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Temporal and Spatial Variations of the Bacterial Diversity in a Deep Alkaline Lake

E. Gozde Ozbayram *, Latife Köker, Ayça Oğuz Çam, Reyhan Akçaalan and Meriç Albay

Department of Marine and Freshwater Resources Management, Faculty of Aquatic Sciences, Istanbul University, Fatih, Istanbul 34134, Turkey

* Correspondence: gozde.ozbayram@istanbul.edu.tr; Tel.: +90-212-440-00-00

Abstract: This study aimed to investigate the effects of thermal stratification and complete mixing conditions on the bacterial diversity of a deep alkaline lake. For this purpose, the water and sediment samples were collected during the winter turnover and stratification periods, and bacterial communities were assessed by metabarcoding. The results showed that temperature shaped the bacterial community patterns in the lake. While the bacterial communities of the water samples showed high similarity during the mixing period, communities had distinctive patterns in the epilimnion and hypolimnion during stratification. The diversity and evenness of the bacterial communities increased with depth, whereas the bacterial communities of sediments were more even and diverse than in water. Proteobacteria members dominated the sediment communities representing 41% to 62% of the total reads in the samples. Particularly, Gammaproteobacteria was the major class found in the sediment; higher abundances were recorded in the mixing period representing 33–51%. Additionally, Actinobacteria species were more abundant in the water samples representing 22–52% of all reads during the stratification period. Due to the complete mixing conditions in the lake, a homogenized bacterial community structure was observed in the lake with minor spatial changes, and a clear divergence was observed between epilimnion and hypolimnion. On the other hand, the sediment bacterial community showed a more stable profile.

Keywords: bacterial composition; high throughput sequencing; metabolic potential; sediment; water quality; temporal variation; Lake Iznik

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1. Introduction

Freshwater ecosystems, lakes, and reservoirs are among the most important aquatic environments, having a part in the biogeochemical cycles and providing ecosystem services such as water supply, irrigation, recreation, etc. [1,2]. Within these habitats, there are dynamic ecological processes in which microorganisms are involved in organic material degradation, nutrient remineralization, and energy flow [2,3]. These processes and microbial diversity are highly affected by changes in environmental conditions [4,5].

In temperate climates, stratification occurs in most lakes during warmer periods in which three thermal layers, epilimnion, metalimnion, and hypolimnion, are formed [6,7]. During the mixing period, a complete homogeneity occurs throughout the water column showing similar physicochemical features as well as community profiles vertically. On the other hand, stratification results in a shift in bacterial diversity [7], and stratified water bodies are considered one of the most suitable ecosystems for assessing the effects of environmental factors on community structures [8]. The sampling at different time intervals and from multiple points and gradients will provide comprehensive information about the microbial community interactions in the environment [9]. The extreme environments including alkaline freshwaters represent a great microbial resource for researchers conducting biotechnology studies regarding the organisms adapted to live there [10].

Next-generation sequencing technologies have emerged as powerful platforms for revealing actual microbial communities in various environments with high coverage, and the advancement in bioinformatics has led to a proliferation of studies that use these technologies in several water bodies [5,11,12]. Some bioinformatic tools, such as PICRUSt, also enable the prediction of the functional properties of the microbial communities from 16S rRNA gene-based taxonomic information and contribute to a deeper understanding of the ecosystem [11,13].

This paper explores the influence of stratification and complete mixing conditions on the bacterial community structures of an alkaline lake. The specific objectives of this study were (i) to evaluate the bacterial community profiles in the surface water and sediment of the lake during the winter turnover and stratification periods, (ii) to compare the bacterial communities throughout the water column collected from epilimnion, metalimnion, and hypolimnion layers, and (iii) to reveal the functional potentials and diversity of bacteria in the lake. The findings of this study may provide an important contribution to advancing our knowledge of bacterial community dynamics and environmental changes in an extreme freshwater habitat.

2. Materials and Methods

2.1. Site Description

Lake Iznik is the fifth largest lake in Turkey and is located in the southeast of the Marmara region. It has a surface area of approximately 313 km^2 with an average depth of 40 m (max. depth is 80 m) [14] and a 12,200 million m^3 water volume [15]. The lake is fed by five streams, Karasu, Sölöz, Orhangazi, Kuru, and Ekinlik, and it has only one outlet, the Karsak stream [16]. The lake area is under the Marmara transition climate, which has warmer winters and dry summers [17]. It is an alkaline lake with an average pH around 9.2 [5,18]. Bicarbonate, carbonate, and sodium are major components of the lake, as it is located in the northernmost tectonic division of Turkey [19]. Human activities such as agriculture, irrigation, industry, and recreation have been changing the water quality over the last 30 years [18,20]. There are more than 45 villages around the lake that affect the trophic status of the lake [15,21], which is recorded as mesotrophic [5].

2.2. Sample Collection and Physicochemical Analysis

Sampling sites were selected from densely populated regions, such as Boyalıca (St. 1), İznik (St. 2), Narlıca (St. 3), Sölöz (St. 4), and Orhangazi, which also has an industrial estate (St. 5). Water and sediment sampling were performed in 6 sites (5 located on the shore and 1 in the middle of the lake) in February 2020, when the lake was completely mixed, and August 2020, when the lake was stratified (Figure 1).

