Influence of the Depth and Season on Microbial Community Dynamics of the Black Sea

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Abstract

The Black Sea is a unique environment having a thin layer of oxic-zone above and anoxic-zone below. Seasonal, vertical, and horizontal microbial assemblages were studied in terms of diversity, abundance, community structure using NGS of the 16S rRNA gene. Total of 750 bacteria species from 23 different phyla were identified. The number of species richness increased from the surface to deeper zones. Although microbial community compositions between sampling stations were similar, microbial community compositions were significantly different vertically between zones. Community compositions of the seawater and sediment were also significantly different. Community composition at 5 meters in summer was significantly different from other seasons, while remaining depths appeared similar. Species of nitrite-oxidizing, sulfate-reducing, thiosulfate reducing, Iron-reducing, Fe-Mn reducing and electricity-producing bacteria were reported for the first time in the Black Sea. Proteobacteria dominated all the sampling depths. Proteobacteria, Cyanobacteria, Bacteroidetes, and Verrucomicrobia were present in the whole water column, while Nitrospinae, Chloroflexi, and Kiritimatiellaota were restricted, appearing abundant at 75 meters and deeper layers. Vertical microbial community composition variation is attributable to environmental factors and their adaptations to the various ecological niches.

1. Introduction

Marine microbial communities play a fundamental role in the biogeochemical cycles of elements (Ji et al., 2019; Sonthiphand et al., 2019), food web structure, and energy flow (Li et al., 2019). Understanding the mechanism of bacterial community assembly is one of the most critical objectives of microbial ecology. Detection of dominant groups within the microbial communities is vital in ecological studies as different bacterial groups have different roles in the ecosystem. Microbial community structures at a temporal and spatial scale vary through ecological factors (Yan et al., 2017) and deterministic partitioning of available resources among organisms (Zhang et al., 2009; Yan et al., 2017; Wu et al., 2019). Several studies attempted to elucidate the ecological factors shaping the diversity of bacterial communities in different environments (Langenheder and Ragnarsson, 2007; Zheng et al., 2014; Learman et al., 2016; Román et al., 2019; Zorz et al., 2019). Few studies have focused on the diversity and community composition of bacteria in the Black Sea (Bobrova et al., 2016; Zhang et al., 2020). Moreover, limited information is available on the microbial diversity in seawater and surface sediment.

The Black Sea is one of the largest enclosed and isolated inland water basins (Oguz, 2005) connected to the Mediterranean Sea by narrow straits. Large freshwater input from the rivers, more than 350 km³/year, produces a low-density surface layer, and salty Mediterranean water flow through straits forms a high-density bottom layer (Tomczak and Godfrey, 1994). A steep pycnocline layer between these two layers is the main physical barrier limiting vertical water circulation, causing stratification (Murray et al., 1989). Pelagic life is restricted by complete anoxic waters below approximately 200 m depths. Dissolved oxygen is depleted below 180–200 m depth, resulting in a hydrogen sulfide layer, the largest known anaerobic water body in the world. Despite these challenges, diverse microbial life inhabits different layers of the Black Sea, before the advent of next generation sequencing. The Black Sea includes a spectrum of hydrographic features, seasonal and vertical stratification. According to the water's biochemical characteristics, the Black Sea is divided into five major strata in terms of density. These depth strata are:

- i. Surface mixed layer (SML) is a homogeneous layer with little variation in temperature, density or other oceanographic conditions (Kara et al., 2000). The exception being the South Eastern Black Sea's mixed layer depth which ranged from 67 m to 15 m in December and June, respectively (Agirbas et al., 2015).
- ii. Thermocline/Pycnocline layer (TL) corresponds to sigma theta 14.6 (Tamminen and Kuosa, 1998).
- iii. Mineralization/nitrification zone (MNZ) depth ranges between sigma theta 14.6 to 15.4, where the majority of mineralization and nitrification is known to take place (Tamminen and Kuosa, 1998)
- iv. Oxygen minimum zone (OMZ) corresponds to sigma theta 15.4 to 15.8. The OMZ depth varies both seasonally and spatially depending on the circulation and the eddies' intensity. However, several investigations have shown that the OMZ could be explained better by water density than depth (Saydam et al., 1993; Tugrul et al., 1992).
- v. Anoxic zone (AZ), which begins after OMZ, corresponds to the depth of sigma theta 16.2 (Besiktepe, 2001).

Only a few metabarcoding surveys have been conducted to describe the diversity and community composition of bacteria in the Black Sea. Previously, bacterial diversity of the suboxic and anoxic zone in the western-central gyre (42°30'N, 30°45'E) of the Black Sea was studied by constructing a 16S rRNA gene library (Fuchsman et al., 2011). Members of the SAR11, SAR324, and *Microthrix* groups were the suboxic zone's dominant bacterial groups. Members of the BS-GSO-2, Marine-Group-A, and SUP05 were found as the dominant

bacteria groups of the anoxic zone. Bobrova et al. (2016) found that surface water samples collected from a single location in the Northeastern Black Sea had a high abundance of Proteobacteria (51.4%), Bacteroidetes (17.3%), and Cyanobacteria (11.1%). Similarly, Bobrova et al. (2016) revealed that Cyanobacteria, Proteobacteria, Bacteroidetes, and Actinobacteria dominated the bacteria assemblages in surface waters collected from three nearby estuaries in the Northwestern Black Sea. Zhang (2020) studied the microbial abundance, diversity, and community composition of the Black Sea's different water layers collected from 12 stations (6 in open waters and 6 in coastal waters). It revealed that the microbial communities were dominated by Proteobacteria, Actinobacteria, Bacteroidetes, and Cyanobacteria.

There is no study on the inter-seasonal variation in community compositions. Moreover, the driving force and mechanisms underlying the Black Sea ecosystem's microbial community assembly are still unclear and require further attention. This study aimed to reveal the seasonal variation in microbial communities in the Black Sea through comparative metabarcoding analysis at five density depth strata and surface sediment from coastal and offshore sampling sites. We also attempted to find the main environmental factors affecting the microbial community composition.

2. Materials And Methods

2.1. Sampling

Sampling was conducted using *KTU Denar-I R/V* in the Southeastern Black Sea. Water samples were seasonally collected (November 2018, February 2019, May 2019, and August 2019) from 5 depth strata at four sampling stations: two coastal; K1 (41°00'03.6"N 40°13'19.2"E), K3 (41°10'01.2"N 40°42'43.2"E), and two offshore sites; K0 (41°35'13.2"N 40°21'28.8"E), K2 (41°16'51.6"N 40°12'03.6"E) (Fig. 1), using a Seabird SBE-32-Carousel water sampler (Seabird Scientific, WA, USA) equipped with 5-l Niskin bottles. These five depth strata (surface mixed layer (SML), thermocline layer (TL), mineralization/nitrification zone (MNZ), oxygen minimum zone (OMZ), and anoxic zone (AZ)) were detected based on the CTD profiles using the SBE-37-SMP-ODO CTD instrument (Seabird Scientific, WA, USA) which corresponded to 5, 25, 75, 100, and 200 meters for all stations and seasons. Two liters of water from each depth were filtered through 10, 5, and 0.22 µm pore sized polyethersulfone membrane filters (GVS Filtration Inc.) to eliminate filter clogging on 0.22 µm pore sized filter. All three filters were transferred to a laboratory under cold-chain and stored at - 80°C until processing. Three filters were joined back again for the DNA extraction. Surface sediments (anoxic zone) were seasonally collected at coastal sampling stations (K1 at 360 m depth and K3 at 290 m depth) using Van Veen Grab Sampler (KC Denmark, Silkeborg, Denmark) and 10 g of sediment stored in sterilized plastic containers at - 80°C until processing. 50 ml of ultra-nanopure water were filtered as a negative control.

