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Uncovering the Prokaryotic Diversity of the Bathyal Waters above the Kuril–Kamchatka Trench

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Abstract: The Kuril–Kamchatka Trench (North-West Pacific Ocean) is included in the deepest trenches (>9000 m). This study is the first that aims at uncovering the bathyal prokaryotic diversity (1000–2000 m) of this fascinating extreme environment. The analysis of α-diversity revealed that bacterial communities showed greater diversity than archaeal communities and that both communities were characterized by poor evenness (indicative of the presence of few dominant OTUs). The metabarcoding analysis showed that *Proteobacteria* (65.5–90.7%), *Bacteroidetes* (2.4–10.7%), and *Actinobacteria* (2.5–9.6%) were the highly represented phyla of bacteria, with *Acinetobacter* (21.5–62.5%) as the most abundant genus. Moreover, the recently described *Pseudofrancisella* genus, which has been isolated from estuarine environments, has been found among the major bacterial taxa. This work represents the first report stating the presence of this genus in bathyal waters. The archaeal communities were dominated by the phylum *Thaumarchaeota* (53.6–94.0%), with *Nitrosopumilus* (53.6–94%) as its representative genus. The functional diversity analysis revealed that overall, the bacterial communities had a higher involvement in the carbon and nitrogen biogeochemical cycles, with chemoheterotrophy (mostly aerobic), aromatic compound degradation, and nitrate reduction as the most represented functions. In the archaeal communities, the most represented ecological function was the aerobic oxidation of ammonia (first stage of nitrification), a functional feature characteristic of *Nitrosopumilus*.

Keywords: bacterial communities; archaeal communities; deep sea; bathypelagic zone; Kuril–Kamchatka Trench; metabarcoding

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1. Introduction

The oceans are the largest ecosystem of our planet, characterized by a number of different habitats and a wide diversity of organisms adapted to various environmental conditions [1]. Five zones have been identified following the vertical profile of the oceans: epipelagic (0–200 m), mesopelagic (200–1000 m), bathypelagic (1000–4000 m), abyssopelagic

(4000–6000 m), and hadopelagic (>6000 m) [1,2]. These zones show differences in physico-chemical parameters (e.g., light, pressure, salinity, temperature, and nutrient concentration) that collectively shape distinct conditions, ruling the microbial community composition and structure [3]. For this reason, profiling the microbial communities of the different oceanic zones enables an understanding of their ecological role (being involved in various biogeochemical cycles) and distribution.

The diversity of prokaryotic communities is normally specific to their geographical situation and can differ between trenches at different depths and/or different latitudes [4]. Hence, the availability of information regarding the prokaryotic diversity of different trenches would provide a broader knowledge of its inter- and intra-trench variations and its possible implications for ecosystem functioning. The investigations of trench prokaryotic communities have principally involved the Mariana Trench (e.g., [3,5–14]). Few works have regarded other trenches, including Japan, Kermadec, New Britain, Puerto Rico, Atacama, Yap, Mussau, and Izu-Ogasawara trenches [7,8,10,14–20]. Still, many trenches remain poorly characterized or definitely uncharacterized from the microbiological point of view.

The Kuril–Kamchatka Trench (KKT) is listed as the sixth deepest trench (having a maximum depth of 9604 m) [21,22]. It is a slightly convex linear depression (length: ~2089 km; surface area: 130,985 km^2) situated in the NW Pacific Ocean and is connected with the Aleutian Trench in the northeast and the Japan Trench (generating a continuum of this hadal area) toward the southwest [21,23]. The interconnected KKT trench and Kuril Basin are placed within one of the most productive oceanic zones worldwide [24]. Various oceanic currents, in some cases, with contrary directions (establishing interactions between warm and cold waters), affect the KKT region [25]. In particular, the trench's upper layers are primarily influenced by the East Kamchatka Current and Oyashio Current, while the deep layers above the hadal zone are affected by currents at the bottom (the deepest current reaching the KKT area is the Lower Circumpolar Deep Water, at ~4000 m); no current has been individuated in the V-shaped depression [26,27].

Explorations involving the KKT region started in 1949 (with the Soviet Vitjaz expeditions [28]), but the works have mainly regarded the description of its physico-chemical and oceanographic characteristics, the productivity, and the distribution of plankton and benthonic animal communities [21,29,30]. The diversity of KKT microorganisms is scarcely investigated. One paper dealt with bacteria found in the gills of hadal bivalve [31]; some studies reported that various macrofaunal species of the Kuril Basin and KKT bottoms host endosymbiotic bacteria with chemosynthetic activity, suggesting that the communities at the ocean bottom could be supported by these microorganisms providing the organic carbon [24,32]. Only two works investigated the prokaryotic diversity (the whole prokaryotic community [33] or specifically the ammonia-oxidizing archaea [34]) of some water samples collected at 1000 m and 3000 m in the KKT area or neighboring regions.

Our researches were carried out in the framework of the German–Russian expedition KuramBio II, which was specifically planned to implement the faunistic knowledge about the KKT ecosystem, following the previous oceanic campaign Kurambio and the historic Vityaz expeditions led by Lev Zenkevitch in the last century [35]. The two expeditions were mainly devoted to characterizing the KKT faunal diversity in terms of species composition, distribution pattern, and trophic relationships [36,37]. Our investigations aimed at profiling the prokaryotic diversity of the KKT ecosystem, which is practically unknown. In a very recent study [38], we provided the first description of the prokaryotic communities found in the abyssal-hadal zone of the trench. The investigation was carried out on samples of the benthic-boundary layer (water layer that is directly adjacent to the ocean bottom and can contain particles derived from the resuspension of surface sediments) that were collected at various locations within the trench and in the neighboring abyssal plain (depth range 5146–9540 m). The study indicated that the abyssal-hadal zone of the Kuril–Kamchatka Trench is inhabited by prokaryotic communities consisting primarily of chemolithotrophic archaea and heterotrophic bacteria (being *Acinetobacter*, *Zhongshania*,

Colwellia, and *Nitrosopumilus* the most abundant genera), which were detected at all depths and did not exhibit a distinctive zonation.

The present study aims to improve the knowledge of the KKT prokaryotic diversity by investigating both the bacterial and archaeal communities in the bathyal zone of the trench, considering a depth range of 1000–2000 m.

2. Materials and Methods

2.1. Collection of Samples and Their Characterization

For the survey, eight samples of water (KKT1-KKT8) were collected from different stations within the KKT area (NW Pacific Ocean) (Figure 1), aboard the Research Vessel (RV) "*Sonne*". Sampling was carried out during the German–Russian expedition KuramBio II (Kuril–Kamchatka Biodiversity Studies II; 16 August–26 September 2016).