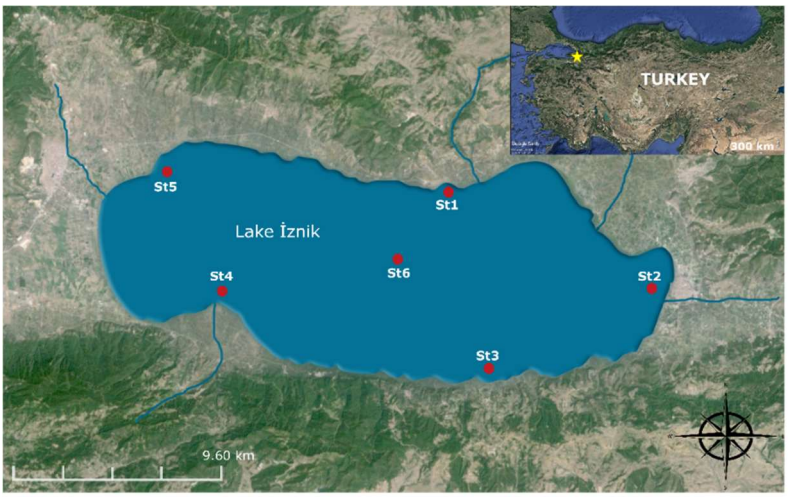

Figure 1. Sampling sites of Lake İznik.

The water samples collected from the surface (0.1 m) and bottom (40 m) layers of the lake were filtered from a 0.22 µm filter on site, and the top layer of sediment samples was taken using an Ekman grab and put into sterile falcon tubes from the lake ground. Water temperature, pH, dissolved oxygen (DO), and conductivity were measured in situ via a portable multiparameter (650 MDS, YSI, Yellow Springs, OH, USA) at each sampling site. All samples were kept under dark and cold conditions and brought to the laboratory immediately. Total nitrogen (TN), the nitrogen species, namely ammonium (NH_4^+-N), nitrate (NO_3^--N), nitrite (NO_2^--N), and total phosphorus (TP), as well as soluble reactive phosphorus (SRP), were analyzed following the procedures of the American Public Health Association [22]. Chlorophyll-*a* (chl-*a*) was measured using the method of ISO 10260 [23].

In order to determine the trophic status of the lake, the Carlson model was used based on the Secchi disk depth, chl-*a*, and total phosphorus [24].

2.3. DNA Extraction and Amplicon Sequencing

The total genomic DNAs were isolated from sediment and filter papers using the NucleoSpin® Soil Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The concentrations of the extracted DNAs were quantified by NanoDrop 1000 (Thermo Fisher Scientific Inc., Waltham, MA, USA). The DNAs were stored at −20 °C for further analysis.

The bacterial community profiles of the samples were analyzed via 16S rRNA gene-targeted sequencing using Illumina® MiSeq™. First, the amplicon sequencing library was prepared using bacteria-specific primers 341F (5′-CCTACGGGNGGCWGCAG-3) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) targeting the V3-V4 region of the 16S rRNA gene [25]. The purified libraries were then quantified and sequenced on the MiSeq™ instrument (Illumina®, San Diego, CA, USA) using 300 bp paired-end chemistry. CASAVA, data analysis software, was used for demultiplexing and clipping of sequence adapters from raw sequences (Illumina®, USA).

The raw sequences were deposited in the EMBL-EBI database under accession number PRJEB44909.

2.4. Sequence Analysis

The bioinformatics analysis was carried out using the amplicons sequencing workflow of QIIME2 v2020.2 [26]. PCR primers were removed from sequences using the cutadapt plugin [27]. Then, paired-end reads were joined (vsearch join-pairs), quality filtered (quality-filter q-score-joined), and the sequences were denoised using deblur (deblur denoise-16S) [28]. Taxonomy was assigned to each amplicon sequence variant (ASV) using the 'feature-classifier classify-sklearn' plugin against the pre-trained naive Bayes classifier specific for 16S rRNA (classifier_silva_132_99_16S_V3.V4_341F_805R.qza) [29]. The final ASV tables were used to calculate alpha diversity metrics including Shannon, Faith's PD, and Pielou's evenness. The beta-diversity index was calculated to reveal the similarity of bacterial communities between the stratification and mixing period.

The bacterial metabolic functions were predicted via PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of the Unobserved States, Galaxy version 1.0.0) and annotated via KEGG (Kyoto Encyclopedia of Genes and Genomes) [13]. For this analysis, the sequence data were analyzed using the Greengenes database (version 13.5) and further processed in the Galaxy platform. The functions were categorized under three subgroups and delivered as metabolism, cellular processes, and environmental processing based on different KEGG [30].

To identify the significant differences between the abundances of bacterial phyla, Statistical Analysis of Metagenomic Profiles (STAMP) software was used [31]. Two groups were determined as mixing and stratification, and the Welch's test was performed for these groups, setting the *p*-value at 0.05.

Correlations between the physicochemical parameters measured in the mixing and stratification periods were calculated using Pearson's correlation method with the level of

significance as $p < 0.001$ and $p < 0.05$. The analyses were carried out in R version 4.0.3 using the "corrplot" package [32].