2.2. Measuring environmental parameters

Temperature, salinity, dissolved oxygen (O₂), sigma-t (kg/m³), conductivity (µs/m) at each sampling depth were measured *in-situ* using Seabird SBE-37-SMP-ODO CTD instrument (Seabird Scientific, WA, USA). Light Attenuations Values (%) were determined using a Hyperspectral color radiometer (Seabird Scientific, WA, USA).

Concentrations of Chlorophyll-a, ammonia (NH₃), nitrite (NO₂), nitrate (NO₃), silicate (SiO₄), phosphate (PO₄), and hydrogen sulfide (H₂S) were measured using a UV-Visible spectrophotometer (Shimadzu UV 2550, Japan) (Bendschneider and Robinson, 1952; Mullin and Riley, 1955; Solorzano, 1968; Cline, 1969). Concentrations were calculated as ng/µL. TOC concentrations in the seawater samples were measured using the combustion catalytic oxidation method (Sharp, 1973).

Normality and homoscedasticity of the environmental data were analyzed using the Shapiro-Wilk test and Levene's test, respectively. Three-way ANOVA was performed to evaluate each environmental parameter based on three factors: depth, season, and station using SigmaPlot v12. 3 (San Jose, CA, USA).

2.3. DNA isolation, PCR, and sequencing

DNA was extracted from the 80 water samples using DNeasy PowerWater Kit (Qiagen, Hilden, Germany) and eight sediment samples using DNeasy PowerSoil Kit (Qiagen), following the manufacturer's protocol. Same procedure was also applied for negative control samples. The DNA samples were assessed using agarose gel electrophoresis and the Nanodrop spectrophotometer (Nano-200, Allsheng Inc. China) and stored at - 20°C before processing. Primers of 314F and 805R (Albertsen et al., 2015) were used to amplify

the V3-V4 hypervariable region of the prokaryotes' 16S rRNA gene. PCR was carried out in a total volume of 25 µL reaction mixture containing 0.75 µL of each primer (0.3 µM each), 0.5 µL of Kapa HiFi HotStart DNA Polymerase (1 u/µL) (Kappa Biosystem, Cape Town, South Africa), 5 µL of 5X HiFi Buffer, 0.75 µL of dNTP Mix (0.3 mM of each), 2–3 µL of DNA template, and PCR-grade water. Nuclease free water as DNA templates was used for negative PCR controls. Applied amplification protocol was as follows: initial denaturation at 95°C for 3 min followed by 24 cycles of denaturation at 98°C for 20 s, annealing at 55°C for 18 s, extension at 72°C for 15 s, and a final extension at 72°C for 10 min. PCR products were assessed on 1.5% agarose gel and purified with NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Dueren, Germany). Indexed adapters were added to the amplicon by using Nextera XT Index Kit V2 (Illumina, California, USA) with 8 PCR cycles. Then PCR products were cleaned-up with AMPure XP beads before DNA libraries quantification with Qubit and real-time PCR (Exicycler 96, Bioneer, Korea). According to the manufacturer's instructions, samples were pooled and loaded onto an Illumina MiSeq analyzer (Illumina, USA). Samples were sequenced in both library directions (2x300 bp) with Illumina Sequencing by Synthesis (Illumina). Sequencing was undertaken by Macrogen (Korea).

2.4. Taxonomic analysis

Adapter sequences were removed, and an error correction was performed for areas where the two reads overlap using the FastP program (Chen et al., 2018). A paired-end sequence was created by assembling via sequencing both directions of the library. Among the assembled reads, the FLASH (v.1.2.11) program was used to remove reads shorter than 400 bp (Magoč and Salzberg, 2011). FastQC (Babraham Bioinformatics, UK) program was used to check each sample sequence quality. For pre-processing and clustering, data containing sequence errors were removed. After removing the ambiguous base and chimeric sequence containing reads, clustering was performed based on sequence similarity. QIIME2 was used for chimera detection, diversity analysis, rarefaction, and taxonomy information. Amplicon Sequence Variant (ASV) was used with 100% similarity for the formation of taxonomic units. Taxonomy was assigned in QIIME 2 by RDP (Ribosomal Database Project) classifier (Wang et al., 2007) against the RDP database. Rarefaction normalization test was used to be able to compare the different samples.

2.5. Statistical analysis

2.5.1. Microbial diversity

All statistical analysis were conducted using R Studio v.1.2.5033. Alpha diversity indexes: Number of Operational Taxonomic Unit (OTU), Species evenness, Chao1, Shannon, Simpson's, and Inverse Simpson's diversity indexes were calculated with the *vegan* package. Rarefaction curves were drawn based on each sample's OTU richness. The accumulation curves for the species richness were drawn and compared with the "specaccum" function using the *vegan* package. Indicator taxa were detected with "multipatt" function in *Indicspecies* package, based on point-biserial correlation index with 999 permutations to identify, which taxa had significantly different detection frequencies in each season, station, depth, and zone. Microbial indicator taxa were defined as a single or group of family taxon representing a specific community association.

2.5.2. Community structure

Phylum and family level OTU data matrices were Hellinger-transformed. Environmental parameters were normalized to z-scores. Non-metric multidimensional scaling (nMDS) was performed using the "metaMDS" function in *vegan* based on Bray-Curtis similarity measure to visualize and compare community compositions' patterns. Furthermore, Permutational multivariate analysis of variance (PERMANOVA) and Kruskal-Wallis test was implemented to check the community composition differences between stations, seasons, and depths. PERMANOVA was applied in *vegan* using "adonis" based on the algorithm of Anderson (2001). Similarity percentage analysis (SIMPER) was performed in *vegan* using the "simper" function and PAST v4. to identify major phyla that contributed to the community difference between different stations, seasons, and depths with 999 permutations. The OTUs with the highest contribution to community differences and environmental parameters were visually represented on the nMDS ordination using the "envfit" function in *vegan*. The environmental parameter's impact on the microbial community composition was assessed using partial redundancy analysis (pRDA) in *vegan* using the "rda" function.