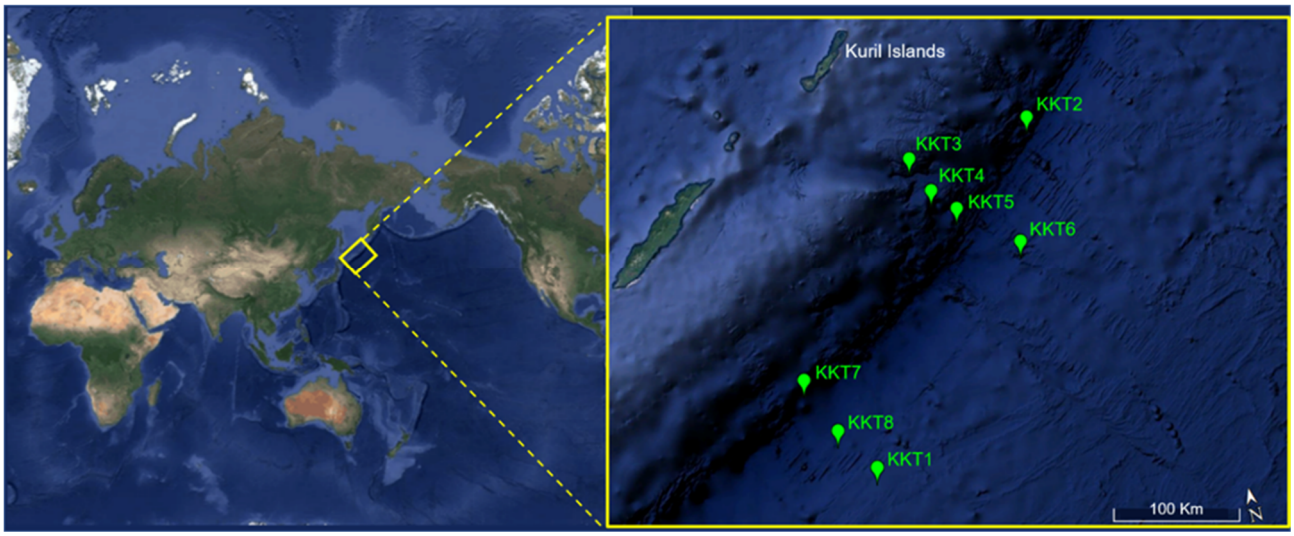

Figure 1. Map of Kuril–Kamchatka Trench with the indication of the sampling sites. Google Earth Pro version 7.3.6 was used to generate the map, which was graphically edited.

The samples (one for each station) were collected from the water column in the bathyal zone at 1000, 1500, and 2000 m depth by an SBE 32 Carousel water sampler (Sea-Bird Electronics, Washington, DC, USA) holding twenty-four 30-L Niskin bottles and equipped with the SBE 9*plus* CTD (for the measurement of temperature, conductivity, and oxygen saturation) (Table 1).

Table 1. Coordinates of the sampling site and related environmental parameters.

Sample	Station	Bottom Depth (m)	Coordinates (Latitude; Longitude)	Sampling Depth (m)	Temperature (°C)	Conductivity (S/m)	Oxygen (V)
KKT1	St.1	5144.4	43° 49.20′ N; 151° 45.60′ E	2000	1.70	3.12	1.89
KKT2	St.2	8126.8	46° 04.62′ N; 153° 58.20′ E	1000	2.72	3.14	1.04
KKT3	St.3	5958.3	45° 57.73′ N; 152° 39.91′ E	1000	2.79	3.14	0.99
KKT4	St.4	6040.1	45° 41.58′ N; 152° 49.49′ E	1000	2.94	3.15	1.01
KKT5	St.5	7757.6	45° 31.35′ N; 153° 02.82′ E	1500	2.33	3.14	1.12
KKT6	St.6	59526	45° 11.00′ N; 153° 36.99′ E	1000	2.72	3.14	0.92
KKT7	St.7	8392.0	44° 31.50′ N; 151° 11.59′ E	1000	2.54	3.13	1.03
KKT8	St.8	6547.0	44° 07.59′ N; 151° 26.58′ E	1000	2.54	3.13	1.04

One liter of water was filtered on sterile membranes (0.22 µm polycarbonate membranes; Millipore, MA, USA) for each sample; filters were maintained in a deep freezer till the extraction of total DNA (which was done on land).

2.2. Extraction of Total DNA, Preparation and Sequencing of the Amplicon Libraries

Total DNA was extracted from each filter by the ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research Corp., Irvine, CA, USA) following the producer's instructions.

For *Bacteria* and *Archaea*, the preparation and sequencing of the multiplexed amplicon libraries were separately carried out. The bacterial community analysis was carried out targeting the V5–V6 hypervariable regions of the 16S rDNA by the 783F and 1046R primers [39,40]. The archaeal community analysis was carried out targeting the V3 hypervariable region of the 16S rDNA by the Arch349F and Arch571R primers [41,42]. The amplicon libraries were prepared by a dual PCR approach, as previously reported by Gorrasi et al. [38]. The sequencing was performed at an external service (Nuova Genetica Italiana SRL, Monza-Brianza, Italy) by Illumina MiSeq (Illumina, San Diego, CA, USA) using a 2 × 250 bp paired-end protocol.

2.3. Processing of Sequences and Data Analysis

The processing of the raw sequences and data analysis were performed as previously reported [43,44]. The demultiplexing of the raw reads was performed according to the indices and internal barcodes. Then, the demultiplexed sequences were processed by the UPARSE pipeline [45]. The merging of paired-end reads (perfect overlapping) and the quality filtering were carried out using the default parameters. Then, the identification and removal of the chimeric sequences and singletons were performed.

Firstly, the Operational Taxonomic Units (OTUs) were determined on the total data set, clustering the sequences at 97% similarity; then, a representative sequence was identified for each cluster. The OTU abundance per sample was estimated through the mapping of the sequences to the OTU representative sequences (at 97% similarity).

The taxonomic annotation was performed using RDP classifier [46], using a confidence cut-off of 50% as suggested for short sequences [47].

All sequences have been submitted to the GenBank database under the following accession numbers PRJNA933908 and PRJNA933911.

The bacterial and archaeal genera shared by all KKT bathyal samples were visualized by bubble plots generated using SRplot online tool (http://www.bioinformatics.com.cn/srplot (accessed on 8 November 2023)).

2.4. Statistical Analyses

Good's method was used to estimate the sample coverage [48].

α-diversity was described using the Number of OTUs, the Chao1 index, the Shannon index, and Gini inequality index. Chao1 provides an estimation of the total number of OTUs in the bacterial/archaeal communities [49]. The Shannon index (from 0 to $+\infty$) increases as the community's evenness and richness increase [50]. The Gini index (from 0 to 1) increasing values indicate an evenness decrease [51]. The indices were calculated on samples rarefied to 42,939 and 27,002 (slightly less than the minimum sequence number in a sample) for *Bacteria* and *Archaea* datasets, respectively.

Analysis of β-diversity was run on non-rarefied samples [52]. For both datasets, the clustering analysis (complete linkage with hierarchical clustering) was performed on the Hellinger distance data. The Redundancy Analysis (RDA) was carried out to identify the environmental parameters structuring the bacterial/archaeal community (excluding collinear parameters; Pearson $|r| > 0.6$). RDA significance was evaluated by 9999 permutations. Afterward, the Generalized Linear Models (GLMs) were used to analyze if the indices of α-diversity and the abundances of taxa significantly changed in relation to the considered environmental variables. GLMs were carried out assuming a Poisson distribution corrected for overdispersion for the most abundant taxa and a Gaussian distribution for the alpha diversity indices; the false discovery rate (FDR) procedure was used to correct the *p*-values for multiple testing [53]. We designated as most abundant the taxa that were most prevalent across the entire dataset, and together, they encompassed more than 99% of the sequences.

The VEGAN, BIODIVERSITYR, and CAR packages (R 4.2.1) were used to perform all the statistical analyses.

The potential functions related to the studied prokaryotic communities were predicted through the functional annotation of the prokaryotic taxa software program (FAPROTAX, Version 1.2.6) [54]. FAPROTAX database contains functional annotations derived from cultured prokaryotes. The OTUs detected across the KKT samples were compared with the FAPROTAX annotation database, and only those that were successfully annotated were used for the community functionality profiling.