3. Results

3.1. Environmental Conditions

The water quality of the lake during the sampling periods is shown in Table 1. Water temperature ranged from 25.1 to 29.1 °C in surface waters and drastically declined and was measured as 9.4 °C at 40 m in the stratification period. The concentration of dissolved oxygen varied between 8.9 and 12.8 mg/L in the surface waters during the study period; however, the lowest value was measured in 40 m (1.04 mg/L) during the stratification period. Electrical conductivity (EC) values varied between 748 and 996 µS/cm and 708 and 1035 µS/cm during the mixing and stratification periods, respectively. pH varied between 8.29 and 9.67. High alkalinity values were observed in all sites in the range of 352–478 mg/L $CaCO_3$. SRP, DIN, and SiO_2 tended to rise in the mixing period and decline in the stratification period. Throughout the sampling periods, sulfate (SO_4) concentrations ranged from 9.13 to 13.95 mg/L, and lower values were detected in the mixing period. The chl-*a* values were significantly higher in the stratification period in the surface water in comparison to the mixing period as a result of the vegetation time of the year. The concentration of all nutrients was relatively high in 40 m at site 6. The Carlson Trophic State Index (TSI) was established according to TP, chl-*a*, and Secchi disk depth and pointed mesotrophic conditions.

Table 1. Water quality of the lake during the sampling periods.

Parameter		Mixing Period							Stratification Period						
		St_1	St_2	St_3	St_4	St_5	St_6	St_6_40 m	St_1	St_2	St_3	St_4	St_5	St_6	St_6_40 m
Temperature	°C	9.38	10.17	9.54	10.23	10.12	9.99	9.19	25.28	29.11	27.79	29.22	25.06	25.76	9.37
EC	µS/cm	748	996	928	937	760	748	734	875	756	746	761	708	1035	398
pH		8.29	9.26	9.05	9.64	8.56	8.58	8.39	8.74	9.39	9.57	9.67	9.19	9.1	7.7
DO	mg/L	10.3	10.12	10.77	11.65	10.53	11.08	9.72	8.92	12.76	11.48	9.49	9.01	9.26	1.04
SRP	µg/L	22.97	13.35	16.90	10.48	17.91	18.25	19.77	2.12	2.27	1.53	1.68	3.15	1.09	71.2
TP	µg/L	34.21	38.63	31.75	30.89	32.49	29.91	30.77	28.43	50.78	32.31	34.72	27.49	25.75	119.7
DIN	mg/L	0.35	0.35	0.25	0.39	0.34	0.31	0.35	0.17	0.15	0.14	0.17	0.19	0.14	0.25
SiO_2	mg/L	1.59	1.48	0.93	2.92	1.51	1.58	1.79	1.33	0.89	1.22	1.16	1.28	1.32	3.05
Chl-*a*	µg/L	9.32	8.44	8.88	8.44	2.66	5.33	5.33	14.21	14.80	14.21	13.32	14.21	11.84	2.22
SO_4	mg/L	9.92	10.26	9.34	13.95	9.13	9.52	9.39	11.30	10.97	10.86	10.84	10.46	10.62	9.42
Alkalinity	mg/L $CaCO_3$	455	453	451	454	478	475	443	366	357	358	354	355	352	325

The results of the correlational analysis for the water quality parameters are illustrated in Figure 2. The temperature was strongly and negatively correlated with nitrogen species, NH_4^+-N and NO_2^--N, alkalinity, and phosphate. On the other hand, a positive correlation was found between temperature and chlorophyll-*a* ($p < 0.001$). There was a significant negative correlation between chlorophyll-*a* and the chemical parameters NH_4^+-N, NO_3^--N, alkalinity, and phosphate. pH was only correlated with phosphate ($p < 0.1$), and no statistically significant difference was observed between pH and other parameters. Among all physicochemical parameters, dissolved oxygen was only significantly correlated with total phosphorus levels ($p < 0.1$). There was a clear separation of the samples according to the sampling period. While the samples collected during the mixing period grouped closely on one part of the graph, the samples, taken during the stratification period, were discrete on the other side (Figure 2B). Furthermore, nutrients and alkalinity levels had a clear effect during the mixing period compared with that of stratification.

3.2. Bacterial Community Compositions

Figure 3 illustrates the beta diversity displayed as a two-dimensional plot of a three-dimensional principal component analysis. There was a clear separation of the bacterial communities inhabiting the water and sediment. While the sediment samples were grouped regardless of the sampling period, there was a clear clustering in the water samples. The bacterial communities of the water samples showed high similarity during the mixing period in which the surface waters and bottom water had similar bacterial community

profiles. On the other hand, during the stratification period, the data point for the bottom layer of water was located distantly showing distinctive community structures from surface waters and sediment communities.

