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Bacteria Community Vertical Distribution and Its Response Characteristics to Waste Degradation Degree in a Closed Landfill

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Abstract: The diversity, community structure and vertical distribution characteristics of bacteria in the surface and subsurface soil and water samples of a closed landfill in Shanghai Jiading District were investigated to reveal the relationships between natural waste degradation degree and the succession of bacterial community composition. High-throughput sequencing of bacteria 16S rDNA genes was used to analyze the bacterial community structure and diversity. The results showed that the diversity of bacteria in the surface samples was higher than that in the deep samples. Proteobacteria was the dominant phylum in all the samples, and the percentage increased with depth. At the genus level, *Thiobacillus*, *Pseudomonas*, *Aquabacterium*, and *Hydrogenophaga* were the dominant genera in surface, medium, deep and ultra-deep soils, respectively. The Bray–Curtis dissimilarity of the soil bacterial communities in the same layer was small, indicating that the community composition of the samples in the same layer was similar. The RDA result showed that ammonium, nitrate, pH and C/N significantly influenced the community structure of soil bacteria. This is of great relevance to understand the effect of natural waste degradation on bacterial communities in closed landfills.

Keywords: bacteria; vertical distribution; waste degradation; ammonium; closed landfill

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Citation: Wang, P.; Dai, H.; Sun, B.; Che, C.; Zhu, R. Bacteria Community Vertical Distribution and Its Response Characteristics to Waste Degradation Degree in a Closed Landfill. *Appl. Sci.* **2022**, *12*, 2965. https://doi.org/10.3390/app12062965

Academic Editor: Vicente Mariscal

Received: 25 January 2022
Accepted: 11 March 2022
Published: 14 March 2022

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1. Introduction

Landfill is one of the common methods for the treatment of municipal solid waste in China [1]. With rapid urbanization in China, cities are expanding in scale and suburban land is being developed. The landfills in suburban areas will be closed gradually [2,3]. In a closed landfill, bacteria play an important role in promoting the natural degradation and removal of waste [4]. Therefore, microbial response to natural waste degradation in closed landfills around urban areas should be analyzed to ensure urban environmental safety.

The composition of municipal solid waste is complex, including glass, metal, construction waste, organic material and other components. Compared with direct combustion, the operational and disposal costs associated with landfills are lower [5]. Municipal solid waste is rich in nutrients and conducive to bacterial growth. Bacteria can utilize the available components in municipal solid waste and decompose them into inorganic salts, water and gases to reduce waste [1]. Previous studies investigated the community structure of bacteria in landfill leachate and simulated landfill reactors [6–8]. Proteobacteria was the predominant phylum in closed landfill, followed by Firmicutes, Acidobacteria and Actinobacteria phyla [7,8]. Previous study also found that Proteobacteria reached the maximum value in the middle stage of waste degradation, while Firmicutes and Actinobacteria increased with prolonged degradation time [8]. However, there are many types of waste in landfill, and the types of degradation products of organic matter are complex. It is difficult to fully

reflect the succession rules for bacterial communities in the vertical direction of a landfill based on a simulated reactor. The composition of microorganisms in the leachate does not easily represent the bacterial community in a landfill.

On the other hand, microorganisms are sensitive to the environment in which they live, and their community composition and functional structure often change significantly when affected by factors such as contaminants [9,10]. The response of soil microorganisms to various environmental impact activities such as heavy metal pollution and the accumulation of domestic waste has been investigated [11,12]. These studies have mostly focused on surface soils, with less attention to the subsurface environment. Landfills can directly affect the surface and subsurface environments and are ideal sites for the study of microbial vertical distribution patterns and the characteristics of their response to varying degrees of waste degradation.

In this study, 16S rDNA high-throughput sequencing was conducted to determine the bacterial community composition of soil and water samples in a closed landfill in Shanghai, and various analytical methods were used to investigate similarities and differences between bacterial communities in the samples and the main influencing factors. Our main objectives were to reveal the vertical distribution patterns and relationships of subsurface microorganisms and their response to varying degrees of waste degradation in landfill near an urban area.