Mantel test evaluates environmental drivers of microbial community composition based on taxonomic data. Euclidean distance was used for environmental data, while Bray-Curtis distance for taxonomic data. The Mantel test calculated the correlation between ecological and taxonomic data with 9999 permutations in *ggcor* based on calculated environmental and taxonomic distances. Pairwise-Pearson's correlation analysis was implemented to evaluate the significance of the relationship between environmental parameters in *ggcor* using the "corr.test" function.

3. Results And Discussion

A total of 80 seawater samples were collected seasonally at five different depths: 5, 25, 75, 100, and 200 meters, which corresponds to 5 depth strata, from four stations. A total of 8 sediment samples were collected seasonally from two coastal stations (K1 and K3).

3.1. Environmental Parameters

Environmental parameters are presented in Table S1 and Figure S1. Clear vertical stratification was detected in some environmental parameters with different gradients between zones. The surface mixed layer (SML, 5 m) and upper thermocline (TL, 25 m) was characterized by seasonal changes in temperature, whereas the deeper zone was relatively stable. Water temperature and salinity did not differ between sites or with increasing distance from shore. The temperature at SML and TL were warmer during the summer and spring, reflecting seasonal patterns. The temperatures at the mineralization/nitrification zone (MNZ, 75 m) and below were within the same range in all seasons. Salinity and density increased with increasing depth in all seasons. Light dramatically declined between SML and TL. Depths below MNZ appeared to be aphotic. Drastic changes in Chlorophyll-a and dissolved oxygen were observed between TL and MNZ.

The highest silicate concentration was measured in MNZ, significantly different than SMZ. Seasonally, the lowest silicate concentration was measured in summer, and the highest concentration was measured in autumn. The availability of silica limits diatom production. Decreased silicate in summer may be related to the increased abundance of diatoms. The highest nitrate concentration was detected in winter, whereas the lowest in summer. Vertically, in terms of nitrate, SML was the poorest, and TL was the richest zone. Spatially and vertically, nitrate concentration significantly ($P < 0.01$) differed in coastal stations and OMZ. Seasonally, nitrate concentration in autumn and winter was significantly different from spring and summer. Comparatively higher nitrate and lower nitrite in this study indicated the rapid conversion of ammonia to nitrate. The highest phosphate concentration was measured in autumn, which significantly differed from other seasons. Phosphate concentration increased with depth, with the highest concentration in AZ. The lowest ammonia concentration was calculated in autumn, which showed a significant seasonal difference. Vertically highest ammonia concentration was calculated for OMZ, whereas the lowest for TL. Dissolved oxygen concentrations decreased dramatically after TL in all seasons, and hydrogen sulfide concentrations increased exponentially after Oxygen minimum zone (OMZ) and remained relatively constant in all seasons. The highest total organic carbon (TOC) concentrations were measured in surface waters during the summer months, attributed to increased water temperature and phytoplankton growth. Although water quality parameters measured at stations close to the shore (K1 and K3) were considerably higher in most of the cases compared to the offshore stations, differences were insignificant ($p < 0.05$).

3.2. Metabarcoding and Microbial Diversity

The MiSeq paired-end sequencing of the libraries yielded a total of 2,358,752 reads. After assembly of paired-reads, quality filtering, and chimera removal, a total of 2,019,141 valid reads were retrieved from 88 samples. The total number of sequence reads ranged between 157,998 (Summer-K3–25 m) and 285,952 (Spring-K1-SED), with an average of 207,960. The number of raw reads for each library was relatively uniform. Average Phred quality score for Q30 was 98.66.

Metabarcoding successfully detected 23 phyla (Table S2), 45 classes, 102 orders, 208 families (Table S3), 527 genera, and 750 species (Table S4) from a total of 88 samples collected seasonally from 4 different stations at five different depths in each station in the Black Sea. Rarefaction curves of sampling sites were similar, and most reached a plateau, excluding sediment samples, indicating that sampling effort was sufficient to detect microorganisms in the water community (Figure S2). The rarefaction curves of the sediment samples did not form plateau which might indicate that sediment samples is not a good representation of the microbial community. Thus, the accumulation curves failed to reach a plateau (Figure S3). Sampling different layers of sediment would give a better representation of the sediment microbial community. Alpha diversity indices are presented in Table S5 and Figure S4. There are a few metabarcoding studies performed in the Black Sea. Bobrova (Bobrova *et al.*, 2016) detected the presence of 8 bacterial phyla from the surface waters of three estuaries. Zhang *et al.* (2020) conducted the most comprehensive metabarcoding study in which water samples were collected at four vertical layers from 12 stations for once and detected 141 prokaryotic species. Our finding differed from previous studies in which comparatively more diverse microbial communities were found, with higher-level at deeper stations. This could also be due to methodological differences especially in the bioinformatics. Also, the previous study did not have sediment samples which would increase diversity. In this study, prokaryotic reads were assigned to 750 bacteria species. The percentage of taxonomically classified reads was high, which is the expected outcome of seasonal sampling from different water

layers. The present study's annotation results reflected the Black Sea's microbial communities' composition based on the current knowledge and information contained in reference databases. Our findings indicated that the number of observed OTUs and species richness increased from the surface to deeper zones reflecting diversified species adapted to a broader range of ecological niches. An increase in species richness from the surface to deeper layers was also reported previously (Pommier *et al.*, 2010; Haro-Moreno *et al.*, 2018; Xue *et al.*, 2020). However, our findings reflect the global relevance of species richness pattern. The deeper sites (100 and 200 m) had the highest diversity. An increase in bacterial diversity with depth could be explained by the increased availability of different inorganic substances (Arnosti, 2014; Haro-Moreno *et al.*, 2018). Instable environmental parameters, high light intensity, and temperature fluctuations at the surface layer can be extreme for some bacterial taxa, resulting in fewer taxa's survival with higher abundance.

At the phylum level, almost half of the phyla (9 out of 25) represented in each sample (Figure 2a). Overall, the majority of the reads and OTUs belonged to Proteobacteria (40% reads and 58.4% OTUs), Cyanobacteria (19.5% reads and 2.8% OTUs), Planctomycetes (12.2% reads and 2.5 OTUs), Bacteroidetes (10.9% reads and 13.8% OTUs), Verrucomicrobia (9.6 reads and 2.9 OTUs), and Actinobacteria (2.3% reads and 5.6% OTUs) (Table 1, Figure 2b and 2c). The major phyla composition, which represented the highest number of reads and OTUs, was uniform among seasons (Figure 2d) and stations (Figure 2e). Meanwhile, the composition of most phyla differed among depths. Almost 20% of lineages (5 out of 25) were dominant in SML and TL, 48% lineages (12 of 25) in MNZ and OMZ, and 32% lineages (8 of 25) were more abundant in AZ (Figure 2f).