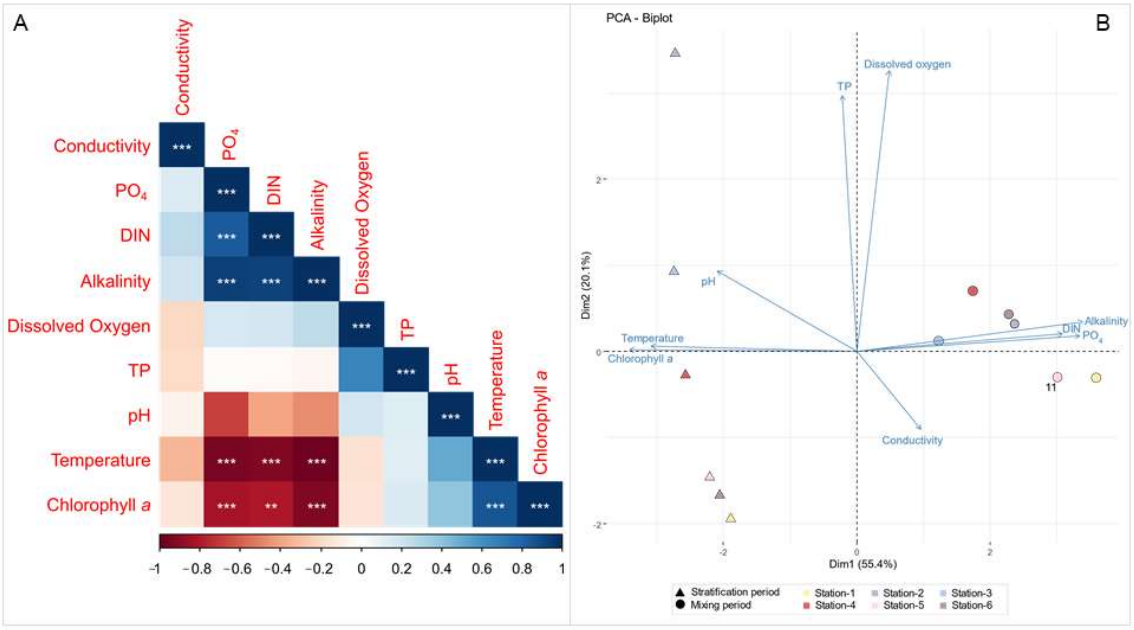

Figure 2. (**A**) The correlations for the environmental parameters. Blue and red colors stand for the positive and negative correlations, respectively, in which the intensity is proportional to the correlation coefficient (asterisks indicate significant correlations: ** $p < 0.05$. *** $p < 0.001$). (**B**) Variable-factor map (colors indicate the sampling sites, shapes indicate the sampling periods).

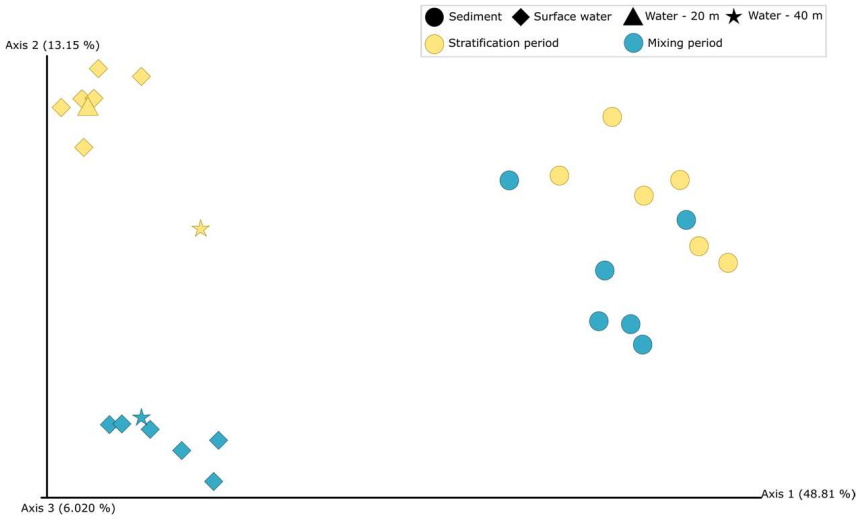

Figure 3. Two-dimensional plot of a three-dimensional principal component analysis based on the beta diversity of bacterial communities (calculated using Bray–Curtis, colors indicate the sampling periods, shapes indicate the sample types).

The bacterial community patterns of the water and sediment samples during the mixing and stratification period are presented at the phylum level in Figure 4A. In general, while Proteobacteria was the most dominant phylum in the sediment samples, Actinobacteria and Bacteroidetes species were more abundant in the water samples.

Figure 4. (**A**) The relative abundance of the main bacterial phyla during the mixing and stratification period of the lake (w: water, s: sediment), in which the taxa with a relative abundance <1% are merged and named as other. Differential analysis indicates the significant phyla during the mixing and stratification periods: (**B**) comparison of the water samples; (**C**) comparison of the sediment samples at the confidence interval of 95% (p-value < 0.05) (* indicates significant difference).

Proteobacteria represented 26–33% of all bacterial communities of the surface water during the mixing period, and the relative abundances ranged between 18% and 46% during the stratification period. Whereas the relative abundance showed similarity between the surface water and bottom water layers during the mixing period (33%), a significant gradual increase throughout the water column was observed during the stratification period (18%, 26%, and 46% in the surface water, 20 m, and 40 m, respectively). In general, higher abundances of Bacteroidetes were observed during the mixing period compared to the stratification period. While the greatest Bacteroidetes abundances were recorded in the surface waters in site 1 (34%) and site 3 (32%), 27% of the total reads were assigned to Bacteriodetes species in the water samples taken from 40 m during mixing conditions. Actinobacteria represented 21–32% and 22–52% of all reads during the mixing and stratification period, respectively, and a decreasing trend in the abundance of Actinobacteria species was observed through the water column during the stratification period. Firmicutes members were generally not abundant in water samples. In all, 20% of the bacterial reads were assigned to Firmicutes in site 2 and site 4 during the mixing period. On the other hand, the highest percentage was observed in site 3 in the stratification period (10%). Cyanobacteria represented $\leq$1% of all reads during the mixing period; it reached 4% in site 1 and 6% in site 4 during the stratification. The members of Verrucomicrobia were enriched during the

stratification, and the highest abundance reached 11% in site 1. Whereas Acidobacteria was not determined in water during the stratification period, it comprised 1–2% of the bacterial communities in the mixing period.

