

Article

Effects of Atmospheric Particulate Matter on Microbial Communities in Wetland Ecosystems

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Abstract: As an important component of urban ecosystems, changes in microbial communities in urban wetland ecosystems have a profound impact on human beings. In this paper, we studied the changes in microbial communities in urban wetland ecosystems (three major interfaces: atmosphere, foliage and water) under the background of atmospheric pollution by high-throughput techniques. The α -diversity of microorganisms at each interface showed that the species richness of the sample communities did not differ significantly at different levels of contamination and it was all at a high level. And the β -diversity showed a significantly larger between-group gap than within-group gap between the samples. The functions predicted a higher metabolic function in water samples and atmospheric samples, and a higher function of microorganisms harmful to humans in the microbial community on the leaf surface. Further analysis of the correlation between atmospheric particulate matter and environmental microorganisms revealed that the atmospheric microbial communities that were strongly negatively correlated with TSP, PM₁₀, PM_{2.5}, and PM₁ were *Actinobacteriota*, *Cyanobacteria*, and *Verrucomicrobiota*. Among the microbial communities on the leaf surface, only *Bacteroidota* was strongly positively correlated with total suspended particle (TSP), particles with a diameter of 10 micrometers or less (PM₁₀), particles with a diameter of 2.5 micrometers or less (PM_{2.5}) and particles with a diameter of 1 micrometers or less (PM₁). As for the microbial communities in the water column, *Firmicutes*, *Bacteroidota*, *Campilobacterota*, and *Deferribacteres* were strongly and positively correlated with the different particle sizes. There was no significant correlation between the functions of the three interfacial microorganisms and the particle size of the atmospheric particles. This paper studies the structure and function of microbial communities within three interfaces at three pollution levels and explores the resulting changes with the aim of providing directions for monitoring urban wetland ecosystems and for species diversity conservation.



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Keywords: atmospheric particulate matter; microbial diversity; wetland ecosystems

1. Introduction

With the rapid social and economic development, environmental problems are becoming more and more prominent, among which the most direct environmental problem faced by human beings is atmospheric pollution [1–3]. Scholars now have a detailed understanding of the composition of atmospheric particulate matter, the sources of matter, and the physicochemical properties of particulate matter [4–7], and clarify its participation in ecological processes such as global energy balance, hydrological cycle, and atmospheric

cycle. At the same time, atmospheric particulate matter also affects other living organisms in the environment [8,9], including environmental microorganisms. The environmental microorganism is a microbial community composed of several populations through symbiosis, mutual benefit, coexistence and competition, which plays an important role in the process of material cycle and energy transformation [10,11].

By conducting numerous studies on the ecological effects between atmospheric particulate matter and environmental microorganisms [12–14], a correlation between atmospheric particulate matter and environmental microorganisms has been found. The relationship between the metabolic characteristics and metabolic functions of atmospheric particulate matter and atmospheric microorganisms has been identified [15–18]. In addition, it has also been shown that atmospheric microorganisms are one of the major sources of human pathogenic pathogens [19]. The effect of atmospheric particulate matter on another environmental microorganism, the interleaf microorganism, has also received some attention [20–25]. Interleaf microorganisms have multiple functions and have important roles in nitrogen fixation, promoting plant growth [26], suppressing pathogenic microorganisms [27,28], improving plant stress resistance [29], and degrading environmental pollutants [30,31], while numerous studies have explored the possible side effects of interleaf microorganisms on leaves [32]. Studies have shown that airborne particles adhering to the surface of plant leaves through sedimentation or adhesion will reduce leaf stomatal conductivity [33] and cause spots on the surface of plant leaves and lead to a decrease in leaf chlorophyll content and photosynthesis. It has also been shown that atmospheric particle deposition also has an effect on water microorganisms [34]. Microorganisms can adapt to changes in environmental factors by indicating changes in environmental factors to reflect the relationship between the microbial community structure in the water body and the surrounding environment [35]. As a decomposer in water bodies, microorganisms in water bodies mainly decompose organic substances such as nitrogen and phosphorus into inorganic substances through nitrification and denitrification of nitrogen, ammonification and decomposition of organic phosphorus, thus regulating the balance of material cycle and energy flow in river ecosystems, reducing the concentration of nutrients in water, promoting the effective use of nitrogen and phosphorus elements by aquatic plants and animals, and playing the role of water purification [36].