3. Results and Discussions

3.1. α- and β-Diversity of the KKT Prokaryotic Communities

Table 2 reports the α-diversity indices calculated for the *Bacteria* and *Archaea* datasets. The Number of OTUs (N. OTUs) ranged from 709 to 1055 and from 252 to 441 for the *Bacteria* and *Archaea*, respectively. The Chao1 index ranged from 1031.864 to 1266.739 and from 313.600 to 488.264 for the bacterial and archaeal libraries, respectively. The Shannon diversity index ranged from 2.693 to 4.474 and from 3.155 to 4.090 for the bacterial and archaeal libraries, respectively. The Gini inequality index ranged from 0.908 to 0.972 and from 0.914 to 0.966 for *Bacteria* and *Archaea*, respectively.

Table 2. α-diversity indices of the KKT prokaryotic amplicon libraries.

	Bacteria				*Archaea*			
Sample	**N. OTUs**	**Chao1 Index**	**Shannon Index**	**Gini Index**	**N. OTUs**	**Chao1 Index**	**Shannon Index**	**Gini Index**
KKT1	950	1165.458	3.442	0.938	327	367.167	3.774	0.942
KKT2	1055	1228.257	4.474	0.908	441	488.264	4.090	0.914
KKT3	994	1266.739	4.122	0.922	417	437.638	3.868	0.928
KKT4	902	1107.271	3.938	0.937	267	313.600	3.155	0.964
KKT5	867	1031.864	3.932	0.940	252	342.417	3.243	0.966
KKT6	1019	1248.700	3.969	0.922	340	397.122	3.380	0.955
KKT7	944	1175.207	3.910	0.936	401	475.094	3.816	0.927
KKT8	709	1035.237	2.693	0.972	395	458.133	3.902	0.927

Overall, the α-diversity analysis revealed that the bacterial communities showed greater diversity (indicative of a more complex community composition) than archaeal ones and that both communities were characterized by poor equitability. Actually, the high Gini values estimated for the two libraries were suggestive of low community evenness and, consequently, of the occurrence of few dominant OTUs. KKT8 sample harbored the bacterial community characterized by the lowest equitability, species richness, and diversity. Instead, KKT4 and KKT5 archaeal communities were those showing the lowest species richness, diversity, and evenness. Finally, KKT2 bacterial and archaeal communities were those having the highest species richness, diversity, and equitability.

For *Bacteria*, the cluster analysis identified four main groups (Figure 2a). The obtained arrangement cannot be explained considering the different sampling depths; in fact, KKT1 (2000 m) clustered together with samples taken at 1000 m, as well as KKT5 (1500 m) grouped with KKT4 (1000 m). Overall, KKT2 and KKT8 samples showed the most divergent communities. Unexpectedly, KKT1 and KKT6 as well as KKT7 and KKT3 harbored very similar bacterial communities, although the samples were taken at stations located more than 200 km from each other. As for *Archaea*, the analysis identified three main groups (Figure 2b), with KKT1 grouped apart (showing the most divergent community).

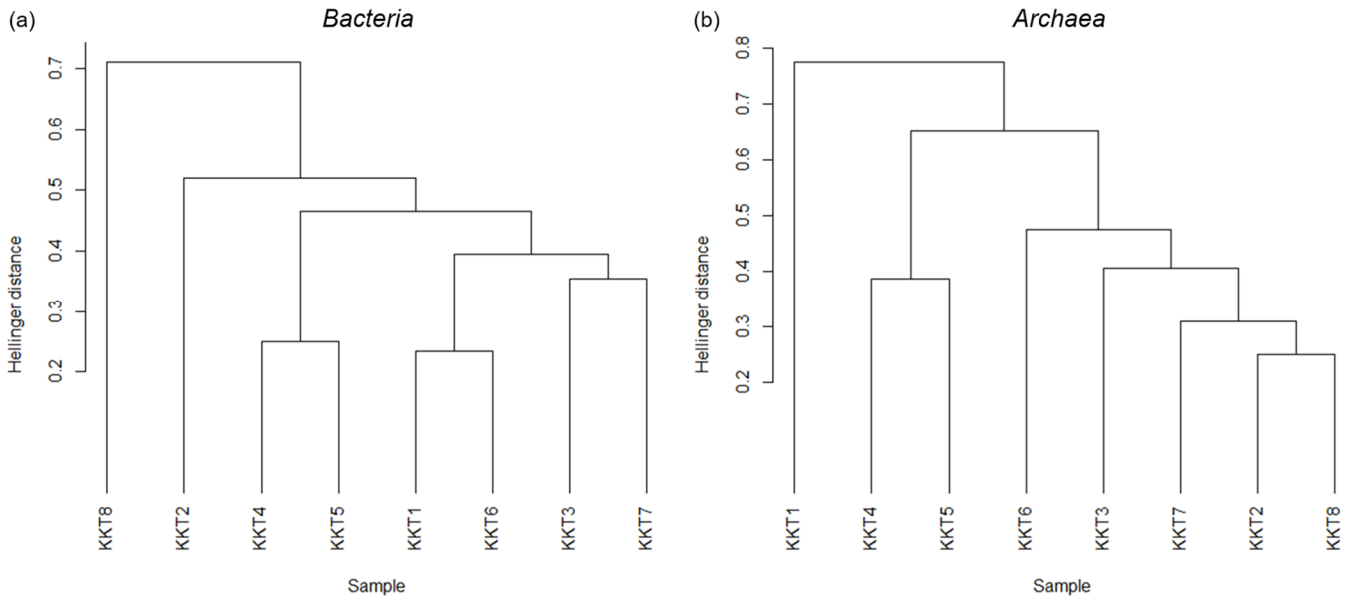

Figure 2. Dendrograms showing KKT sample clustering for *Bacteria* (**a**) and *Archaea* (**b**) based on their OTU composition. Clustering (complete linkage) was run using Hellinger distance data.

Overall, considering the two analyses, KKT4 and KKT5 definitely had the most similar prokaryotic communities (revealing closely related assemblages for both bacteria and archaea).

The RDA was performed to identify the parameters driving the bacterial and archaeal community structuring. Temperature and oxygen (output voltage) were dismissed as highly collinear (Pearson $|r| > 0.9$), whereas depth and conductivity were used as predictors. Regarding the *Bacteria*, none of the parameters entered in the RDA significantly explained the variance of the data ($F_{2,5} = 1.385$, $p = 0.182$). Instead, conductivity significantly affected the archaeal community structure (Table 3). Overall, samples collected at 1000 m were more closely associated with the parameter vector, being characterized by a higher conductivity; KKT6 and KKT1 archaeal communities were those influenced by a higher and a lower conductivity, respectively (Figure 3).

The Generalized Linear Models (GLMs) were performed to explore if the indices of α-diversity and the archaeal/bacterial taxa significantly changed in relation to depth and conductivity, and no significant trend was evidenced. Going into detail, for both *Bacteria* and *Archaea*, α-diversity indices did not significantly vary according to the considered parameters (*Bacteria*: $F_{2,5} > 0.0449$, $P_{FDR} > 0.215$; *Archaea*: $F_{2,5} < 0.153$, $P_{FDR} > 0.141$). As for the bacterial taxa, GLMs were performed on the most abundant phyla/genera (together including more than 99% of the sequences), and none showed a significant abundance variation according to the depth and the conductivity (phyla: $F_{2,5} > 0.0726$, $P_{FDR} > 0.307$; genera: $F_{2,5} > 2.303$, $P_{FDR} > 0.164$). Finally, regarding *Archaea*, the GLMs were performed on all phyla/genera; even in this case, the taxa abundance did not significantly change in relation to the parameters (phyla: $F_{2,5} > 0.503$, $P_{FDR} > 0.05$; genera: $F_{2,5} > 0.00286$, $P_{FDR} > 0.0959$).