In the sediment, Proteobacteria species represented more than 50% of all sequences in the mixing period (ranged 51–62%), and relatively lower abundances were observed during the stratification (ranged 41–53%). Noticeably, Acidobacteria and Chloroflexi species were enriched in the stratification period, and the abundances were almost doubled compared to the mixing conditions. Bacteroidetes members comprised 5–12% of the bacterial community of the sediment. Similarly, the relative abundance of Actinobacteria was 4–12%. Nitrospirae species were abundant in the sediment collected from the deepest point of the lake (site 6) and represented 10–11% of the total reads during the mixing and stratification conditions, respectively. By contrast, in the other sampling points, the relative abundance ranged from 1% to 6%. Interestingly, a higher abundance of Cyanobacteria was detected (11% in the mixing and 6% in the stratification periods) in the sediment of site 4. The highest abundances of Verrucomicrobia were observed in the samples collected from site 3, representing 15% and 6% of the total reads during the mixing and stratification conditions, respectively.

The taxonomic profile during the mixing and stratification periods was compared for water and sediment samples (Figures 4B and 4C, respectively) at the phylum level. The biomarkers for the water samples were identified as Acidobacteria, Nitrospirae, Verrucomicrobia, and Cyanobacteria ($p < 0.05$). By contrast, more taxa were determined with significant differences in sediment, and Acidobacteria was identified as a biomarker similar to the water samples. Further, Chloroflexi, Latescibacteria, Nitrospinae, Planctomycetes, Rukobacteria, Kiritimatiellaeota, Patescibacteria, and Proteobacteria revealed significant differences during the mixing and stratification period in bacterial communities of sediment.

The dominant classes differed according to the sample type and period (Figure 5). During the mixing conditions, the bacterial community of the water was dominated by Bacteroidia, Gammaproteobacteria, Acidomicrobia, Actinobacteria, and Alphaproteobacteria. Ignavibacteria species were abundant in the water samples collected from site 1 and site 2, representing 12% and 13% of the total reads, respectively, while the abundance was in the range of 2–7% in the other sites. Whereas the abundance of Bacilli species was lower than 1% in the water samples, 15–17% of the sequences assigned to this class were in the water samples taken from sites 2 and 4. On the other hand, Gammaproteobacteria was by far the most abundant class, representing 33–51% of the total reads in the sediment bacterial communities. Deltaproteobacteria, Alphaproteobacteria, and Bacteroidia species were also abundant in the sediment in the mixing period.

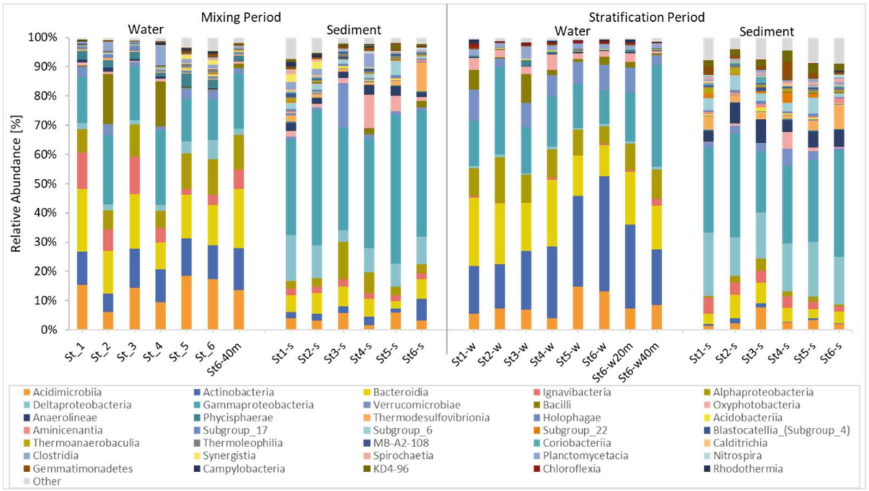

Figure 5. The relative abundances of the bacterial classes during the mixing and stratification period of the lake. The taxa with a relative abundance <1% are merged and named as the other.

During the stratification conditions, Actinobacteria and Verrucomicrobiae abundances went up in the water samples representing 15–39% and 3–11% of the total reads, respectively. Gammaproteobacteria and Alphaproteobacteria abundances were still high during this period. However, Ignavibacteria abundance decreased. In the sediment, although the bacterial community was dominated by Gammaproteobacteria, the abundance decreased compared to the mixing period. On the other hand, Deltaproteobacteria, Thermodesulfovibrionia, Anaerolineae, and species were enriched in the sediment. The highest abundance was recorded for site 6 both in the mixing and stratification conditions.