2. Materials and Methods

2.1. Site Description and Sample Collection

The landfill, next to a sewage treatment plant, is about 380,000 m^2 in area and located in Jiading District, Shanghai. The Jiading District is located at the northern margin of the northern subtropics. The average annual temperature is 15.5 °C, and the average annual precipitation is 1105.9 mm. The landfill was mainly used to bury construction waste. After manual sorting, paper, plastic and metal were collected and transported to social recycling facilities. Concrete rubble, brick rubbish and gravel were the main composition in the landfill. There was about 120,000 m^2 of municipal solid waste from 2008 to 2017. The maximum thickness of the landfill was about 7 m. The landfill was closed in 2017, but part of the original planned river on the east side was not included in the closed area. Black HDPE impermeable membrane was used to prevent the infiltration of leachate into the soil. In the vertical direction, cement bentonite mixing piles with a diameter of 650 mm and an average depth of 15 m were used to build impermeable walls around the landfill. Due to high investment costs, horizontal seepage control was not applied. Landfill leachate was collected and discharged to the sewage treatment plant through related sewage pipes. However, due to the lack of systematic drainage measures, leachate leaked and polluted the surrounding environment. The landfill gas was collected and discharged by vertical collecting well and horizontal drainage blind ditch, but there was still malodorous gas. There is a north-to-south river in the surrounding area, and the nearest distance is only about 5 m (Figure S1). Changes in the landfill water level are correlated with the river.

The landfill sampling points W1, W2, W3 and W4 were selected near the river. The distance between sampling points W1 to W4 and the river gradually decreases (Figure S1). The drilling depth of sampling points W1, W3 and W4 was 6 m, and that of sampling point W2 was 15 m. The soil samples from W1, W3 and W4 were collected every 2 m along the vertical depth, and the collection depths were 2, 4 and 6 m, respectively. The soil samples from W2 (D) were collected from 6~7.5, 9~10.5 and 13.5~15 m. All soil samples were collected using sterile sampling bags and sterilized plastic shovels in July 2019 (Figure S1). The groundwater samples were collected at 6 m using sterile groundwater collectors and sterile plastic water bottles after drilling at four sites using a Geoprobe drilling vehicle from Kejr [13]. The river water was collected as a reference sample. Soil and groundwater samples were frozen on dry ice after collection and sent to the laboratory for subsequent experiments. The soil samples were mixed in the laboratory and divided into two parts;

one part was frozen and prepared for subsequent DNA extraction, and the other part was frozen and stored for analyses of physical and chemical properties.

2.2. Analyses of Soil Physicochemical Properties

Soil ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen were determined by extraction with KCl solution using a continuous flow analyzer (Skalar Analytical B.V., Breda, The Netherlands) [14]. Soil organic matter content (TOC) was determined by loss on ignition protocol at 550 °C for 2 h using a muffle furnace [15]. Ten milligrams of dry soil sample were weighed in a tin boat to determine soil CNS using a carbon, nitrogen, and sulfur analyzer (vario MACRO, Elementar, Langenselbold, Germany) [16,17]. Soil pH was measured by mixing 10 g soil and 25 mL Milli-Q water.

2.3. DNA Extraction and High-Throughput Sequencing

For soil samples, 0.25 g of fresh soil was used for DNA extraction using the DNeasy Powersoil Kit from Qiagen (QIAGEN, Hilden, Germany). For groundwater samples, 200 mL of water samples were filtered through a disposable 0.22 μm polyethersulfone membrane filter funnel, and then DNA extraction was performed using the DNeasy Powerwater Kit from Qiagen. The PCR amplification and high-throughput sequencing experiments were performed on the 16S rDNA V3–V4 region of the bacteria. The primers used for PCR were 341F: CCTACGGGNGGCWGCAG and 805R: GACTACHVGGGTATCTAATCC [18]. After two rounds of PCR amplification, the PCR products were examined by agarose electrophoresis. The amplification products were sent to Sangon Company (Shanghai, China) for further experiments. All sequences have been deposited in the NCBI under the accession number PRJNA769245.

2.4. Statistical Analysis

Sequencing data were bioinformatically analyzed on the laboratory server using QIIME2 version 2021.8 [19]. Specifically, the Demux plugin was used to perform data splitting (demultiplex) and sequencing quality assessment, and then the DADA2 plugin was used to obtain an operational taxonomic unit (OTU) table and the representative sequences [20,21]. The representative sequences were used to classify and identify bacteria using the Naïve Bayes classifier based on the Greengenes 13.8 99% OTU database trained by scikit-learn provided in QIIME2 software using the q2-feature-classifier plugin [22]. The processed sequence data were analyzed in the vegan (2.5–7) package in R language for alpha diversity. Percentage stacked bars, box line plots, and PCoA plots were plotted in Origin 2021. RDA was plotted using Canoco v5.0 software [23].