Table 3. Descriptive statistics of the RDA analysis inspecting the environmental parameters driving the archaeal community structuring.

Variable	df	Variance	F	*p*
Conductivity	1	0.0322	2.455	0.027
Depth	1	0.0211	1.613	0.147
Residuals	5			
$F_{2,5} = 2.102$, $p = 0.032$, $R^2_{adj} = 0.239$				

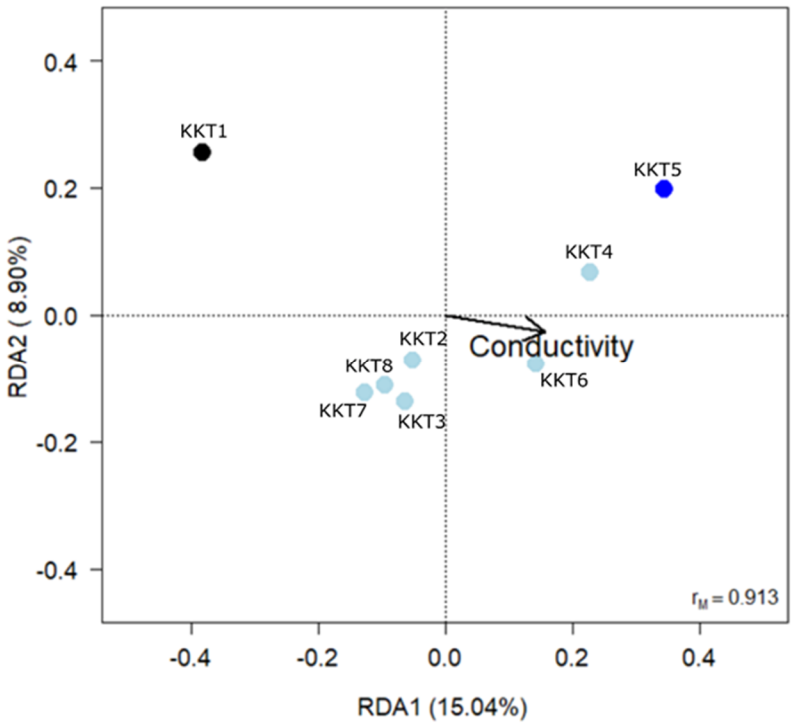

Figure 3. RDA biplot indicating the relationship between the samples (based on archaeal OTU profiles) and the conductivity. Variance explained by the two axes is shown in brackets. Samples are colored according to the sampling depth (cyan: 1000 m; blue: 1500 m; black: 2000 m). r_M represents the Mantel correlation coefficient between the Hellinger distances (between samples) and the Euclidean distances (between the sample symbols in the biplot). Values close to 1 indicate that the plot properly lays out the distance between samples.

3.2. Bacterial Community Profiling

A total of 410,832 bacterial sequences were gained (with Good's index values $\geq$ 98, indicative of good library coverage). The sequences were clustered in 1523 OTUs, which were assigned to 24 phyla, 48 classes, 92 orders, 181 families, and 297 genera. The fraction accounted by unassigned OTUs varied between 1.1 and 3.6%, 3.8 and 14.0%, 6.0 and 21.0%, 7.2 and 24.8%, and 9.6 and 34.4% at phylum, class, order, family, and genus level, respectively.

As for the phyla, seven major taxa (relative abundance (Ra) $\geq$ 1% in at least one sample) were recorded: *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Marinimicrobia*, *Firmicutes*, *Verrucomicrobia*, and *Planctomycetes* (Figure 4a). *Proteobacteria* (65.5–90.7%) represented the dominant taxon in all samples, followed by *Bacteroidetes* (2.4–10.7%) and *Actinobacteria* (2.5–9.6%). *Marinimicrobia* was a major phylum in all samples, with Ra_s in the range of 1.0–3.6%. *Firmicutes* (0.5–4.1%) was recorded in all samples with a mean Ra of 1.7%; it represented a minor taxon in KKT5, and it showed the highest Ra in KKT2. Among the major taxa, *Verrucomicrobia* and *Planctomycetes* were the least represented, with Ra_s in the ranges of 0.6–2.0% and 0.5–1.6%, respectively. In the oceans, these seven phyla are ubiquitous and broadly detected in the bathyal zone [17,20,55–58].

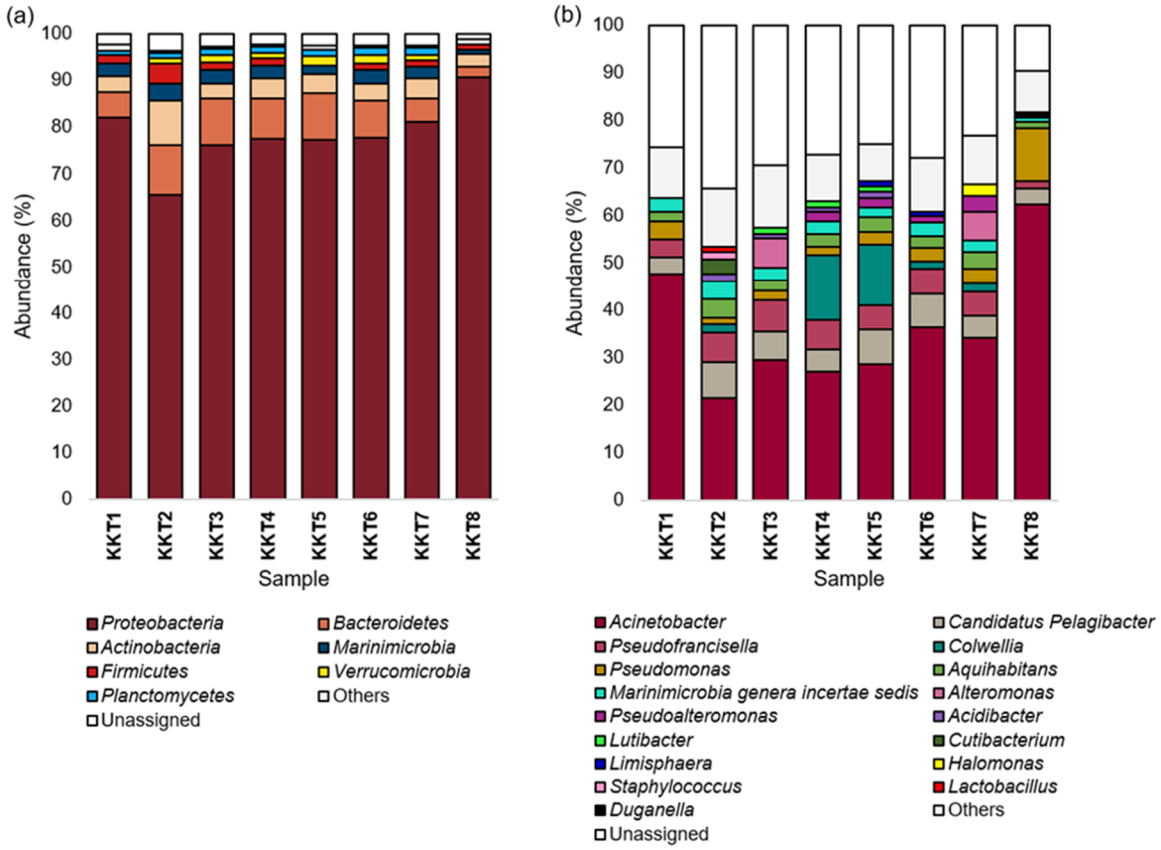

Figure 4. Composition of the bacterial communities of KKT bathyal zone at the phylum (**a**) and genus (**b**) level. The bar plots report the distribution of the major taxa (Ra $\geq$ 1% in at least one sample); taxa with Ra < 1% were grouped in "Others".