After excluding OTUs with low reads at the family level, 29 out of 76 OTUs were presented in each station (Figure 3a). Overall, the bacterial community was dominated by Prochloraceae, a family of Cyanobacteria (16.9% reads) represented with a single species, *Prochlorococcus marinus*. The remaining majority of the reads and OTUs belonged to Rhodobacteraceae (11.6% reads and 11% OTUs), Planctomycetaceae (10% reads and 0.9% OTUs), and Flavobacteriaceae (7.5% reads and 13.2% OTUs), which is the most diverse family represented with 60 OTUs (Figure 3b and 3c). The composition of the major families represented with the highest number of reads and OTUs was uniform among seasons (Figure 3d) and stations (Figure 3e). Most of the community composition differed with depth at the family level (Figure 3f). Some of the lineages' distribution was non-uniform among different depths with asymmetrical allocations of reads (Figure S5-S10).

A total of 12 families were identified as indicator taxa for the seasonal changes in microbial communities. A detailed list of indicator family taxa for stations, seasons, depths, and zones is tabulated in Table S6. In particular, Cyanobacteria families of Chamaesiphonaceae and Spirulinaceae were among the strongest indicator taxa in the Autumn and Spring season. Anaerolineaceae and Spirulinaceae families were recovered as indicator taxa for station groups of K1 and K1-K3, respectively. However, not all sampling sites belonging to these groups are included in these taxa. We observed zonation in the microbial communities belonging to Desulfobacteraceae, Vicinamibacteraceae, Ectothiorhodospiraceae, *Dehalogenimonas* (Genus), Desulfuromonadaceae, and *Methyloligella* (Genus) being commonly recovered from water samples below MNZ (Sewell *et al.*, 2017; Kato *et al.*, 2019; He *et al.*, 2020), while Lentimicrobiaceae and Desulfobulbaceae were identified as indicator taxa for AZ. The absence or low recovery of Caldilineaceae appeared as an indicator for surface SML. Families of Rhodothermaceae and Mycobacteriaceae were identified as indicator taxa for SML, TL, MNZ, and OMZ, respectively, with low restriction to these zones. On the other hand, four taxa were assigned as an indicator for an anoxic zone. The strongest indicator taxon for AZ was OTUs assigned to Anaerolineaceae. The presence of OTUs belonging to the families, Desulfobacteraceae, Caldilineaceae, and Vicinamibacteraceae were strongly associated with aphotic and anoxic zones (Table S6).

3.3. Variation in microbial community composition

Microbial community composition difference between sampling sites was insignificant ($p > 0.05$). K3 station was chosen to assess whether the copper mining discharge system in that specific region at a depth of 180 meters affected the microbial community structure and composition. However, there was no significant difference between sampling stations (PERMANOVA, $F = 0.927$, $R^2 = 0.031$, $p = 0.538$). Similar communities could be found between long distances as long as no oceanographic front was crossed. A similar absence of horizontal stratification for microbial communities in the Black Sea was recently reported (Zhang *et al.*, 2020). Meanwhile, significant differences were found in community compositions between depths (PERMANOVA, $F = 0.889$, $R^2 = 0.376$, $p = 0.001$) and seasons (PERMANOVA, $F = 3.054$, $R^2 = 0.098$, $p = 0.002$). Vertically, microbial community compositions were significantly different except for SML-TL, MNZ-OMZ, MNZ-AZ, and OMZ-AZ. Additionally, community compositions of the seawaters and sediments were significantly different ($p < 0.0001$). Limited studies have shown vertical stratification of microbial communities in the seawater column

using the metabarcoding technique (Qian *et al.*, 2011; Haro-Moreno *et al.*, 2018; Xue *et al.*, 2020). Seasonally, only winter samples were significantly different from spring samples when all the depth groups were considered whole for each station ($p = 0.0201$). Specifically, community composition in SML in summer was significantly different than in other seasons. Temperature appeared to be a leading cause of the difference between shallow depths. Community composition at remaining zones was insignificant seasonally ($p > 0.05$). Sampling fast-changing unstable surface layer can be problematic. Besides fluctuations in environmental parameters, sampling at different sites and seasons may result in microbial community differences. The grouping of samples (based on Bray-Curtis dissimilarity) displayed on nMDS plots showed similarity in microbial community composition between the four sampling sites (stress: 0.1161) (Figure 4A). In contrast, dissimilarities were observed between seasons and depths (Figure 4B and C). There was a strong influence of sampling depth on microbial community composition, where samples from SML-TL, MNZ-OMZ, and AZ were separately clustered (Figure 4D). Clustering samples from SML and TL were expected since both depths were within the mixed layer. Samples from 75 and 100 m did not join the surface cluster. Moreover, these samples were more similar to the deeper than the surface samples. The grouping of microbial communities at the various season, depths, and zone in each sampling station are presented in Figure S11. Seasonally, microbial community composition in autumn was less tightly clustered within seasonal clustering.

SIMPER analysis revealed that the AZ was differentiated from the other zones by an increase in abundance of taxa belonging to the family Helicobacteraceae. In contrast, decreased abundance of Rhodobacteraceae, Prochloraceae, Verrucomicrobiaceae, and increased abundance of Planctomycetaceae and Nitrospinaceae in the MNZ and OMZ were the major contributor to the dissimilarity between the communities of SML-TL and MNZ-OMZ. The major contributor phyla and families to the dissimilarities between seasons, stations, depth, and zones are tabulated (Figure 4, Table S7-S10).

Among the most abundant phyla, Proteobacteria, Cyanobacteria, Bacteroidetes, and Verrucomicrobia were present in the whole water column. Conversely, Nitrospinae, Chloroflexi, and Kiritimatiellaeota were more restricted, appearing abundantly at 75 meters and deeper layers. While the abundance of Planctomycetes, Acidobacteria, and Firmicutes increased with depth, the abundance of Cyanobacteria, Bacteroidetes, and Balneolaeota decreased with depth. Ecologically distinct microbial taxa occupy different niches. Nevertheless, information about most of the bacterial taxa in the marine environment is limited. The Proteobacteria members' adaptation (Planctomycetes, Bacteroidetes, Verrucomicrobia, and Acidobacteria group) to most water depths spanning the entire chemical gradient could indicate an adaptation to a wide range of chemical stressors and an efficient survival strategy. SML and TL were dominated by Cyanobacteria that perform photosynthesis and nitrogen fixation with light and oxygen. Cyanobacteria were found in all water column and sediment, but their abundance decreased in the deeper layers. A comparatively lower but still high abundance of Cyanobacteria in AZ might be due to the precipitation of dying bacteria from the water column into the sediment. The abundance of Nitrospinae, Chloroflexi, Kiritimatiellaeota, and Planctomycetes were high in MNZ and below, inconsistent with the previous report on the abundance of the Chloroflexi taxa below the chlorophyll maximum zone (Morris *et al.*, 2002; Qian *et al.*, 2011). Iron-reducer (*Geoalkalibacter ferrihydriticus*) and Iron-Manganese reducer (*G. subterraneus*) of the genus *Geoalkalibacter* (Greene *et al.*, 2009) were found at 75 m and below, including sediment. *Geobacter sulfurreducens* was mostly found in the water column where H_2S is present. Since *G. sulfurreducens* produce electricity (Poddar and Khurana, 2011), environmentally friendly microbial fuel cells can be produced (Bond and Lovley, 2003) to create electricity by the degradation of waste products using *G. sulfurreducens* (Poddar and Khurana, 2011).