3. Results

3.1. Soil Properties of Closed Landfill

The physicochemical properties of 15 soil profile samples were determined (Table 1). The soil pH was predominantly weakly alkaline, ranging from 7.06 to 8.52. The average pH in the surface soil (0~2 m) was 7.64, while in the deeper layers (4~6 m), pH reached 8.03. The soil nitrogen content of all samples was only between 0.01% and 0.08%. The soil carbon (0.78% to 1.63%) and sulfur (0.06% to 0.30%) content were higher than that of soil nitrogen. In the topsoil, the C/N ratios of samples W1-4 were close to each other. The C/N ratios of W1-4 in subsurface soil were higher than that in topsoil, and there was a difference between the C/N ratios of W1-4 in subsurface soil. The ammonia nitrogen content in the topsoil was low (0.07–2.64 mg/kg), while the subsurface soil ammonia nitrogen was generally high, with high values for ammonia nitrogen (>20 mg/kg) in the middle layer in W3-2 and in the deep layer in W2-3 and W4-3. As for the samples from W2D, the ammonia nitrogen content in the samples from 6 to 7.5 m was low (3.12 mg/kg), while the content in the samples from 9 to 10.5 m and 13.5 to 15 m was high (23.97 and 51.16 mg/kg, respectively). This trend also appeared in the loss on ignition (LOI) test; soil from 6 to 7.5 m exhibited low LOI (1.87% to 2.70%), while in ultra-deep soils from 9 to 15 m, it reached 4.60% to 6.71%.

Table 1. Chemical and physical characteristics of soil samples.

Sample No	Name	Depth m	$N\text{-}NH_4^+$ mg/kg	$N\text{-}NO_3^-$ mg/kg	$N\text{-}NO_2^-$ mg/kg	N %	C %	S %	C/N	pH	LOI %
S	W1-1	0~2	2.64	2.66	0.39	0.04	0.92	0.15	25.46	7.58	3.71
	W2-1		0.07	0.33	0	0.04	0.91	0.12	25.29	7.67	3.81
	W3-1		0.21	0.53	0.12	0.05	1.02	0.09	21.22	7.62	4.12
	W4-1		0.27	0.88	0.19	0.08	1.63	0.07	21.57	7.69	5.23
M	W1-2	2~4	5.40	1.25	0.52	0.03	0.92	0.15	27.49	7.78	3.47
	W2-2		6.83	0.81	0.27	0.04	1.02	0.14	26.18	7.41	4.12
	W3-2		91.23	0.19	0.01	0.07	1.36	0.15	18.26	7.06	4.55
	W4-2		10.96	0.17	0	0.04	1.13	0.08	30.05	7.89	3.94
D	W1-3	4~6	2.89	0.09	0.01	0.01	0.85	0.14	57.77	8.02	2.22
	W2-3		79.42	0.27	0	0.02	0.80	0.11	38.25	8.16	2.17
	W3-3		4.55	0.13	0	0.02	1.01	0.11	42.51	7.93	2.70
	W4-3		26.78	0.35	0.06	0.02	0.83	0.11	49.56	8.01	1.87
U	W2D-1	6~7.5	3.12	0.17	0	0.01	0.78	0.05	66.49	8.35	1.80
	W2D-2	9~10.5	23.97	0.10	0	0.03	1.02	0.06	32.47	8.52	4.60
	W2D-3	13.5~15	51.16	0.18	0	0.07	1.19	0.30	16.90	7.74	6.71

3.2. Microbial Community Diversity

In this study, high-throughput sequencing of microbial DNA extracted from six water samples and 15 soil samples was performed. In total, 1,192,144 DNA sequences were obtained from double-end sequencing, and after filtering, 739,143 high-quality sequences remained. The coverage of the sequencing results was all higher than 0.99, indicating that the sequencing results were sufficient to represent most of the samples (Table S1).

The box plots of the number of OTUs (richness), Shannon–Wiener index, Simpson index and Pielou's evenness index of the samples from different locations are shown in Figure 1. For the soil samples, the topsoil samples had the highest overall values in all indices, and the diversity decreased with increased depth and reached the lowest value in the deep soil samples. The OTUs, Shannon–Wiener index, Simpson INDEX and Pielou's evenness index of groundwater and deep soil samples were mostly similar. As for the river water samples, the OTUs, Shannon–Wiener index, Simpson index and Pielou's evenness index were similar to those of the topsoil.