As far as we know, only one paper [33] described the prokaryotic community composition related to a couple of samples collected in the bathyal zone of the KKT region. Li et al. [33] collected water samples from stations sited around the Sea of Okhotsk and the NW Subarctic Pacific Ocean, including the KKT portion influenced by the East Kamchatka Current. The majority of samples were collected from the surface; two additional samples were taken at the same station located within the KKT area from deeper layers representing the bathypelagic zone (at 1000 and 3000 m depth). The bacterial community patterns of the KKT bathyal zone displayed in our work were rather consistent with the phyla patterns reported by these authors for the two bathypelagic samples. They reported *Proteobacteria*, *Marinimicrobia*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia*, and *Planctomycetes* as the major phyla, with *Proteobacteria* as the dominant taxon. The main difference arising between our and their survey was in *Marinimicrobia* occurrence, which in the samples collected by Li et al., represented the second most abundant phylum [33].

In addition, a preponderance of *Proteobacteria*, followed by *Bacteroidetes* and *Actinobacteria*, has already been reported by Jing et al. [57] regarding a water sample collected in the NW Subarctic Pacific Ocean (the sampling station was located in the interconnection zone between the KKT and the Japan Trench) at 1500 m depth.

Although differences in both the presence and abundance of phyla can be observed among various trenches, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* are generally found among the most abundant bacterial phyla in the bathyal waters [3,5,7,17,18,20,59]. Nevertheless, despite the quite invariant prevalence of these most abundant phyla across the bathypelagic waters, the distribution and abundance of their members can change [55,60]. Most of the works (involving the Mariana, Japan, New Britain, and Kermadec trenches) reported a general dominance of *Proteobacteria* [3,5,7,17,18,59], whereas Zhao et al. study [20]

evidenced a higher abundance of *Firmicutes* in the water samples from the Atacama Trench bathyal zone (at 1000 and 3000 m depth). However, it is worth noting that the prokaryotic communities of the bathypelagic zone may vary (in particular, at the genus/species level, contributing to the observed difference in phylum abundances) from one region to another across the oceans, suggesting some level of biogeography. This variation may be influenced by factors such as the food supply, environmental factors, and the complex interactions among the microbial taxa (including predation, competition, and cooperation), which can result in community dynamics that change spatially [55,60]. The environmental conditions, nutrient availability, and microbial interactions can spatially vary both across the bathyal waters and within the same bathyal zone. The bathypelagic zone encompasses a wide range of depths (1000–4000 m), and the physical and chemical conditions can change with depth, therefore, prokaryotic communities may be adapted to specific depth ranges, leading to variations in the composition of prokaryotic taxa with depth. In addition, the composition and diversity of prokaryotic communities in the bathypelagic zone can be influenced by the unique properties of each water mass. Different water masses have distinct characteristics, such as temperature, salinity, and nutrient levels, which create specific environmental conditions for microbial communities, leading to variations in community structure across different regions [55]. Moreover, the processes of particle colonization that occur in surface waters may influence the composition and biogeography of the communities in the bathypelagic zone. It has been postulated that sinking particles serve as vectors carrying viable particle-attached surface microorganisms into the deeper layers, significantly determining the composition, activity, and geographical distribution of the bathypelagic prokaryotic communities [61].

The bacterial OTUs were assigned to 297 genera (Table S1), of which 17 were major taxon in at least one sample (Figure 4b). Some of these major genera (e.g., *Acinetobacter*, *Pseudomonas*, *Alteromonas*, *Pseudoalteromonas*, and *Halomonas*) have been recognized among major taxa across other oceanic bathyal waters [55,62]. *Acinetobacter*, *Candidatus* Pelagibacter, *Pseudofrancisella*, *Colwellia*, *Pseudomonas*, *Aquihabitans*, and *Marinimicrobia genera incertae sedis* were the most retrieved taxa. Except for *Colwellia*, which was a rare taxon (Ra < 1%) in KKT1, KKT3, and KKT8, all the other genera were found as major taxa in all samples. Among them, *Acinetobacter* (21.5–62.5%) was the most abundant taxon in all samples and represented the dominant fraction of the bacterial community in KKT8.

Acinetobacter is widely found in a number of ecosystems, and it is a highly heterogeneous genus, including various species with different metabolic/physiological features and ecological roles (e.g., pathogeny and biodegradation of xenobiotics), which actively participate in nutrient cycles [63–65]. It was found in oceanic waters and sediments, from the superficial layers to the bottom (also in some trenches) [19,66–72]. The metabolic characteristics and the possible ecological traits of some *Acinetobacter* members recognized in the deep sea indicated their possible involvement in hydrocarbon degradation, sulfur, and metal oxidation [66,69,71,73].

Candidatus Pelagibacter is a cultivable member of SAR11 [74], a family of heterotrophic bacteria found throughout the oceans; this genus has been recognized from the superficial waters to the hadal ones, and its significant role in the ocean carbon recycling and microbial food web is related to the metabolization of dissolved organic matter (DOM) and volatile organic compounds (VOCs) [6,9,11,75,76].

Aquihabitans is a genus of bacteria found in intestinal microbiota or in associations with animals and in various natural (terrestrial and aquatic) environments [77–80], which are reported to be denitrifying (nitrate-reducing) microorganisms [77,81].

Members of *Pseudomonas* and *Colwellia* are often recognized throughout the oceanic environments (both in waters and sediments) [6,7,55,82–85] and play important roles in nutrient cycles; oceanic representatives of these two genera include denitrifiers [82,84,86,87] and potential hydrocarbon degraders [73,88].

Interestingly, the presence of *Pseudofrancisella*, a recently described genus that includes only one species isolated from estuarine seawater [89], was revealed in KKT bathyal com-

munities. Currently, data related to *Pseudofrancisella* occurrence in the oceanic environment and information about its ecology and/or a possible role in ecosystem functioning are not available. Therefore, our survey represents the first recognition of this genus in the oceanic realm.

As for the genera, it is not possible to compare their occurrence with previous data due to their unavailability. It should be noted that the only study that has examined the bathyal prokaryotic communities in the Kuril–Kamchatka Trench area is that carried out by Li et al. [33]; however, the authors did not provide a taxonomic profiling of the communities at the genus level. The only comparison that can be made is with the abyssal-hadal communities of the KKT that we have recently described [38]. Nevertheless, it is important to highlight that the samples analyzed in our previous study differ significantly from those of the current one. In the previous study, the samples were collected at depths ranging from 5146 to 9540 m and were representative of the benthic boundary layer, which also includes microorganisms from the sediment surface resuspension. We are unable to thoroughly discuss the similarities/differences between the bathyal and abyssal-hadal communities of the KKT due to the absence of suitable environmental data (in particular regarding the available nutrients) that, in addition to the difference in hydrostatic pressure and in the inherent nature of the samples, could meaningfully explain the differences in the occurrence of the taxa (in terms of absence/presence and abundance) between the two zones. Therefore, the comparison will be limited to some general observations only. Comparing the data obtained from the taxonomic analyses of the bacterial communities of the KKT bathyal and abyssal-hadal zones, it can be noted that among the 17 genera found as major taxa in the bathyal samples, 9 were also detected as major in the abyssal-hadal samples. These genera (*Acinetobacter*, *Candidatus* Pelagibacter, *Colwellia*, *Pseudomonas*, *Aquihabitans*, *Marinimicrobia genera incertae sedis*, *Pseudoalteromonas*, and *Staphylococcus*) showed significant presence across different depths within the trench. This suggests that they most likely encompass members that have developed distinctive adaptations enabling them to thrive at different conditions across different depths and that these genera potentially play crucial roles in maintaining the stability of the trench ecosystem. In addition, it is worth noting that *Pseudofrancisiella* was not found in the KKT abyssal-hadal zone. Its absence in these remote trench regions might reflect an adaptation or distribution specificity, which highlights the need for additional investigation in order to identify the factors affecting *Pseudofrancisiella* distribution. A list of the bacterial genera found in the bathyal zone (current work) and/or in the abyssal-hadal zone (previous work; [38]) of the KKT was supplied to provide a more detailed comparison of their occurrence among the two trench regions (Table S2).