Phylum Nitrospinae is represented with five species in the Black Sea. *Nitrococcus mobilis* was detected in high abundance in the aphotic and anoxic zones, whereas *N. gracilis* was only detected in the sediment. *N. mobilis* and *N. gracilis* are obligate chemoautotrophic nitrite-oxidizing bacterial species. Enrichment of *N. mobilis* indicates prevalent nitrite cycling in the aphotic and anoxic zones (Watson and Waterbury, 1971). Although the other three obligately anaerobic, thermophilic, sulfate-reducing bacterial species (*Thermodesulfovibrio hydrogeniphilus*, *T. aggregans*, and *T. thiophilus*) were previously isolated from thermophilic environments (Haouari *et al.*, 2008; Sekiguchi *et al.*, 2008), their presence was detected in AZ with water temperature around 8°C.

Sulfate-, sulfur- and sulfite- reducing bacteria produce hydrogen sulfide by reducing different inorganic and organic materials. Sulfur-oxidizing and anoxic bacteria groups were enriched in AZ due to the high H_2S and low O_2 content in these deep waters in the Black Sea. Sulfate-reducing bacteria use sulfate as an electron acceptor during the breakdown of organic material to produce hydrogen sulfide (Rückert, 2016). Sulfate-reducing bacteria families (Helicobacteraceae, Granulosicoccaceae, Desulfobacteraceae, and Desulfobulbaceae) of the phylum Proteobacteria dominated the AZ. Enrichment of these groups is a clear indication of prevalent sulfur cycling. *Shewanella hafniensis* and *Shewanella vesiculosa* that produce H_2S from thiosulfate were found in all water column and sediment. Metabolic activities of identified sulfide reducing bacteria taxa distributed abundantly in sediment and 100–200 meters

might be the source of H₂S in the Black Sea. The presence of strict anaerobic bacterial groups in high abundances, such as the Anaerolineaceae family of the phylum Chloroflexi in AZ, reflects the anoxic properties of these layers. Members of Fusobacteria, Gemmatimonadetes, and Lentisphaerae phyla were absent in the sediment. Distribution and presence of bacterial phyla in AZ were similar, except for Lentisphaerae, found only in waters, and Fibrobacteres found only in sediment.

Based on abundance, the most common five species found in each depth and sediment of the Black Sea were *Prochlorococcus marinus*, *Gimesia maris*, *Kineobacterium sediminis*, *Azotobacter beijerinckii*, and *Planctopirus limnophila*. Genus of *Prochlorococcus* has a single species called *Prochlorococcus marinus*, common in oligotrophic oceans (Partensky *et al.*, 1999). *P. marinus* contains both chlorophyll-a and chlorophyll-b, which supports its growth in deeper waters with low light (Ralf and Repeta, 1992). Chlorophyll-b absorbs blue light and carries out photosynthesis even at 200 m depth as long as blue light penetrates (Zinser *et al.*, 2007). This might be the main reason behind the distribution of photosynthetic cyanobacteria, *P. marinus*, abundantly in all of the Black Sea's sampled depths or environmental DNA of the dead individuals might be another reason behind the presence of photosynthetic cyanobacteria in anoxic zone and aphotic zone. Although *P. marinus* adapt in low phosphate waters by replacing phospholipids membranes to sulfolipids membrane (Van Mooy *et al.*, 2006), phosphate concentrations were not scarce, ranging between 0.02 µM in summer at surface water and 2.5 µM in fall in AZ.

Members of the Planctomycetes are known to colonize both oxic and anoxic layers (Dedysh and Ivanova, 2019). Planctomycetes are an important phylum for carbon and nitrogen cycles in the environment found in fresh, brackish, and marine water (Van Niftrik *et al.*, 2004). *Gimesia maris*, previously known as *Planctomyces maris*, a species of the phylum Planctomycetes, was first isolated from the shallow waters at USA (Ferreira *et al.*, 2016). *Planctopirus limnophila* is a member of the Planctomycetaceae family that do not contain peptidoglycan in their cell wall (Jeske *et al.*, 2015). *G. maris* and *P. limnophila* were recorded in each sampling depth abundantly, yet the research on these species' function is limited. *Kineobacterium sediminis* was first described and isolated from marine sediment (Chang *et al.*, 2019). *K. sediminis* was found in each sampling depth and sediment in the Black Sea. The Genus of *Azotobacter* is mostly a soil bacterium that play a role in the nitrogen cycle by binding atmospheric nitrogen and converting it to ammonium. *Azotobacter beijerinckii* is found in soils and water associated plants (Tejera *et al.*, 2005). It was the fourth most abundant bacterial species in the Black Sea. Although *Azotobacter* fixes atmospheric nitrogen in the soil, its function in the water column is unknown. All the five most abundant species were also found in the sediment of AZ. Since we used molecular techniques to identify microorganisms, both the live and debris of dead organisms could be identified. Thus, their actual presence in the sediment cannot be ascertained.

3.4. Influence of Environmental Parameters on Microbial Community Structure

When the entire water column was considered, the permutation test indicated that temperature, salinity, sigma-t, conductivity, light, chlorophyll-a, O₂, NO₃, NH₃, PO₄, Si, and H₂S appeared to have a significant effect on the vertical bacterial community richness ($p < 0.05$). In contrast, TOC and NO₂ did not have any significant impact ($p > 0.05$) on vertical bacterial community compositions following Bonferroni type correction (Table S11). Among the significant environmental factors, temperature ($r^2 = 0.605$), PO₄ ($r^2 = 0.634$), and O₂ ($r^2 = 0.33$) were found to have the most influence on the overall bacterial community composition. Correlation between each environmental parameter with the microbial community composition at each zone is presented in Figure S12. Correlation between environmental parameters and vertical distribution of bacterial families are presented in Figure 5. Varied correlations were detected between environmental variables and the community structures of samples. Partial redundancy analysis (Figure S13) was performed to assess further the relationship between environmental variables, which revealed a similar set of environmental variables that statistically influenced the microbial community. Environmental parameters are represented by arrows that point toward the direction of variation.

Correlation between environmental parameters (Figure S13) and microbial communities appeared to be different in each zone. In SML and TL, the temperature was positively correlated with conductivity (Pearson's test $R^2 > 0.95$, $p < 0.001$), and salinity negatively correlated with TOC. Mantel test indicated that temperature and conductivity were strongly related to microbial composition (Mantel's $R > 0.5$, $p < 0.01$) (Figure 6A). In MNZ and OMZ, conductivity was positively correlated with salinity and dissolved oxygen (Pearson's test $R^2 > 0.95$, $p < 0.001$). Conversely, oxygen was negatively correlated with conductivity and H₂S. Salinity and H₂S were positively correlated with conductivity. Mantel test indicated that NO₂, NO₃, PO₄, and Si had an influence on community composition (Mantel's $R > 0.25$, $p < 0.01$) (Figure 6B). In AZ, NO₃ was negatively correlated with temperature, salinity, conductivity, and NH₃, whereas conductivity was

positively correlated with temperature and salinity (Pearson's test $R^2 > 0.95$, $p < 0.001$). Mantel test indicated that PO_4 was strongly related to microbial composition (Mantel's $R > 0.5$, $p < 0.01$) (Figure 6C).