At the family level, Ilumatobacteraceae, Sporichthyaceae, and Burkholderiaceae predominated in the bacterial community of water in both periods (Supplementary Figures S1 and S2). However, their abundances varied due to the sampling period and location. The sediment community patterns in the sampling points showed high variation under mixing conditions; there was not a common family that dominated the community. While Competibacteraceae represented 18% of the total reads in site 2, the abundance was quite low in the other sites. Rubritaleaceae was only found in site 3, and 13% of the bacterial community was assigned to this family. While the community in site 4 was dominated by Xenococcaceae species (9%), Nitrosococcaceae was abundant in site 5 (13%). In the stratification period, Burkholderiaceae and Desulfobacteraceae were found as common families in the sediment.

The summary of the alpha-diversity analysis is depicted in Table 2. In general, the water samples had lower Shannon and Faith's PD values compared to the sediment, indicating less diverse communities. Moreover, the bacterial communities in sediments were more even. The highest richness and evenness were detected in the sediment sample collected from site 3 during the stratification period. On the other hand, the highest phylogenetic diversity was found in the water sample collected from the same sampling point and time (site 3—stratification).

Table 2. Summary of the alpha diversity of the bacterial communities in water and sediment samples.

Sampling Period	Sample Type	Sample	Number of OTUs	Shannon	Pielou's Evenness	Faith's PD
Mixing Period	Water	St_1	466	6.74	0.76	50.11
		St_2	548	7.52	0.83	53.81
		St_3	429	6.47	0.74	41.92
		St_4	765	7.82	0.82	67.34
		St_5	653	7.56	0.81	61.49
		St_6	663	7.43	0.79	63.79
		St_6_40 m	766	7.80	0.81	66.72
	Sediment	St_1	801	8.64	0.90	70.69
		St_2	815	8.48	0.88	71.84
		St_3	885	8.44	0.86	80.68
		St_4	723	8.23	0.87	70.79
		St_5	791	7.90	0.82	71.25
		St_6	653	7.62	0.81	57.17
Stratification Period	Water	St_1	212	6.13	0.79	29.14
		St_2	390	7.08	0.82	42.55
		St_3	1068	8.47	0.84	91.46
		St_4	334	6.79	0.81	38.52
		St_5	324	6.47	0.78	37.93
		St_6	160	5.54	0.76	25.07
		St_6_20 m	345	6.66	0.79	41.21
		St_6_40 m	434	7.43	0.85	45.56
	Sediment	St_1	699	8.13	0.86	66.65
		St_2	544	8.05	0.89	49.95
		St_3	859	8.83	0.91	81.32
		St_4	607	7.74	0.84	61.15
		St_5	892	8.63	0.88	86.35
		St_6	666	7.88	0.84	63.36

3.3. Predicted Functions of Bacterial Communities

The results of the PICRUSt analysis, i.e., predicted KEGG pathways at level 2, are shown in Figure 6. Most of the functions belonged to metabolism (0.50 ± 0.01), genetic information processing (0.17 ± 0.002), and environmental information processing (0.13 ± 0.005).