3.3. Bacterial Community Structure

The taxonomic data on bacteria in the samples was obtained from the taxonomic analysis of the OTU data. The taxonomy was specified as a total of 47 phyla and 736 genera. The percentages of species at the phylum level are shown in Figure 2a, and the phyla with less than 0.5% were combined as Others. Proteobacteria was the dominant phylum in the soil samples, and the percentage of species in Proteobacteria increased with depth, ranging from 58.8% to 96.0%. The other dominant phyla in the surface soil samples were more evenly distributed, with 7.4% of species in Actinobacteria, 6.2% in Bacteroidetes, 6.2% in Acidobacteria, 5.8% in Gemmatimonadetes and 4.4% in Chloroflexi. In the middle and deep samples, most of the phyla were inhibited except Proteobacteria, Bacteroides (5.5% and 2.7%, respectively), Firmicutes (4.3% and 5.4%) and Chloroflexi (3.7% and 1.1%). Proteobacteria, Bacteroides and Chloroflexi accounted for relatively higher percentages than other phyla in the middle and deep samples, but were still decreased compared to the topsoil. However, the percentage of species in Firmicutes increased from the surface layer to the deep layer. In the ultra-deep samples, only Proteobacteria, Bacteroides (1.6%) and Firmicutes (1.0%) accounted for more than 1.0%. In the groundwater (GW) sample, only Proteobacteria and Bacteroides accounted for more than 1.0%. The α-diversity of river water were higher than that of groundwater; Proteobacteria, Bacteroides, Acidobacteria, Chloroflexi, Cyanobacteria and Planctomycetes were more evenly distributed in the river water compared to groundwater.

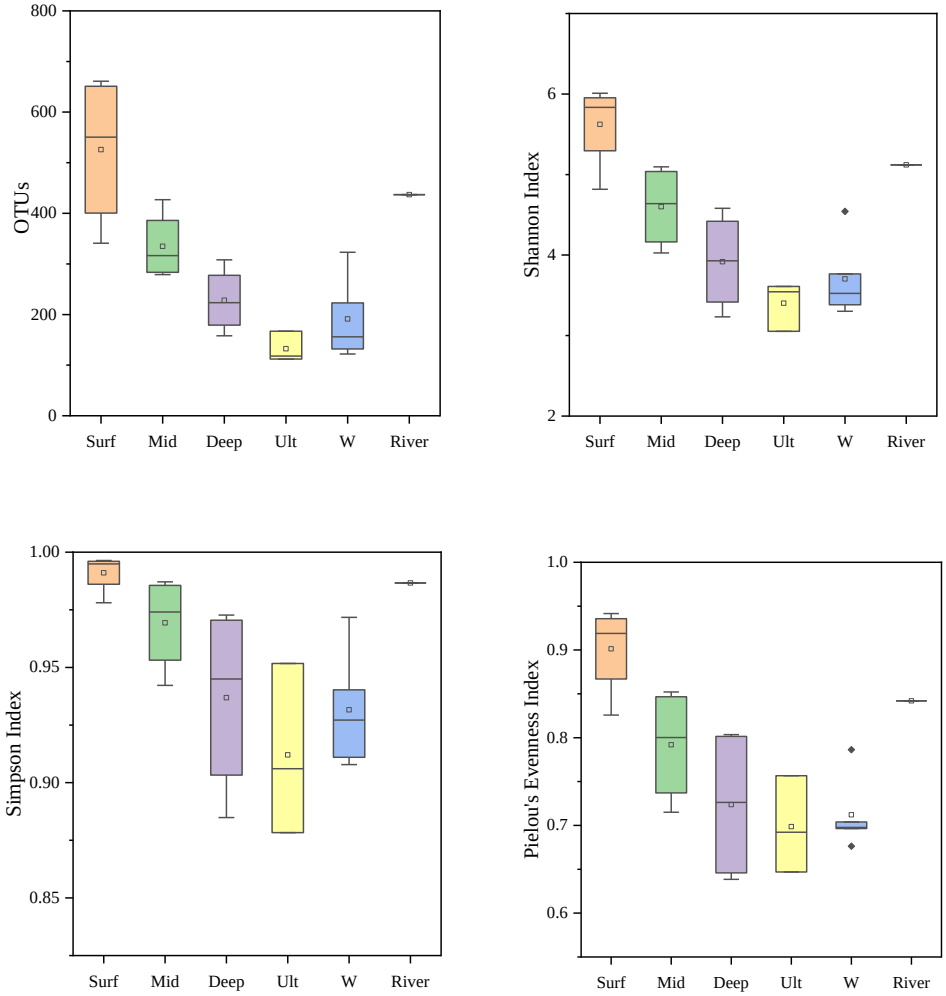


Figure 1. α-Diversity of bacterial communities in different depths.