However, previous studies are mostly limited to the association between environmental microorganisms and atmospheric particulate matter at a single interface, and although differences in substratum can make great differences in the structure of environmental microbial communities, no attention has been paid to the variability in the effects of environmental changes on environmental microorganisms in the whole ecosystem. Therefore, this study's aim is to expand the study area to focus on a typical urban wetland ecosystem. In most urban wetlands, the atmosphere, forest and water are the three main interfaces in the area, and it is these three components that form the stable ecological structure of urban wetlands. Within the forest–water composite ecosystem, atmospheric particulate matter gradually settles downward, first passing through the atmosphere, then partially adhering to the surface of leaves, and finally reaching the water surface. This study reveals changes in the structure and function of environmental microbial communities within three interfaces under atmospheric deposition conditions: 1. exploring changes in the structure and function of microbial communities in different interface environments; 2. comparing the differences in the microbial community structure at multiple interfaces; 3. analyzing the correlation between atmospheric particulate matter particle size and environmental microorganisms. This study aims to provide basic data on the link between atmospheric deposition and environmental microorganisms within urban artificial wetland ecosystems, and to support further studies to explain the principles of environmental microbial action.

2. Materials and Methods

2.1. Study Area

The experimental area (Figure 1) was selected from the Millennium Watchtower Forest (39°53′35.33″ N, 116°42′48.35″ E) located at Yunfan Road (about 250 m northwest of the Grand Canal Forest Park) in Tongzhou District, Beijing, covering an area of 34.2 ha. The climate of the area is typical of temperate monsoon, with an average annual temperature of 13.8 °C, and abundant sunshine and heat, and it is a typical urban artificial wetland. The rich species diversity in the garden provides high-quality sampling conditions for this experiment.

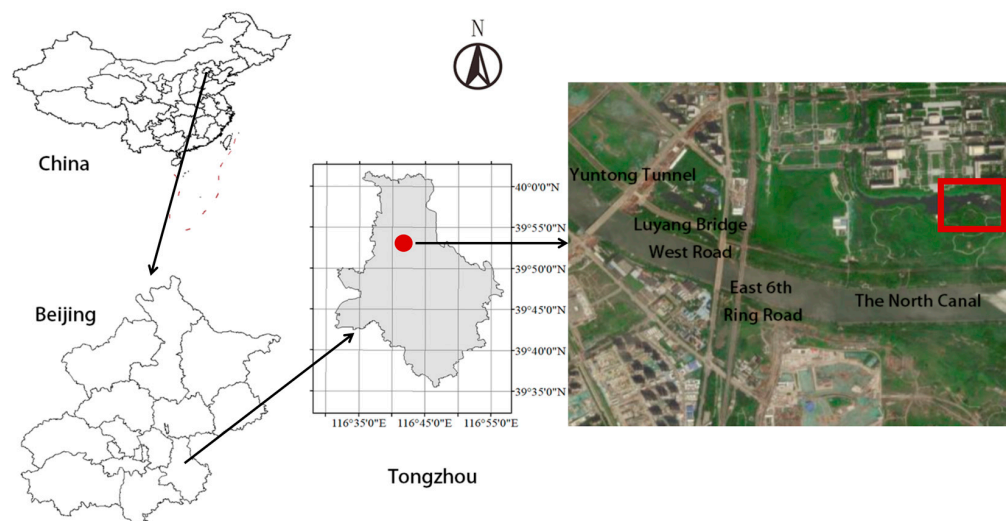


Figure 1. Location of the experimental area.

2.2. Sample Collection

2.2.1. Collection of Atmospheric Particles

Atmospheric particle concentration data include total particle concentration, particles with a diameter of 10 micrometers or less (PM10) concentration, particles with a diameter of 2.5 micrometers or less (PM2.5) concentration and particles with a diameter of 1 micrometers or less (PM1) concentration. The collection instrument was a high-precision handheld dust detector, DUSTMATE, with a setting height of about 1.5 m and a collection time of 12 h. The data collected by DUSTMATE and the air quality index (AQI) found on the website were combined to determine the level of haze pollution (PM2.5, the main pollutant), including good (AQI < 100), light pollution (101 < AQI < 150) and moderate pollution (151 < AQI < 200). Air samples were collected at a single centralized location.