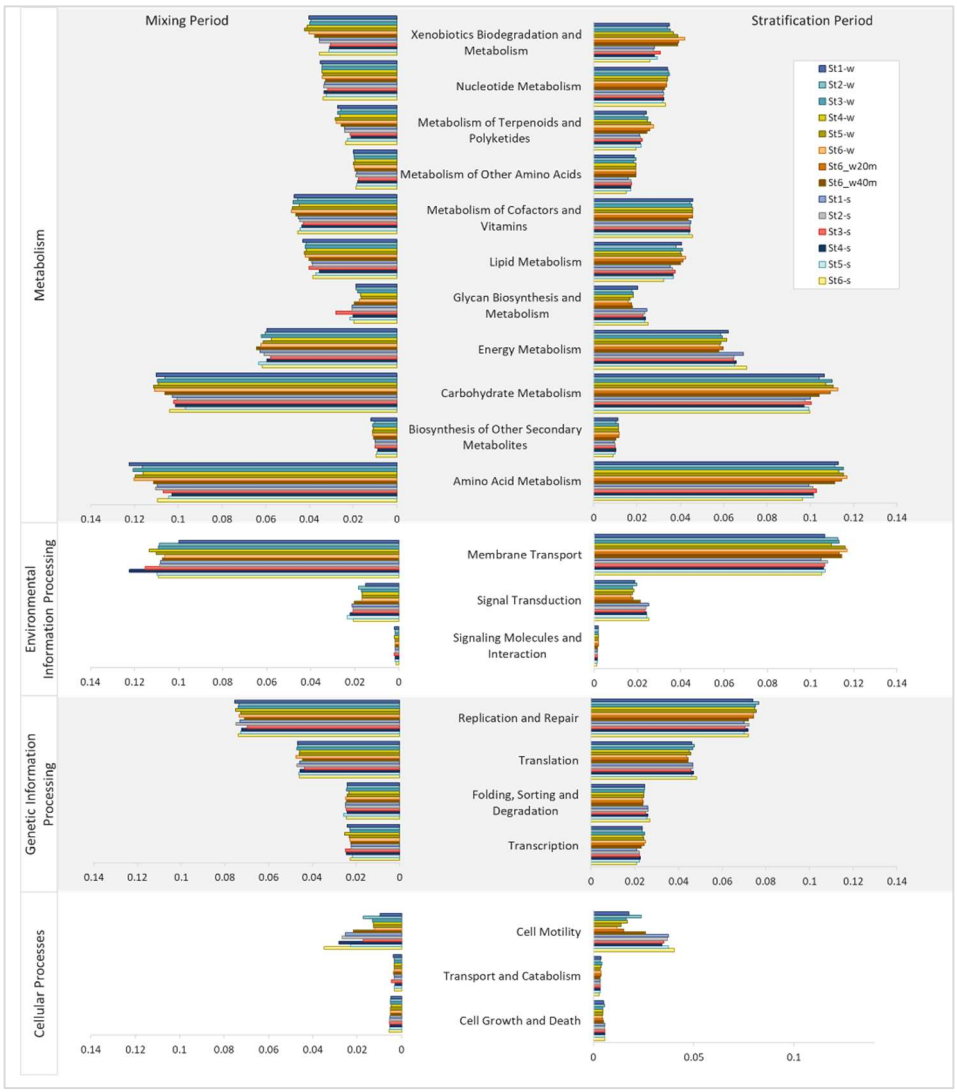

Figure 6. The predicted functional structure of the bacterial communities in the stratified lake.

Amino acid and carbohydrate metabolisms were the most abundant functional categories followed by energy metabolism in the metabolism cluster. In general, metabolisms were higher in the water samples, except for glycan biosynthesis and metabolism during both sampling periods and energy metabolism during the stratification period. In the environmental processing cluster, by far the greatest share of the predicted sequences was assigned to membrane transport (0.11 ± 0.005), in which the highest prediction was assigned to sediment samples collected during the mixing period from site 4. Signal transduction share was higher in the sediment samples (0.023 ± 0.002) compared to the surface water samples (0.017 ± 0.001) in both periods. Moreover, it increased through the water column. In the genetic information cluster, replication and repair showed the highest abundance followed by translation. In the cellular processes cluster, higher abundances of cell motility were detected in the sediment samples and bottom layer water samples.

4. Discussion

This paper attempts to show the bacterial community distribution in the stratified alkaline lake in mixing and stratification conditions. The bacterial community diversity is highly dependent on physical conditions based on the mixing and stratification period of the year [33]. In stratified water bodies, lake mixing is one of the factors influencing the chemical and physical properties of the lake as well as the community structure [34]. Thus, deep lakes are considered important environments to provide insights into relations between environmental variations and microbial community successions [35].

A comparison of the findings with those of other studies revealed deteriorations in trophic conditions of Lake Iznik. While the lake was in mesotrophic status in 2013–2014 [14], our study showed that it shifted through eutrophic status. Even though our results are based on a very limited period of time, the nutrient inputs through rivers and agricultural runoff, anthropogenic pressure from an industrial estate, and various urban areas are important factors in the trophic status of the lake. Thermal stratification had a significant impact on vertical water quality, which is similar to the findings of Chimney et al. [36]; the slow water exchange between layers resulted in water quality deterioration at the bottom of the lake. Due to the disappearance of thermal stratification, the nutrient at the hypolimnion is vertically mixed and may enrich the surface waters. Similarly, hypolimnion will be oxygenated and biogeochemical processes will be affected together with the composition of the bacterial community [37]. Furthermore, it is known that temperature is a major factor shaping the bacterial community diversity [38]. Since air temperature and water temperature differed between the sampling periods of our study, it can be said that it is one of the key parameters shaping the community structure.

While microbial communities are homogeneous during mixing conditions as a result of the physicochemical features of the water body, stratification reveals distinctive community patterns between the nutrient-poor warmer epilimnion and nutrient-rich, mostly anoxic hypolimnion [34]. In accordance with this information, the similarity of the bacterial community patterns of the epilimnion and hypolimnion in this study points to complete mixing conditions in Lake Iznik and revealed a straightforward homogenization. This result is also supported by Garcia et al. (2013), in which the communities were quite uniform in the lake during the mixing period [39].

On the other hand, the stratification caused habitat heterogeneity. During the stratification period, bacterial diversity showed a distinctive structure between the epilimnion and hypolimnion. This contrast can be the result of the temperature and dissolved oxygen gradient as well as the nitrogen, phosphorous, and organic matter concentrations through the water column, which also revealed restriction of bacterial community dispersion along with depth [8]. Furthermore, the diversity was higher in the hypolimnion compared to the epilimnion, which was also similar to the findings of Llorens-Marès et al., which might be explained by the interactions between the bacterial community of sediment and hypolimnion [40]. Another possible explanation for the higher diversity in the hypolimnion may be the organic matter reaching from the upper waters and/or nutrient release from the sediment [7]. While the community of metalimnion was quite similar to that of the epilimnion, the bacterial diversity of hypolimnion varied widely. These results are likely to be related to the hypothesis explained by Yu et al. [35]. Firstly, habitat divergence can be a result of energy and nutrient presence. Secondly, even though the local community can tolerate intermediate changes in the environmental conditions, they may not recover from the extreme differences. The stratification period is relatively long in the lake starting from April and the whole water column mix in November. During stratification, the hypolimnion is isolated due to its nature of being colder and denser; thus, it is separated from the other oxygen flows and remains anoxic. While the environmental conditions between the epilimnion and hypolimnion were quite divergent, it can be assumed that the physical disturbances were also high, which resulted in different bacterial patterns.

Even though only two samples were analyzed in this study, it could be concluded that the bacterial composition may be affected by the physical conditions of the water.