The percentage of the community at the genus level is shown in Figure 2b. Genera with less than 0.5% were combined as Others. Others accounted for >60% of the community in topsoil and river water samples—63.2% and 72.6%, respectively. The dominant genus in the topsoil was *Thiobacillus* of Proteobacteria, accounting for 11.8%, and which was also widely present in the subsurface soil samples. In the river water samples, the dominant genus was an unclassified genus under the ACK-M1 family of Actinomycetes, which accounted for 17% of river water. In this study, this genus only appeared in the river water samples. The sum of the *Pseudomonas*, *Aquabacterium*, *Hydrogenophaga*, *Comamonas*, *Acinetobacter*, *Lysobacter* and *Perlucidibaca* genera was only 4.17% in the surface soil, while in the medium, deep and ultra-deep samples, the sum was 34.5%, 58.4% and 70.3%, respectively. The genera *Pseudomonas, Aquabacterium, Hydrogenophaga, Perlucidibaca, Sulfuricurvum, Azoarcus* and *Rhodobacter* were the main facultative anaerobic or anaerobic bacteria in the samples. The proportion of these facultative anaerobic or anaerobic bacteria increased with depth, accounting for 3.3%, 26.7%, 45.4% and 41.4% in the surface, medium, deep and ultra-deep samples, respectively. In the groundwater samples, Others accounted for a smaller proportion, similar to that of the deep soil samples. *Novosphingobium*, accounting for 19.0%, and *Methylomonas*, accounting for 10.8%, were dominant only in the groundwater samples.