The Black Sea is an inland water body with permanent vertical stratification. In terms of water density, there are five well-defined strata in the Black Sea. In this study, seasonal fluctuations and heterogeneity of environmental parameters between the zones led to differences in the microbial community. Microbial diversity and density showed trends consistent with vertical stratification and environmental parameters for some of the taxa. High temperature, light, and dissolved oxygen in the upper zones facilitated the growth of photosynthetic bacteria in high density. However, lack of dissolved oxygen and light and high concentrations of hydrogen sulfide in the anoxic zone facilitated heterotrophic and anaerobic microorganisms in great abundance. OMZ and MNZ appeared to be the most diverse zones. Numerous studies addressed the environmental factors (Langenheder and Ragnarsson, 2007; Zheng *et al.*, 2014) and deterministic partitioning of available resources (Zhang *et al.*, 2009; Yan *et al.*, 2017; Wu *et al.*, 2019), shaping the microbial community compositions. Previous studies showed a strong impact of temperature, light, and dissolved oxygen on vertical stratification of microbial communities rather than geographic position and/or other environmental factors (Sunagawa *et al.*, 2015). Nevertheless, environmental parameters did not appear as an apparent regularity for the entire community assemblages as previously reported (Niño-García *et al.*, 2016).

4. Conclusions

Marine microorganisms influence environmental dynamics and play a key role in marine productivity and biogeochemical processes by the combined effect of countless interactions at a single-cell level. Microbial communities in the Black Sea are under-studied. In this study, 88 metabarcoding libraries were generated to assess the seasonal, vertical, and horizontal variation of microbial community compositions in the Northeastern Black Sea. Our results revealed that microbial communities differ with depth and fluctuate seasonally at upper layers. This study is the first to assess microbial communities seasonally and vertically in the Black Sea and provide insight into microbial ecology in one of the world's unique marine ecosystems. Contrary to popular belief, extreme conditions in the deep sea, such as the absence of light, low dissolved oxygen level, and low temperature, do not limit microbial diversity, confirmed by the comparatively higher diversity in deeper zones.

Declarations

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Conflict of Interest

We do declare that no conflict of interest in financial aspects and no personal relationships in publishing this research work.

Data Availability Statement

The original data are present in the article.

References

1. Agirbas, E., Feyzioglu, A.M., Kopuz, U., and Llewellyn, C.A. (2015) Phytoplankton community composition in the south-eastern Black Sea determined with pigments measured by HPLC-CHEMTAX analyses and microscopy cell counts. *J Mar Biol Assoc United*

2. Albertsen, M., Karst, S.M., Ziegler, A.S., Kirkegaard, R.H., and Nielsen, P.H. (2015) Back to basics - The influence of DNA extraction and primer choice on phylogenetic analysis of activated sludge communities. *PLoS One***10**: e0132783.
3. Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Eco***26**: 32–46.
4. Arnosti, C. (2014) Patterns of Microbially Driven Carbon Cycling in the Ocean: Links between Extracellular Enzymes and Microbial Communities. *Adv Oceanogr***1**: 706082.
5. Bendschneider, K. and Robinson, R.J. (1952) A New Spectrophotometric Method for the Determination of Nitrite in Sea Water. *J Mar Res***11**: 87–96.
6. Besiktepe, S. (2001) Diel vertical distribution, and herbivory of copepods in the south-western part of the Black Sea. *J Mar Syst***28**: 281–301.
7. Besiktepe, S. and Unsal, M. (2000) Population structure, vertical distribution and diel migration of *Sagitta setosa* (Chaetognatha) in the south-western part of the Black Sea. *J Plankton Res***22**: 669–683.
8. Bobrova, O., Kristoffersen, J.B., Oulas, A., and Ivanytsia, V. (2016) Metagenomic 16s rRNA investigation of microbial communities in the Black Sea estuaries in South-West of Ukraine. *Acta Biochim Pol***63**: 315–319.
9. Bond, D.R. and Lovley, D.R. (2003) Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Appl Environ Microbiol***69**: 1548–1555.
10. Chang, Y.Q., Meng, X., Du, Z.Z., and Du, Z.J. (2019) *Kineobacterium sediminis* gen. Nov., sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol***69**: 2395–2400.
11. Chen, S., Zhou, Y., Chen, Y., and Gu, J. (2018) Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics***34**: 884–890.
12. Cline, J.D. (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol Oceanogr***14**: 454–458.
13. Dedysh, S.N. and Ivanova, A.A. (2019) Planctomycetes in boreal and subarctic wetlands: Diversity patterns and potential ecological functions. *FEMS Microbiol Eco***95**: 227.
14. Ferreira, C., Soares, A.R., Lamosa, P., Santos, M.A., and da Costa, M.S. (2016) Comparison of the compatible solute pool of two slightly halophilic planctomycetes species, *Gimesia maris* and *Rubinisphaera brasiliensis*. *Extremophiles***20**: 811–820.
15. Fuchsman, C.A., Kirkpatrick, J.B., Brazelton, W.J., Murray, J.W., and Staley, J.T. (2011) Metabolic strategies of free-living and aggregate-associated bacterial communities inferred from biologic and chemical profiles in the Black Sea suboxic zone. *FEMS Microbiol Eco***78**: 586–603.
16. Greene, A.C., Patel, B.K.C., and Yacob, S. (2009) *Geoalkalibacter subterraneus* sp. nov., an anaerobic Fe(III)- and Mn(IV)-reducing bacterium from a petroleum reservoir, and emended descriptions of the family Desulfuromonadaceae and the genus *Geoalkalibacter*. *Int J Syst Evol Microbiol***59**: 781–785.
17. Haouari, O., Fardeau, M.L., Cayol, J.L., Fauque, G., Casiot, C., Elbaz-Poulichet, F., et al. (2008) *Thermodesulfovibrio hydrogeniphilus* sp. nov., a new thermophilic sulphate-reducing bacterium isolated from a Tunisian hot spring. *Syst Appl Microbiol***31**: 38–42.
18. Haro-Moreno, J.M., López-Pérez, M., de la Torre, J.R., Picazo, A., Camacho, A., and Rodríguez-Valera, F. (2018) Fine metagenomic profile of the Mediterranean stratified and mixed water columns revealed by assembly and recruitment. *Microbiome***6**: 128.
19. He, P., Xie, L., Zhang, X., Li, Jiang, Lin, X., Pu, X., et al. (2020) Microbial Diversity and Metabolic Potential in the Stratified Sansha Yongle Blue Hole in the South China Sea. *Sci Rep***10**: 5949.
20. Jeske, O., Schüller, M., Schumann, P., Schneider, A., Boedeker, C., Jogler, M., et al. (2015) Planctomycetes do possess a peptidoglycan cell wall. *Nat Commun***6**: 7116.
21. Ji, B., Liang, J., Ma, Y., Zhu, L., and Liu, Y. (2019) Bacterial community and eutrophic index analysis of the East Lake. *Environ Pollut***252**: 682–688.
22. Kara, A.B., Rochford, P.A., and Hurlburt, H.E. (2000) An optimal definition for ocean mixed layer depth. *J Geophys Res Ocean***105**: 16803–16821.
23. Kato, S., Hirai, M., Ohkuma, M., and Suzuki, K. (2019) Microbial metabolisms in an abyssal ferromanganese crust from the Takuyo-Daigo Seamount as revealed by metagenomics. *PLoS One***14**: e0224888.
24. Langenheder, S. and Ragnarsson, H. (2007) The role of environmental and spatial factors for the composition of aquatic bacterial communities. *Ecology***88**: 2554–2561.