Among the 297 genera that we recognized in the KKT bathyal zone, 110 (accounting for 63.9–90% of the bacterial communities) were detected in all samples, representing the core taxa of the KKT bathyal bacterial communities (Figure S1). The core community typically consists of those taxa that are consistently present, well-adapted to the local conditions, and contribute to the stability and functioning of the studied environment [90]. As expected, among the KKT bacterial core taxa there were all the most retrieved genera (listed above), which presumably act as keystones and play essential roles in maintaining ecosystem processes (e.g., in nutrient cycling) that occur within the KKT bathyal zone. Their possible contributions were argued above. In addition, among the core taxa, 71 genera were detected only in the trench bathyal zone; it is worth noting that they were not found in the KKT abyssal-hadal region [38]. These 71 taxa accounted for from 8.4% to 25.3% of the bathyal communities and included nine major genera (*Pseudofrancisella*, *Aquihabitans*, *Marinimicrobia genera incertae sedis*, *Acidibacter*, *Cutibacterium*, *Limisphaera*, *Lutibacter*, *Staphylococcus*, and *Duganella*). Among these nine major genera exclusively found in the bathyal samples, some of them are reported to show important metabolic features, such as *Aquihabitans* and *Duganella,* which can have nitrate-reducing capabilities, and *Acidibacter,* which is reported to be an iron-reducing microorganism [81,91,92].

3.3. Archaeal Community Profiling

A total of 296,440 archaeal sequences were gained (with Good's index values ≥99, indicative of good library coverage), which were clustered in 572 OTUs. As for the taxonomic assignation, only the summary of OTU classification obtained at phylum and genus levels was reported, being the lineage of some genera currently not fully defined. The OTUs were assigned to 4 phyla and 11 genera; at the phylum and genus level, unassigned OTUs accounted for 0.7–3.4% and 6–41.2% of the archaeal communities, respectively. The identified phyla were *Thaumarchaeota*, *Euryarchaeota*, *Woesearchaeota*, and *Crenarchaeota* (Figure 5a). *Thaumarchaeota* (53.6–94.0%) was the dominant taxon in all KKT bathyal samples, representing the predominant portion of the communities in KKT4 (85.7%) and KKT5 (94.0%) samples. *Euryarchaeota* (4.6–40.1%) represented the second most abundant taxon over all archaeal communities. This was consistent with what was previously observed by Li et al. [33], who reported that *Thaumarchaeota* and *Euryarchaeota* were the principal archaeal phyla of their samples collected at 1000 and 3000 m within the KKT region. However, a high presence of these two archaeal phyla, with *Thaumarchaeota* overriding *Euryarchaeota*, is consistent with the archaeal occurrence pattern observed across various oceanic bathyal regions [7,17,55,56,59,93].

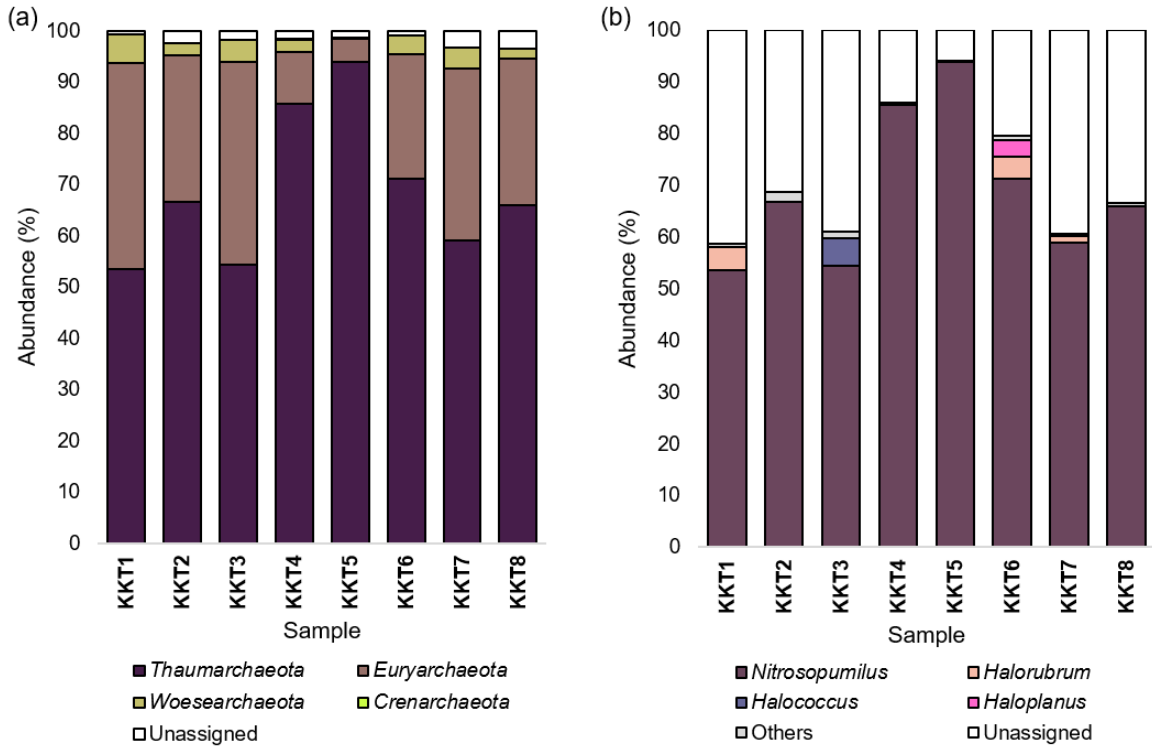

Figure 5. Composition of the archaeal communities of KKT bathyal zone at the phylum (**a**) and genus (**b**) level. The bar plots report the distribution of the major taxa (Ra ≥ 1% in at least one sample); taxa with Ra < 1% were grouped in "Others".

Marine *Thaumarchaeota* (previously known as Marine Group I, MGI) are well-recognized widespread chemolithoautotrophs, having significant roles in ocean carbon and nitrogen cycling for their ability to fix carbon and perform the first nitrification step (the ammonia oxidation to nitrite) [94].

Marine *Euryarchaeota* are subdivided into three main groups (MGII, MGIII, and MGIV): MGII are more abundant in shallower water and decrease with depth increase, while MGIII and MGIV represent rare members of deep-sea communities and are more frequently detected in meso- and bathy-pelagic regions [94]. *Euryarchaeota* ecological impact is primarily on the carbon biogeochemical cycle; MGII and MGIII could be involved in organic matter re-

cycling. In addition, the phylum includes some taxa of methanogenic and methanotrophic archaea [94,95].