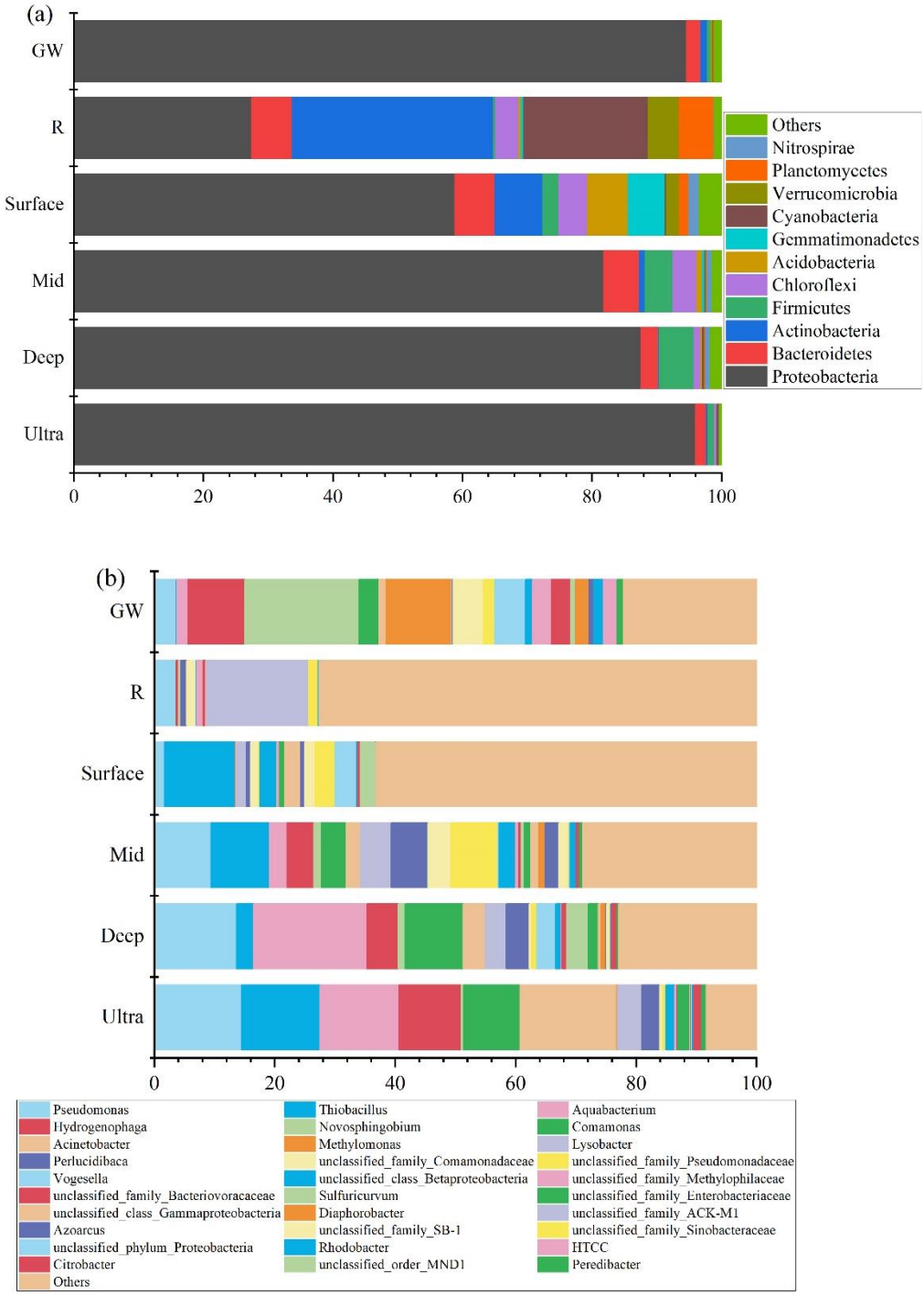


Figure 2. Bacterial community composition at (**a**) the phylum level and (**b**) genus level.

3.4. Relationships between Bacterial Community Structure with Environmental Variables

Principal coordinates analysis (PCoA) of the sample OTU data based on the Bray–Curtis dissimilarity matrix is shown in Figure 3. The points representing groundwater, topsoil and deep and ultra-deep soil samples were clustered into one category each. Overall, the positive direction of the vertical axis was mostly soil samples and the negative direction of the vertical axis was mostly water samples, indicating that PCoA2 represented the difference between water and soil samples. The positive direction of the horizontal axis was mostly shallow samples and the negative direction of the horizontal axis was mostly deep samples, indicating that PCoA1 represented the vertical distribution of samples.

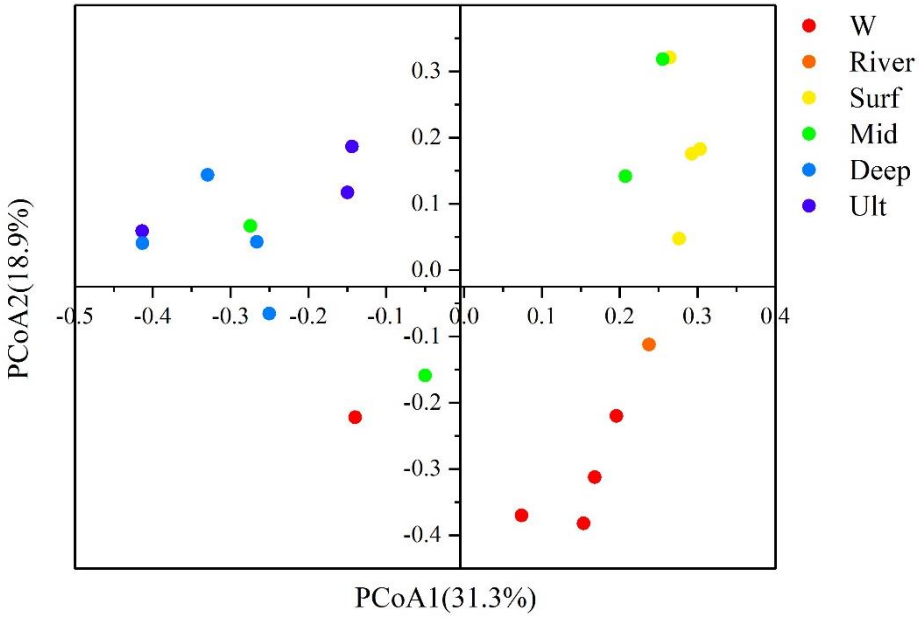


Figure 3. PCoA plot of β-diversity of bacterial communities.