Additionally, lower diversity was observed during the stratification period in the water generally. It seems possible that these biodiversity reductions are also due to the intensity and long duration of UV radiation during warmer periods [41]. The results here also illustrate that spatial variety has negligible effects on the bacterial community structure of water.

The general profile of the bacterial communities in the freshwater lake is in accord with the findings of similar research indicating the dominance of Proteobacteria, Actinobacteria, and Bacteroidetes species in the water samples [5,6,39,42]. Proteobacteria was also found as the dominant phyla in lakes with alkaline characteristics [10,43,44]. Since there are not any significant differences in pH measurements between the sampling periods, the pH has negligible effects on the community structure. The major bacterial phyla did not differ despite seasonal variation, which is a common feature of lentic freshwater ecosystems [41]. A number of factors are known to affect the bacterial community structures in lakes in which precipitation has clear impacts on the primary production [45]. However, bacterial communities have resilience to extreme weather conditions which can return to pre-storm states immediately [45].

During the sampling periods, Cyanobacteria proliferation was not observed, which can explain the lower abundances of Cyanobacteria species in the bacterial community. However, in an early study, *Chrysosporum ovalisporum* proliferation was observed in the surface waters of Lake İznik in August 2013 [14]. Even though the temperature was around 27–29 °C in the lake during our sampling in August 2020, there was no Cyanobacteria bloom in the lake. Since their appearances are dependent on many factors, including nutrient inputs, weather conditions, water flows, etc., the overall conditions of the lake were likely not suitable for their proliferation.

Actinobacteria species are ecologically very diverse [46]. Although Actinobacteria species generally inhabit soils, they are also abundant in the surface waters of reservoirs [35]. The results revealed a decreasing trend for Actinobacteria abundance which went down throughout the water column. The observed decrease could be attributed to the decreasing oxygen concentrations in which Actinobacteria species are suggested to flourish in the upper layers [35,47]. The results of our study also revealed that the prevalence of Actinobacteria species was not affected by mixed water environments similar to [48].

On the other hand, Proteobacteria, especially Gammaproteobacteria and Alphaproteobacteria, abundances increased along with the water column. Proteobacteria was also abundant in the sediment. Higher Proteobacteria abundance is generally detected in nutrient-rich environments, and Proteobacteria members are among the major contributors to material cycles [49]. Previous research also revealed that Proteobacteria species can tolerate various toxicities and have a role in the decomposition of organic matter in the sediment of lakes [50]. The ecology of Gammaproteobacteria species is very diverse. While some of them can live under anaerobic conditions, others are strictly aerobic and species can be involved in the adsorption and degradation of organic materials, ammonia, and sulfide [12]. The members include both chemoautotrophs and photoautotrophs [51]. This wide ecological spectrum enables the proliferation of respective Gammaproteobacteria species in the environment. Wang et al. stated a link between the sediments with high organic components and Gammaproteobacteria presence [50]. Furthermore, Gammaproteobacteria and Alphaproteobacteria share a number of key features, such as sulfur-oxidizing and being a part of sulfur-related processes [52]. Regarding the environmental conditions, respective species can enrich the water environment, and they may contribute to the continuity of ecological processes in the lake.

The sediment bacterial community pattern was in line with those previous studies, which highlighted the dominance of Proteobacteria [6,53,54]. It is speculated that Proteobacteria species are involved in the degradation process in lake sediment [48]. Furthermore, Chloroflexi species were enriched in the stratification period in the sediment, in which the members of Chloroflexi are suggested to degrade recalcitrant organic materials [52]. Anthropogenic factors such as wastewater discharge, agricultural runoffs, excessive water

abstraction, and nutrient inputs create pressure on freshwater ecosystems [55]. Contamination of lakes with various pollutants such as heavy metals and pesticides may result in community changes. Since organochlorinated pesticides were found in the sediment of Lake at high concentrations [56], these species can contribute to their degradation.

In comparison to the biodiversity of water, bacterial diversity in the sediment was higher. It is a general phenomenon which is also in agreement with previous observational studies [57,58]. Higher diversity is probably related to the heterogeneity of the sediment [58]. Furthermore, similar to Diao et al., bacterial communities in the sediment showed more stability to seasonal variations than that in the water [6].

Prior studies were cautious about the prediction of functions from amplicon sequencing data, but it should be noted that a general overview of the metabolisms can be provided out of metagenomics [59], and with the DNA-based approach, the potential can be revealed [40]. Carbohydrate metabolisms were the most abundant functional category among all clusters and were found to be higher in the water samples. It is indicated that the water compartment had a greater carbon utilization rate than the sediment.

5. Conclusions

Overall, this study was undertaken to assess the impact of thermal stratification on the bacterial community profile along with the water column and sediment during the mixing and stratification periods. The results highlighted that stratification reveals a distinctive community pattern throughout the water column, and the bacterial community of water showed higher variation than that of sediment. The bacterial communities were homogeneous during winter turnover, demonstrating complete mixing conditions in Lake Iznik. On the other hand, the community patterns varied between the warmer epilimnion and anoxic hypolimnion in the stratification period, in which higher diversity was observed in the hypolimnion due to the interactions with the bacterial community of sediment. While Actinobacteria species were abundant in the upper layers, Proteobacteria dominated the bacterial community in the sediment, and the sediment harbored a more diverse bacterial community than that of the water compartments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14244097/s1.

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