Woesearchaeota and *Crenarchaeota* were the least represented phyla over all bathyal samples. *Woesearchaeota* was recorded as a major taxon in KKT samples, except in KKT5. In marine environments, it has been found mainly in sediments, while it has been scarcely detected in waters; in addition, it has been reported in hydrothermal vent ecosystems [96,97]. Its ecology and metabolic functions remain largely unknown. Genomic studies have suggested that most *Woesearchaeota* may mainly have an anaerobic heterotrophic metabolism, featuring metabolic deficiencies that imply the establishment of potential syntrophic/mutualistic partnerships with other microorganisms [96]. It was reported that *Woesearchaeota* tends to show a consortial co-occurrence with *Methanomicrobia* and *Methanobacteria* (euryarchaeotal classes of methanogenic archaea) [96]. Although archaeal class distribution across the KKT bathyal samples is not reported and discussed in this study (being not meaningful since the lineage of some archaeal genera is presently not completely defined), we detected the presence of *Methanomicrobia* in all samples, even if as minor taxon (Ra 0.03–0.9%). Since in KKT samples *Woesearchaeota* and *Methanomicrobia* were concurrently found, a possible syntrophic relationship between them could be supposed.

As for *Crenarchaeota*, it was not detected in KKT5 and represented a rare taxon in all other KKT samples. Members of this taxon were reported in several oceanic environments and can represent an abundant moiety of the archaeal communities, mainly in surface sediments and in oxygenated deep waters [98].

The archaeal OTUs were assigned to 11 genera (Table S3), with *Nitrosopumilus* (53.6–94%), *Halococcus* (0–5.3%), *Halorubrum* (0.007–4.5%), and *Haloplanus* (0–3.3%) found as major taxa in at least one sample (Figure 5b). The archaeal diversity of the trench bathyal zone was dominated by *Nitrosopumilus*, a genus with high biogeochemical significance, particularly in carbon and nitrogen cycling. Members of this genus feature a chemolithoautotrophic metabolism and are deemed key primary producers, especially in the absence of photoautotrophic activity [20,99,100]. In addition, *Nitrosopumilus's* key ecological trait is the ability to oxidize ammonia to nitrite [99]. As known, the first step of nitrification is the oxidation of ammonia carried out by ammonia-oxidizing archaea and bacteria (AOA and AOB), followed by the nitrite-oxidation step that is performed by nitrite-oxidizing bacteria (NOB, which are recognized as syntrophic partners of AOA/AOB) [101]. It was reported that in the oceans, AOA, including *Nitrosopumilus*, are the principal ammonia oxidizers. Then nitrite is further oxidized by NOB, mostly belonging to the phylum *Nitrospinae* and, to a lesser degree, to the genera *Nitrococcus* and *Nitrospira* [101–103]. Although with Ra $\leq$ 1%, we detected the presence of NOB belonging to *Nitrospira* (Table S1), suggesting a potential syntrophic relationship with *Nitrosopumilus*.

Comparing the archaeal communities of the KKT bathyal and abyssal-hadal zones (our present and previous investigations [38]), it can be observed that all the 11 genera detected in the bathyal samples were also found in the abyssal-hadal ones. However, among the taxa found as major in the bathyal samples, *Nitrosopumilus*, *Halococcus*, and *Halorubrum* were major genera in the abyssal-hadal ones, even with *Nitrosopumilus* the dominant archaeal representative in both regions. This indicates that this taxon represents an essential component for the structuring and stability of the KKT archaeal communities.

Among the 11 genera identified in the bathyal samples, *Nitrosopumilus*, *Halorubrum*, and *Woesearchaeota Incertae Sedis AR20* were those detected in all samples, representing the core taxa of the archaeal communities of the KKT bathyal zone (Figure S2).

3.4. Bacterial and Archaeal Ecological Function Prediction

FAPROTAX was used to explore bacterial and archaeal functional characteristics.

The potential ecological functions predicted by the tool were primarily associated with the biogeochemical cycles of carbon, nitrogen, and sulfur (Figures 6 and 7).

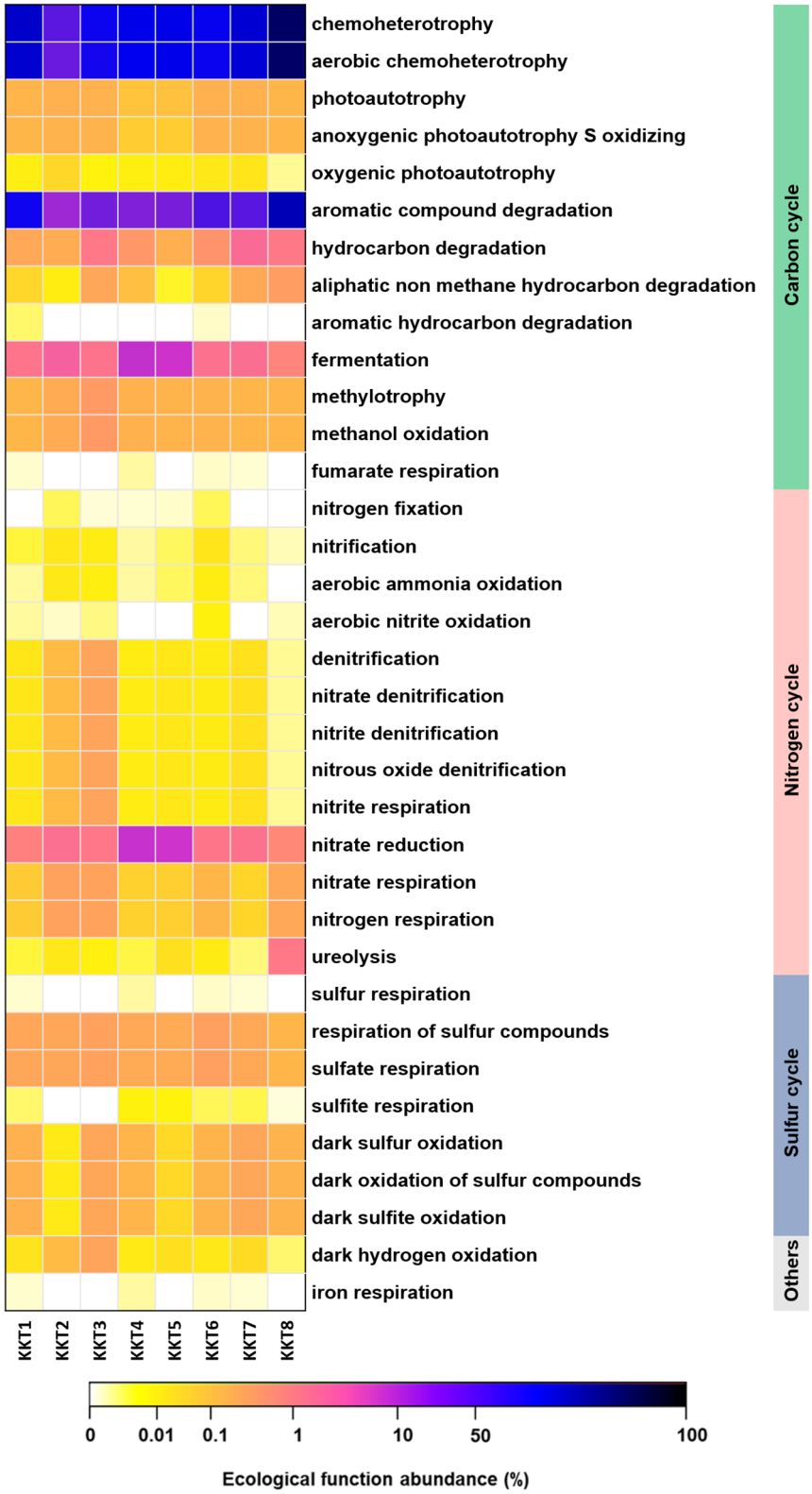

Figure 6. FAPROTAX prediction of the ecological function related to the KKT bathyal bacterial communities.