The redundancy analysis (RDA) method was used to represent the relationships between bacterial community at the genus level and physicochemical factors such as pH, LOI, CNS, C/N ratio, ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen (Figure 4). The first axis of RDA explained 35.52% of the variation, and the second axis explained 20.72% of the variation. For the different samples, the deep samples were mostly distributed in the positive half-axis of the RDA1, while the surface and middle samples were in the negative half-axis of the RDA1. The bacterial community structure mainly correlated with ammonia nitrogen, nitrate nitrogen, pH and C/N.

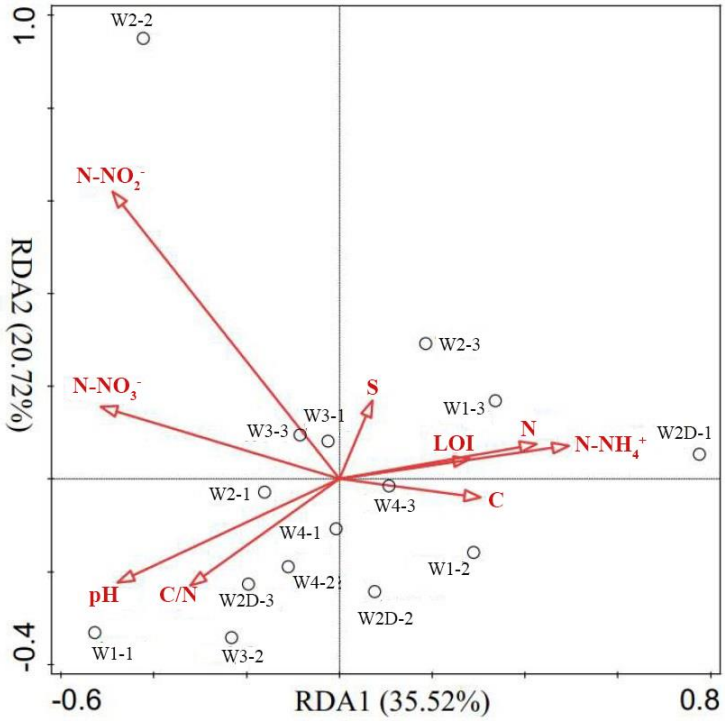


Figure 4. Redundancy analysis of bacterial community and physicochemical properties.

4. Discussion

In this study, the physicochemical properties and bacterial community of landfill soils were investigated. The total nitrogen content was low in all soil samples, probably because the landfill was mainly formed by construction waste, in which the biologically relevant nitrogen nutrient content was low [24]. The dynamic variations of groundwater led to divergence of subsurface soil properties and uneven distribution [25]. The subsurface soil ammonia nitrogen was higher than that in the topsoil, and the highest values appeared in samples W2-3, W3-2 and W4-3, which probably reflected the pathway of leachate migration. The ammonia nitrogen in samples W2D (9~15 m) was high (23.97 and 51.16 mg/kg), which indicated the subsurface soil in the ultra-deep layer had been contaminated due to seepage [26]. Previous study found that soil carbon and nitrogen ratios reflected the origin and components of the soil [16]. In this study, the C/N in topsoil was similar, indicating that the topsoil of samples W1-4 was homogeneous and closer in properties. The C/N in subsurface soil was higher than that in topsoil, probably because the subsurface soil biomass was lower.

The bacterial diversity of topsoil and river water were higher than that of subsurface soil and groundwater, indicating that the growth of bacteria had been inhibited in the deep samples due to the harsh growth conditions and ammonia contamination [1]. The diversity of the groundwater samples was similar to that of the deep soil samples from 4~6 m, which laterally reflected the groundwater samples and deep soil samples taken from the same layer.