2.2.2. Collection of Atmospheric Microbial Samples

The collection point was set up in the park's weather station flux tower. A model DL-6100D intelligent high-flow-rate particulate sampler was selected for atmospheric microbial collection, and the sampling filter membrane was glass fiber membrane. The sampling airflow rate was controlled at 1.18 m³/min, the sampling time was 10 h, and the instrument was set to automatically record one set of data every 5 min. A total of three groups were set.

The sampling membrane was autoclaved (121 °C, sterilized for 30 min) before sampling, and then put into a sterile self-sealing bag for slow drying. Alcohol wipe sterilization of experimental equipment before collection of particulate matter was carried out as follows: removal of residual large particles with double-distilled water and 75% alcohol; removal of the glass fiber filter membrane from the sterile self-sealing bag into the collector using sterile forceps; and loading the filter membrane into the filter cartridge, ensuring that the

membrane is in the collector and starting the collection. After the samples were collected, the filter membranes were clamped with sterilizing forceps and quickly placed in sterile self-sealing bags, marked with the sample number and placed on dry ice in a constant temperature sterile box for initial storage and transport.

2.2.3. Collection of Microbial Samples on Leaf Surface

The phyllosphere microbial collection point was set up within the oleander forest at the periphery of the flux tower. Medical sterile cotton swabs were used for sampling. A sterile mask and gloves were used and the attached material from the plant leaves was gently wiped. Needles generally wipe 50–100 leaves; the leaves where the height was consistent were wiped, 1.3–1.5 m was deemed appropriate, and a total of three groups were collected. The swab for wiping the adherent was placed in a sterile self-sealing bag, and finally the collected samples were marked with the sample number and placed on dry ice in a constant temperature sterile box for initial storage and transport.

2.2.4. Collection of Microbial Samples in Water

The water microorganism collection point was set in the open water north of the flux tower. Collection was performed using sterile water collection bags. Sterile gloves were used, and surface water of the river was collected. Each sample is 1.5 liters in triplicate. The 0.45 µm and 0.22 µm filter membranes were retained, placed in sterile self-sealing bags, marked with sample numbers, and placed on dry ice in a constant temperature sterile chamber for initial storage and transport. The samples were labeled according to contamination level and sample type (Table 1).

Table 1. Collection sample number.

	Good (AQI < 100)	Light Pollution (101 < AQI < 150)	Moderate Pollution (151 < AQI < 200)
Atmospheric microorganism sample	L.A1	QA1	Z.A1
Water microorganism sample	L.W1	QW1	Z.W1
Phyllosphere microorganism samples	L.L1	QL1	Z.L1

2.3. Analysis of 16S rRNA Gene Amplicon

The reagents used in part of this method were obtained from Takara.

2.3.1. Genomic DNA Extraction and PCR Amplification

The genomic DNA of the samples was extracted by the CTAB or SDS methods, and then the purity and concentration of the DNA were detected by agarose gel electrophoresis (Cleaver Scientific, Rugby, Warwickshire, UK). An appropriate amount of the sample DNA was put into a centrifuge tube, and the sample was diluted to 1 ng/µL with sterile water. Using diluted genomic DNA as the template, PCR (Analytik Jena, Jena, Germany) was performed using specific primers with Barcode and efficient high-fidelity enzymes according to the selection of sequencing regions to ensure amplification efficiency and accuracy. Corresponding regions of the primers: 16S V4 primers (515F:GTGCCAGCMGCCGCGGTAA and 806R:GGACTACHVGGGTWTCTAAT): to identify bacterial diversity.

2.3.2. Mixing and Purification of PCR Products

PCR products were detected by electrophoresis with agarose gel of 2% concentration. The qualified PCR products were purified by magnetic beads, and quantified by enzyme labeling (Varioskan LUX, Thermo Scientific, Waltham, MA, USA). The same number of samples were mixed according to the concentration of PCR products. After full mixing, 2% agarose gel electrophoresis was used to detect the PCR products.

2.3.3. Library Construction and Computer Sequencing

Truseq[®] DNA PCR-Free Sample Preparation Kit library construction Kit was used for library construction. After Qubit and Q-PCR quantification, the constructed libraries were qualified, and then Novaseq 6000 was used for computer sequencing. There was a certain proportion of Dirty Data in the Raw Data obtained by sequencing. In order to make the results of information analysis more accurate and reliable, the Raw Data were first spliced and filtered to obtain the Clean Data. Then, OTUs' (Operational Taxonomic Units) clustering and species classification were analyzed based on effective data.