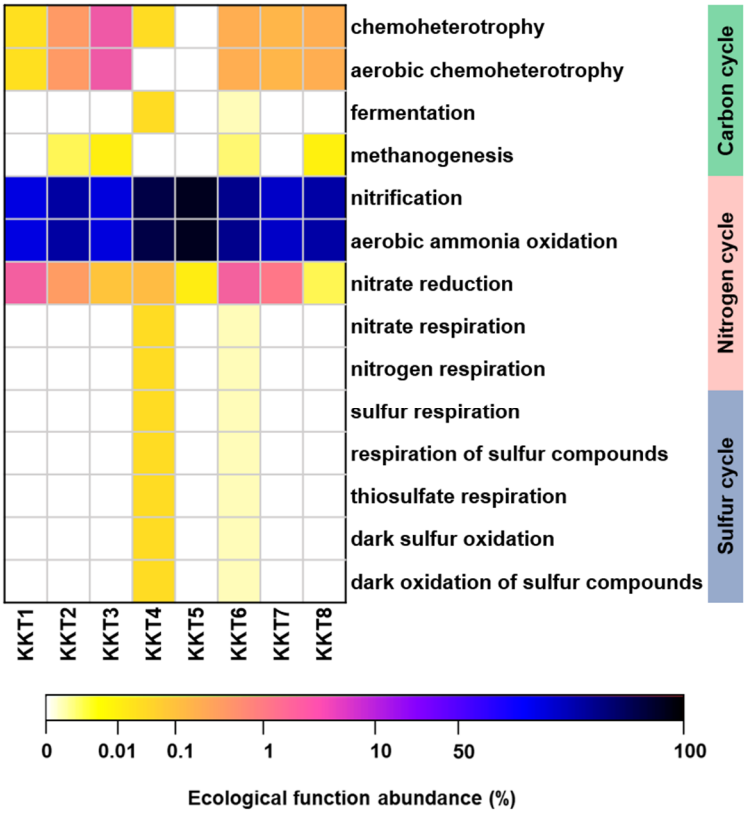

Figure 7. FAPROTAX prediction of the ecological function related to the KKT bathyal archaeal communities.

The analysis revealed that chemoheterotrophic metabolism was the main strategy adopted by the bacterial community members; this ecological trait was associated with 124 genera (principally aerobic chemoheterotrophs), also including taxa detected as major across the KKT samples (e.g., *Acinetobacter*, *Colwellia*, *Shewanella*, and *Pseudomonas*) (Figure 6; Table S1). Interestingly, the presence of ecological functions referable to the ability to metabolize recalcitrant compounds was evidenced. A high moiety (in particular in KKT1 and KKT8, where it accounted for 47.6% and 62.5%, respectively) of the bacterial communities showed competence for the aromatic compound degradation, a trait mostly related to *Acinetobacter* but also to *Rhodococcus* and *Nocardioides* (Figure 6; Table S1). In addition, a smaller fraction was able to metabolize hydrocarbons, including obligate hydrocarbonoclastic microorganisms such as *Alcanivorax* members (Figure 6; Table S1). However, it must be said that the occurrence of obligate hydrocarbonoclastic bacteria has also been reported in pristine and remote oceanic areas where apparent hydrocarbon pollution was never revealed, sustained by the presence of small quantities of aromatic and aliphatic molecules of biotic and abiotic origin (including chlorophyll A and long-chain alkanes) [104,105]. Amongst the ecological functions predicted in relation to the nitrogen cycle, nitrate reduction was the most represented (especially in KKT4 and KKT5, associated with ~13–14% of the community), involving 14 genera but principally owed to *Colwellia* (Figure 6; Table S1). Considering the sulfur cycle, respiration of sulfur compounds (primarily of sulfate) and dark oxidation of sulfur and sulfur compounds were the most predicted metabolisms, which involved just minor taxa. Sulfate respiration pertained to *Desulfacinum*, *Desulfofaba*, *Desulfofrigus*, *Desulfobulbus*, and *Desulfatiglans*, whereas dark oxidation of sulfur/sulfur compound to *Sulfitobacter* (Figure 6; Table S1).

As for the archaeal communities, as expected, considering the high prevalence of *Nitrosopumilus*, the ammonia oxidation in aerobic conditions was the principal ecological function (Figure 7). Also the reduction of nitrate was amongst the widely predicted functions attributable to the nitrogen cycle, related to *Thermodiscus* and *Halorubrum* (Figure 7; Table S3). Finally, all other metabolic traits related to the nitrogen cycle, alongside those

related to the sulfur cycle, showed the same pattern over KKT samples, all owing to *Thermodiscus* (Figure 7; Table S3).

4. Conclusions

The current work represents a worthwhile contribution to the characterization of the understudied KKT area prokaryotic biodiversity. It is important to consider that there are no future plans for further investigations in this unique environment, which makes this study even more valuable. The investigation allowed to uncover the taxonomical and functional prokaryotic diversity of the KKT bathyal zone. Indeed, while others have investigated the prokaryotic communities of just a couple of samples collected at 1000 and 3000 m from the same station within the KKT area, the authors did not provide a more detailed description of the communities till the genus level. Thus, the current study is the first to provide a more comprehensive insight into the bathyal zone communities within the KKT trench, shedding light on the identified genera and their potential ecological roles. Our survey evidenced that *Proteobacteria*, *Bacteroidetes,* and *Actinobacteria* were the most represented phyla in the bacterial communities, with *Acinetobacter* as the most abundant genus. The archaeal communities were dominated by *Thaumarchaeota*, being *Nitrosopumilus* its representative genus. In addition, we identified the core taxa (detected in all samples) across the bathyal prokaryotic communities, represented by 110 bacterial and 3 archaeal genera, including the most retrieved taxa *Acinetobacter*, *Candidatus* Pelagibacter, *Pseudofrancisella*, *Colwellia*, *Pseudomonas*, *Aquihabitans*, *Marinimicrobia genera incertae sedis*, *Nitrosopumilus*, and *Halorubrum.* Overall, the prokaryotic communities harbored mostly heterotrophic and chemolithoautotrophic genera, characterized by potential metabolic traits with important implications in carbon, nitrogen, and sulfur biogeochemical cycles. In particular, among the most retrieved taxa which are part of the core microbiota, there are microorganisms that play important roles in the nitrogen cycle, such as *Colwellia*, *Pseudomonas*, *Aquihabitans,* and *Halorubrum,* that have been documented to own nitrate-reducing capabilities and *Nitrosopumilus*, a well-known genus of AOA (involved in nitrification) that participates in the conversion of ammonia to nitrite, contributing to the availability of nitrogen-based nutrients.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse11112145/s1, Table S1: Bacterial genera found across the KKT bathyal samples; Table S2: Bacterial genera found in the bathyal zones of the KKT and other trenches (at depths in the range 1000–2000 m); Table S3: Archaeal genera found across the KKT bathyal samples; Figure S1: Bubble plot showing the distribution of the bacterial core taxa across the KKT bathyal samples; Figure S2: Bubble plot showing the distribution of the archaeal core taxa across the KKT bathyal samples.

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