Bacterial community structure results indicated that Proteobacteria was the dominant phylum in the soil samples and groundwater samples, ranging from 58.8% to 96.0%. Moreover, the percentage of species in Proteobacteria increased with depth, which indicated that the subsurface samples were not suitable for the growth of most phyla of bacteria. Similarly to Proteobacteria, the percentage of species in Firmicutes also increased with depth, probably because Firmicutes was often found in contaminated environments such as landfills [27–29]. At the genus level, the dominant genera in the topsoil were Others, accounting for 60%. The second most dominant genus was *Thiobacillus*, which uses sulfide as substrates. In this study, the landfill gas was collected and discharged without treatment, resulting in the generation of malodorous gas, in which sulfide and nitride were important components. The sulfide in malodorous gas may have been the main reason for the high abundance of *Thiobacillus* in the topsoil. A large number of genera accounting for less than 0.5% of the total contributed to the high α-diversity of topsoil. Similar results were found in river water, indicating that surface construction waste did not significantly change the diversity of bacteria. The unique dominant genus in river water was unclassified_ family_ACK-M1, which was a characteristic genus of the local river. The absence of unclassified_ family_ACK-M1 in soil samples and groundwater samples indicated that the river water did not recharge the groundwater. In the subsurface soil, the percentage of Others decreased with depth, probably because most of the genera accounting for small percentages of the total could not adapt to the survival conditions in the subsurface environment. However, the genus adapted to subsurface anaerobic oligotrophic conditions occupied the corresponding ecological niche and could become the dominant genus [6,28].

Principal coordinates analysis results showed that there were large differences between the different soil layers while the community composition from the same layer was similar (Figure 3). The community composition between water and soil samples was separated on PCoA2, because the dominant bacteria in soil and water were different [30]. Previous studies show that bacterial communities in soils are usually affected by variation in physicochemical factors [31–33]. In this study, ammonia nitrogen, nitrate nitrogen, pH and C/N were the main physicochemical indices affecting the diversity and distribution of the bacterial community. Ammonia nitrogen was an important source of available nitrogen; however, excessive ammonia nitrogen would degrade the soil and was harmful to bacterial growth. Previous study has found that ammonia nitrogen gradually decreased vertically with reaction attenuation in the process of vertical infiltration [34]. However, the

subsurface soil ammonia nitrogen was higher than that in the topsoil due to the migration of high concentrations of ammonia nitrogen in leachate [4]. In addition, AOB could oxidize ammonia to nitric acid and further reduce the concentration of ammonia nitrogen in the topsoil [17]. The high concentration of ammonia nitrogen indicated that the degree of waste degradation was low in subsurface soil, which reduced the diversity and changed the distribution of the bacterial community. The nitrate nitrogen also had a significant influence on the bacterial community structure in this landfill. Denitrifiers can reduce nitrate under anaerobic conditions and reduce the concentration of nitrate in subsurface soil [15]. Compared with the high concentration of ammonia nitrogen, nitrate nitrogen had less effect on bacterial community structure in this study. Moreover, it has been proved that the optimal growth pH range of bacteria is narrow [31,32]. Therefore, top and middle soil layers with pH ranging from 7.06–7.89 may support the coexistence of diverse bacteria. The C/N ratio is also reported to be a key factor affecting the diversity and structure of the bacterial community [15,17]. The structure of the bacterial community was also influenced by C/N in this study. These influencing factors and relationships with bacterial communities provide insights for the management of closed landfills around urban areas.

5. Conclusions

In conclusion, our study investigated the diversity and community composition of soil and water bacteria in closed landfills in Shanghai. The diversity of microbial communities was mainly controlled by the soil layer, and the surface samples were more diverse while the deeper samples were relatively barren. The soil in the middle and deep layers was more seriously contaminated by ammonia nitrogen, which was one of the important factors affecting the vertical distribution of microorganisms. The community composition of samples from the same layer was similar. In the soil samples, Proteobacteria was the most dominant phylum and its percentage increased with depth. Soil ammonia nitrogen, nitrate nitrogen, pH and C/N were the most important factors affecting the composition of local microbial communities. This study demonstrated the important effects of change in landfill environments on soil and water bacteria.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12062965/s1, Figure S1: Map of the sampling sites in the closed landfill in Shanghai, China. W1-4 stand for four wells with the depth 6 m. W2D stands for the deep well based on W2. R stands for river water; Table S1: Bacteria community α-diversities of samples.

Author Contributions: Conceptualization, P.W.; Data curation, B.S. and C.C.; Funding acquisition, R.Z.; Investigation, P.W., H.D. and C.C.; Writing—original draft, B.S.; Writing—review & editing, H.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (grant no. 41776190; 41976220).

Institutional Review Board Statement: The study did not involve humans or animals.

Informed Consent Statement: The study did not involve humans.

Data Availability Statement: The datasets generated in this study were deposited in NCBI under accession number PRJNA769245.

Acknowledgments: We are thankful to Shaoyi Jiang (City University of Hong Kong) for his support and help in data processing.

Conflicts of Interest: The authors declare no conflict of interest.

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