2.4. Data Analysis

The data of atmospheric particulate matter were processed simply by Excel. The Illumina Novaseq sequencing platform was used to sequence the gene library to obtain OTUs' clustering. Species annotation analysis was performed using the Mothur method and the SSU rRNA database of Silva138 (<http://www.arb-silva.de/>) to annotate OTUs' sequences. alpha diversity and beta diversity were analyzed according to the number of OTUs. The calculation of α -diversity indices and β -diversity was based on Unifrac distances using Qiime software (Version 1.9.1). LEfSe analysis was carried out using LEfSe software (Novo Magic, updated 22 December 2024. <https://magic.novogene.com/customer/main#/info-downloadnew/bc9c345abcbe890f8e95cf62c0ebd0b4>) to understand significant differences in microbial community data. Functional annotation using Faprotax software (Novo Magic, updated 22 December 2024. <https://magic.novogene.com/customer/main#/info-downloadnew/bc9c345abcbe890f8e95cf62c0ebd0b4>) was conducted to characterize the functional changes corresponding to the structure of the microbial community. An analysis of the correlation between environmental factors and microbial community structure and function was carried out using the ggcor and ggplot2 packages of the R language software (R 4.1.3, updated 22 December 2024).

3. Results

3.1. Changes in Microbial Community Structure Under Different Pollution Levels

3.1.1. Community Composition and Abundance

Sequences were clustered into OTUs (Operational Taxonomic Units) with 97% identity based on sequencing on the Illumina Nova sequencing platform. Species annotation of OTUs' sequences was carried out against the Silva138 database. Based on the species annotation results, at the phylum level, we found that the dominant ones included mainly *Actinobacteriota*, *Proteobacteria*, *Firmicutes*. The dominant species at the genus level were *Bifidobacterium*, *unidentified_Chloroplast*, *unidentified_Blattabacteriaceae*. Dominant species were *Bifidobacterium_animalis*, *Candidatus_Uzinura_diaspidicola*, *Parabacteroides_merdae*. The top 10 species with genus-level abundance content were selected for species abundance display (Figure 2), from which the distribution percentages of individual species in different samples and the corresponding changes in abundance can be seen. For example, the proportion of *Machin_Chloroplast* in atmospheric samples is relatively high, and the content of *Machin_Chloroplast* increases first and then decreases with the increase in particulate matter concentration. Especially under the condition of moderate pollution, the abundance of this species decreases dramatically. The percentage of *HGCI-clade* in water samples was relatively high, and the abundance of *HGCI-clade* in water samples changed little under mild pollution conditions, but decreased significantly under moderate pollution conditions. The content of *Parabacteroides* in leaf samples also changed obviously, and the species abundance generally increased with the increase in pollution degree. For *Caproiciproducens*, they all existed in the samples of the three interfaces, and showed a relatively stable trend of increasing species abundance with the increase in pollution level.

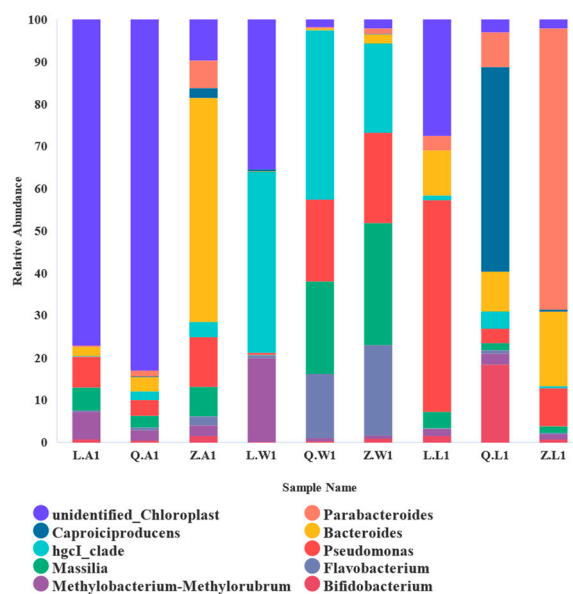


Figure 2. Heatmap of the top 10 species in abundance within each sample (genus level).