25. Learman, D.R., Henson, M.W., Thrash, J.C., Temperton, B., Brannock, P.M., Santos, S.R., et al. (2016) Biogeochemical and microbial variation across 5500 km of Antarctic surface sediment implicates organic matter as a driver of benthic community structure. *Front Microbio***7**: 284.
26. Li, A.-Z., Han, X.-B., Zhang, M.-X., Zhou, Y., Chen, M., Yao, Q., and Zhu, H.H. (2019) Culture-Dependent and -Independent Analyses Reveal the Diversity, Structure, and Assembly Mechanism of Benthic Bacterial Community in the Ross Sea, Antarctica. *Front Microbio***10**: 2523.
27. Magoč, T. and Salzberg, S.L. (2011) FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics***27**: 2957–2963.
28. Van Mooy, B.A.S., Rocap, G., Fredricks, H.F., Evans, C.T., and Devol, A.H. (2006) Sulfolipids dramatically decrease phosphorus demand by picocyanobacteria in oligotrophic marine environments. *Proc Natl Acad Sci U S A***103**: 8607–8612.
29. Morris, R.M., Rappé, M.S., Connon, S.A., Vergin, K.L., Siebold, W.A., Carlson, C.A., and Giovannoni, S.J. (2002) SAR11 clade dominates ocean surface bacterioplankton communities. *Nature***420**: 806–810.
30. Mullin, J.B. and Riley, J.P. (1955) The colorimetric determination of silicate with special reference to sea and natural waters. *Anal Chim Acta***12**: 162–176.
31. Murray, J.W., Jannasch, H.W., Honjo, S., Anderson, R.F., Reeburgh, W.S., Top, Z., et al. (1989) Unexpected changes in the oxic/anoxic interface in the Black Sea. *Nature***338**: 411–413.
32. Van Niftrik, L.A., Fuerst, J.A., Sinninghe Damsté, J.S., Kuenen, J.G., Jetten, M.S.M., and Strous, M. (2004) The anammoxosome: An intracytoplasmic compartment in anammox bacteria. *FEMS Microbiol Lett***233**: 7–13.
33. Niño-García, J.P., Ruiz-González, C., and Del Giorgio, P.A. (2016) Interactions between hydrology and water chemistry shape bacterioplankton biogeography across boreal freshwater networks. *ISME J***10**: 1755–1766.
34. Oguz, T. (2005) Black Sea ecosystem response to climatic teleconnections. *Oceanography***18**: 122–133.
35. Partensky, F., Hess, W.R., and Vaulot, D. (1999) Prochlorococcus, a Marine Photosynthetic Prokaryote of Global Significance. *Microbiol Mol Biol Rev***63**: 106–127.
36. Poddar, S. and Khurana, S. (2011) Geobacter: The Electric Microbe! Efficient Microbial Fuel Cells to Generate Clean, Cheap Electricity. *Indian J Microbiol***51**: 240–241.
37. Pommier, T., Neal, P.R., Gasol, J.M., Coll, M., Acinas, S.G., and Pedrós-Alió, C. (2010) Spatial patterns of bacterial richness and evenness in the NW Mediterranean Sea explored by pyrosequencing of the 16S rRNA. *Aquat Microb Ecol***61**: 221–233.
38. Qian, P.Y., Wang, Y., Lee, O.O., Lau, S.C.K., Yang, J., Lafi, F.F., et al. (2011) Vertical stratification of microbial communities in the Red Sea revealed by 16S rDNA pyrosequencing. *ISME J***5**: 507–518.
39. Ralf, G. and Repeta, D.J. (1992) The pigments of Prochlorococcus marinus: The presence of divinylchlorophyll a and b in a marine procaryote. *Limnol Oceanogr***37**: 425–433.
40. Román, S., Ortiz-álvarez, R., Romano, C., Casamayor, E.O., and Martin, D. (2019) Microbial community structure and functionality in the deep sea floor: Evaluating the causes of spatial heterogeneity in a submarine canyon system (NW Mediterranean, Spain). *Front Mar Sci***6**: 108.
41. Rückert, C. (2016) Sulfate reduction in microorganisms – recent advances and biotechnological applications. *Curr Opin Microbio***33**: 140–146.
42. Saydam, C., Tugrul, S., Basturk, O., and Oguz, T. (1993) Identification of the oxic /anoxic interface by isopycnal surfaces in the black Sea. *Deep Sea Res Part I Oceanogr Res Pap***40**: 1405–1412.
43. Sekiguchi, Y., Muramatsu, M., Imachi, H., Narihiro, T., Ohashi, A., Harada, H., et al. (2008) Thermodesulfovibrio aggregans sp. nov. and Thermodesulfovibrio thiophilus sp. nov., anaerobic, thermophilic, sulfate-reducing bacteria isolated from thermophilic methanogenic sludge, and emended description of the genus Thermodesulfovibrio. *Int J Syst Evol Microbio***58**: 2541–2548.
44. Sewell, H.L., Kaster, A.-K., and Spormann, A.M. (2017) Homoacetogenesis in Deep-Sea Chloroflexi, as Inferred by Single-Cell Genomics, Provides a Link to Reductive Dehalogenation in Terrestrial Dehalococcoidetes. *MBio***8**.
45. Sharp, J.H. (1973) Total organic carbon in seawater - comparison of measurements using persulfate oxidation and high temperature combustion. *Mar Chem***1**: 211–229.
46. Solorzano, L. (1968) Determination of ammonia in natural waters by the phenylhypochlorite method. *Limnol Oceanogr***17**: 799–801.

