and ecological roles. We observed that our Woesearchaeales ASVs exhibited different distribution patterns, with some detected only from 5-15 cm sediments, others among all surface sediments (0-15 cm), and others only in the subsurface. These patterns indicate that these ASVs might belong to different subgroups harboring distinct lifestyles, and further studies are needed to understand the ecological roles of Woesearchaeales (and other DPANN members) in the biogeochemical cycles from the deep SW Atlantic.

Although Thermoplasmata was detected in all surface and subsurface samples, the dominant groups within this phylum in subsurface were Marine Benthic Group D and DHVEG-1, and SG8-5, contrasting with surface layers where Methanomassiliicoccales were prevalent. Marine Benthic Group D (MBG-D) is being described as cosmopolitans in deep-sea subsurface sediments, together with Bathyarchaeia, potentially contributing to the benthic marine biogeochemical cycles (Zhou et al., 2019). They are thought to be benthic anaerobic archaea capable of exogenous protein mineralization and acetogenesis (Lazar et al., 2017). Significant non-random association of MBG-D with Lokiarchaeia and Hadarchaeia has been demonstrated through co-occurrence analyses, suggesting a niche overlap or potential interactions among these archaeal groups (Zhou et al., 2019). Thus, the occurrence of MBG-D and Lokiarchaeia in our subsurface sediment reinforces previous suggestions about their potential synergistic roles for carbon remineralization and production of labile compounds, which other microorganisms can use in the marine subsurface (Zhou et al., 2019; Baker et al., 2020).

COMPARISON WITH OTHER DEEP-SEA BENTHIC ARCHAEOMES

The gap in studies regarding archaeal diversity in the South Atlantic Ocean makes it challenging to compare with the benthic microbiomes within the region. Recently, Bendia et al. (2021) described the microbiome associated with the Alpha Crucis Carbonate Ridge (SW Atlantic), located near our sampling sites. Despite the geographical proximity, they found a different microbial composition in surface and subsurface sediments, including a significantly low abundance of Bathyarchaeia in the subsurface sediment. A study performed in the Southeast Atlantic has also described a different pattern in archaeal composition, where Nitrososphaeria relatives were abundant both on surface and subsurface, and Woesearchaeles only in the subsurface samples, contrasting with the vertical archaeal distribution found in our study (Varliero et al., 2019). Likewise, in our results, these authors found Lokiarchaeia in the subsurface sediments; however, groups found in our samples, such as Bathyarchaeia, Hadarchaeia, and MBG-D, were not detected. Looking at distant locations, the surface and subsurface archaeome of Guaymas Basin showed similar taxa to our study, such as bathyarchaeal lineages, Hadarchaeia, and MBG-D (Ramírez et al., 2020). However, other taxa such as ANME-1 and Terrestrial Miscellaneous Euryarchaea Group (TMEG) were not detected among our samples; additionally, Lokiarchaeia and Woesearchaeles were not found in their study.

POTENTIAL INFLUENCE OF ENVIRONMENTAL SETTINGS ON THE ARCHAEAL DIVERSITY

Several geochemical and oceanographic factors may influence the archaeal distribution in the benthic marine ecosystems, such as water and sediment depths, water masses properties, geological features, location at the continental margin or open ocean, oceanographic currents, organic compounds supply, rates of subseafloor redox processes, labile or recalcitrant organic matter, among many others (Lazar et al., 2017; D'Hondt et al., 2019; Baker et al., 2020; Ramírez et al., 2020). Among our sediment samples, we found a mud carbonate-rich characteristic influenced by the Intermediate Western Frontier Current (IWBC), which strongly flows northeastward (<0.3 m.sec^{-1}), transporting the Antarctic Intermediate Water (AAIW) as a part of the Subtropical Gyre (Viana et al., 1998; Biló et al., 2014), and deposits and mixes sediments in the region (Maly et al., 2019). However, how these geochemical and oceanographic factors locally affect the distribution and diversity of archaeal communities remains unknown.

Along these heterogeneities of environmental settings, the versatile metabolic pathways could allow the archaeal taxa to survive and thrive under a wide range of sedimentary habitats (Oni et al., 2015; Baker et al., 2020). Within the SW Atlantic benthic archaeal communities, it seems that they are fueled by both autotrophic and heterotrophic processes, including the degradation of different labile and recalcitrant organic compounds, likely resulting from sedimentation rates in the continental slope region deposited by IWBC. However, an investigation to elucidate the main geochemical and oceanographic drivers of the archaeome from the deep SW Atlantic Ocean and their metabolic potentials through metagenomic is underway.

CONCLUSION

Using high-throughput sequencing targeting the 16S rRNA gene, we described the archaeal community composition among surface and subsurface sediments from the SW Atlantic upper slope. We found that archaeal composition in the surface sediments presented similar patterns for deep-sea oxic sediments, being dominated mainly by ammonia-oxidizing archaea within Nitrososphaeria class. The Nitrososphaeria ASVs distribution among 0 to 15 cm strata suggests the presence of several species or ecotypes. In the deep SW Atlantic subsurface, we detected several uncultivated anaerobic and still poorly described archaeal taxa, in which the composition showed to be distinct in comparison with the subsurface archaeome from other regions. These subsurface archaea include representatives of all supergroups (Asgard, TACK, DPANN, and Euryarchaeota). They potentially harbor diverse metabolic capabilities, including autotrophic and heterotrophic pathways, such as acetogenesis, methylotrophy, and degradation of several labile and recalcitrant organic compounds. One explanation for these diverse metabolic potentials may be related to IWBC transport and sediment mixing along the continental slope, probably enriching these anaerobic archaeal lineages from the seafloor. These archaea may play an important role in the remineralization of organic matter. Nevertheless, their active roles in marine benthic biogeochemical cycles remain unknown, mainly due to the difficulties in obtaining cultivated lineages. Further, the influence of the main geochemical and oceanographic drivers on the archaeal distribution along the deep SW Atlantic Ocean needs further investigations, in addition to the understanding of their metabolic potentials through a metagenomic approach and using replicates for statiscal analyses. Our study provides the first description of the archaeome in surface and subsurface sediments from the SW Atlantic slope, which are located in a carbonate-rich region considered a vulnerable marine ecosystem that urges a better understanding for future conservation strategies.

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AUTHOR CONTRIBUTIONS

A.G.B.: Conceptualization; Methodology; Investigation; Formal Analysis; Writing – original draft; Writing – review & editing.

F.M.N.; A.C.A.B.; M.C.P.K.; R.B.R.; C.N.S.; R.A.L.; Methodology; Investigation; Formal Analysis; Writing – original draft; Writing – review & editing.

M.M.M.: Methodology; Investigation; Resources; Funding Acquisition; Formal Analysis; Writing – original draft; Writing – review & editing.

P.Y.G.S.: Supervision; Resources; Project Administration; Funding Acquisition; Writing – original draft; Writing – review & editing.

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