47. Sonthiphand, P., Ruangroengkulrith, S., Mhuantong, W., Charoensawan, V., Chotpantarat, S., and Boonkaewwan, S. (2019) Metagenomic insights into microbial diversity in a groundwater basin impacted by a variety of anthropogenic activities. *Environ Sci Pollut Res***26**: 26765–26781.
48. Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K., Salazar, G., et al. (2015) Structure and function of the global ocean microbiome. *Science (80-)***348**: 1261359–1261368.
49. Tamminen, T. and Kuosa, H. (1998) Eutrophication in Planktonic Ecosystems: Food Web Dynamics and Elemental Cycling, Springer Science.
50. Tejera, N., Lluch, C., Martinez-Toledo, M. V., and González-López, J. (2005) Isolation and characterization of Azotobacter and Azospirillum strains from the sugarcane rhizosphere. *Plant Soil***270**: 223–232.
51. Tomczak, M. and Godfrey, J.S. (1994) Adjacent seas of the Atlantic Ocean. In *Regional Oceanography: An Introduction*. Elsevier Ltd., pp. 297–327.
52. Tugrul, S., Basturk, O., Saydam, C., and Yilmaz, A. (1992) Changes in the hydrochemistry of the Black Sea inferred from water density profiles. *Nature***359**: 137–139.
53. W., M.J., Codispoti, L.A., and Friederich, G.E. (1995) Oxidation-Reduction Environments: The suboxic zone in the Black Sea. In *Aquatic Chemistry*. Huang, C.P., O'melia, C.R., and Morgan, J.J. (eds). Washington: American Chemical Society, pp. 157–176.
54. Watson, S.W. and Waterbury, J.B. (1971) Characteristics of two marine nitrite oxidizing bacteria, Nitrospina gracilis nov. gen. nov. sp. and Nitrococcus mobilis nov. gen. nov. sp. *Arch Mikrobiol***77**: 203–230.
55. Wu, X., Wang, Y., Zhu, Y., Tian, H., Qin, X., Cui, C., et al. (2019) Variability in the Response of Bacterial Community Assembly to Environmental Selection and Biotic Factors Depends on the Immigrated Bacteria, as Revealed by a Soil Microcosm Experiment. *mSystems***4**: e00496-19.
56. Xue, C.-H., Liu, J., Lea-Smith, D.J., Rowley, G., Lin, H., Zheng, Y., et al. (2020) Insights into the Vertical Stratification of Microbial Ecological Roles across the Deepest Seawater column on Earth. *Microorganisms***8**: 1309.
57. Yan, Q., Stegen, J.C., Yu, Y., Deng, Y., Li, Xinghao, Wu, S., et al. (2017) Nearly a decade-long repeatable seasonal diversity patterns of bacterioplankton communities in the eutrophic Lake Donghu (Wuhan, China). *Mol Eco***26**: 3839–3850.
58. Zhang, Q.G., Buckling, A., and Godfray, H.C.J. (2009) Quantifying the relative importance of niches and neutrality for coexistence in a model microbial system. *Funct Eco***23**: 1139–1147.
59. Zhang, Y., Pavlovska, M., Stoica, E., Prekrasna, I., Yang, J., Slobodnik, J., et al. (2020) Holistic pelagic biodiversity monitoring of the Black Sea via eDNA metabarcoding approach: From bacteria to marine mammals. *Environ Int***135**: 105307.
60. Zheng, B., Wang, L., and Liu, L. (2014) Bacterial community structure and its regulating factors in the intertidal sediment along the Liaodong Bay of Bohai Sea, China. *Microbiol Res***169**: 585–592.
61. Zinser, E.R., Johnson, Z.I., Coe, A., Karaca, E., Veneziano, D., and Chisholm, S.W. (2007) Influence of light and temperature on Prochlorococcus ecotype distributions in the Atlantic Ocean. *Limnol Oceanogr***52**: 2205–2220.
62. Zorz, J., Willis, C., Comeau, A.M., Langille, M.G.I., Johnson, C.L., Li, W.K.W., and LaRoche, J. (2019) Drivers of regional bacterial community structure and diversity in the northwest atlantic ocean. *Front Microbio***10**: 281.

Tables

Table 1. Sampling, number of phyla, abundance, and number of species of bacteria and archaea sampled at different water column and sediment.

Bacteria					Achaea			
Phylum Abundance (%)					Species (749)	Phylum (2)	Phylum Abundance (%)	Species (3)
Sampling	Phylum (24)	First	Second	Third			Most abundant	
5 m	15	Proteobacteria (34.2)	Cyanobacteria (25.2)	Bacteroidetes (12.5)	397		N.A.	
25 m	16	Proteobacteria (37.4)	Cyanobacteria (23.2)	Bacteroidetes (12.7)	414		N.A.	
75 m	17	Proteobacteria (30.8)	Planctomycetes (13.2)	Cyanobacteria (13.1)	419	1	Thaumarchaeota (0.03%)	2
100 m	19	Proteobacteria (31.2)	Planctomycetes (15.0)	Cyanobacteria (9.5)	498	1	Thaumarchaeota (0.04%)	2
200 m	21	Proteobacteria (33.8)	Planctomycetes (15%)	Cyanobacteria (10.0)	506	1	Thaumarchaeota (0.03%)	2
Sediment	21	Proteobacteria (25.7)	Cyanobacteria (21.6)	Chloroflexi (13.0)	485	1	Euryarchaeota (0.25%)	1

Figures

Figure 1

Sampling stations in the Black Sea

Figure 2

Taxonomic distributions of reads at phylum level. Occupancy (A) is expressed as the number of stations (88 in total) in which the phylum observed. Log-transformed number of reads (B) and Log-transformed OTUs (C). Distribution of most abundant phyla across seasons (D), stations (E) and depths (F).

Figure 3

Taxonomic distributions of reads at family level. Occupancy (A) is expressed as the number of stations (88 in total) in which the family observed. Log-transformed number of reads (B) and Log-transformed OTUs (C). Distribution of most abundant families which are represented with more than 1000 reads across seasons (D), stations (E) and depths (F).

Figure 4

Two-dimensional Nonmetric multidimensional scaling (NMDS) ordination of microbial communities detected at different stations (A), seasons (B), depths (C), and (zones). Black lines represent the bacteria families responsible for causing the major differences. Strong predictors have longer lines than weak predictors.

Figure 5

Two-dimensional Nonmetric multidimensional scaling (NMDS) ordination of microbial communities detected at different depths. Black lines represent environmental parameters responsible for causing the major differences. Strong predictors have longer lines than weak predictors. TOC and NO₂ did not exhibit significant relationships with bacterial community composition following the Bonferroni type correction and excluded.

Figure 6

Pairwise comparisons of environmental parameters and relationships of environmental parameters with water microbial communities in 5-25 meters (a), 75-100 meters (b), and 200 meters (c) by partial mantel tests. The color gradients and circle sizes represent Pearson's correlation coefficient. Red color indicates a positive correlation and blue indicates a negative correlation. Insignificant correlations were not shown. Line thickness corresponds to the Mantel's r statistics and edge color denotes the statistical significance.

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