Based on the number of OTUs and species annotation, α -diversity and β -diversity were further calculated, and comparisons of differences between groups were carried out, revealing differential characteristics of community structure at different concentrations or environments.

3.1.2. Alpha Diversity of the Community

Usually, alpha diversity refers to the diversity within a community or habitat, and focuses on the species diversity within the community, i.e., species richness and evenness. Table 2 shows the α -diversity indices obtained from the analysis of OTU data after homogenization, where observed_species is the number of OTUs after homogenization. The Simpson's Index of Diversity (1-D) was used in this analysis to characterize the diversity and evenness of species distribution within the community; the higher the value, the greater the diversity of species within the community. The chao1 index is used to indicate community richness, not considering the relative abundance of each species, but only the total number of species; the larger the index, the higher the community richness. The goods_coverage index is used to indicate the sequencing depth index. The table shows that the Simpson, chao1, and goods_coverage indices are at a high level for each interface of the samples, indicating that the collected samples are rich in species diversity and have a high degree of homogeneity. The Simpson's Index of Diversity and the goods_coverage index are above 0.90, which indicates that the species richness of the sample communities is at a high level under different pollution levels.

Table 2. Alpha diversity index of microbial communities.

Group	Observed_Species	Simpson	Chao1	Goods_Coverage
L.A1	3664	0.981	4705.922	0.965
L.L1	2405	0.972	3377.787	0.975
L.W1	1280	0.934	2137.853	0.981
Q.A1	3844	0.929	5302.565	0.956
Q.L1	2698	0.953	3723.494	0.969
Q.W1	1713	0.947	2527.951	0.978
Z.A1	3941	0.967	5349.213	0.957
Z.L1	2233	0.916	2660.967	0.984
Z.W1	2107	0.973	3235.727	0.972

The alpha diversity of the community can be determined indirectly based on the rarefaction curve, in addition to the magnitude of the index values. When the rarefaction curves tends to be flat, the number of species corresponding to the observed number is the diversity of each sample. The rarefaction curves of each interface at different pollution levels are shown in Figure 3. For both atmospheric and water samples, the community richness showed an increasing trend with the increase in pollution level, and the richness in water samples increased very significantly. For the leaf samples, the community richness showed a trend of increasing and then decreasing with the deepening of the pollution level. Overall, the microbial community diversity was highest in the atmospheric samples, followed by the leaf samples, and the lowest in the water samples among the three interfaces.

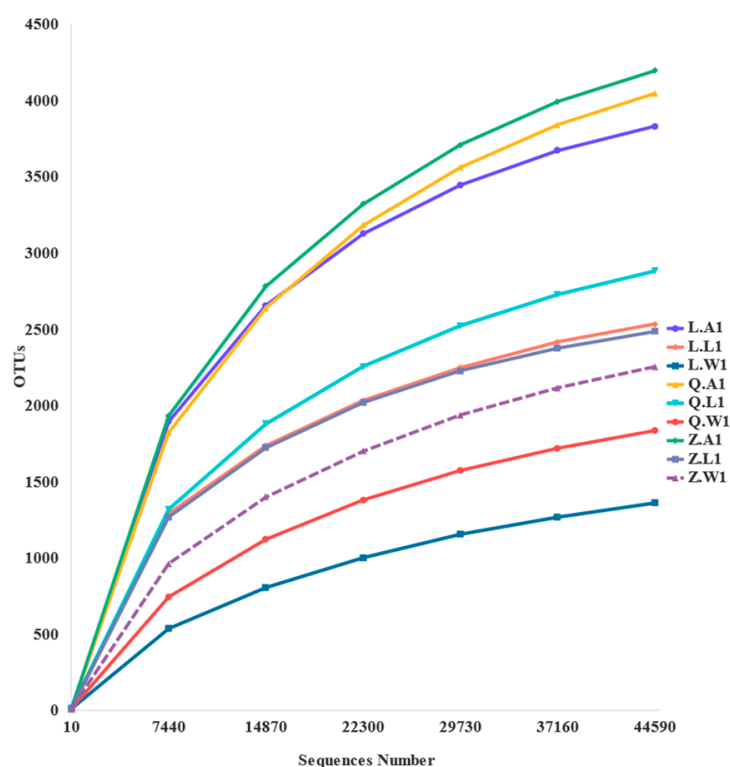


Figure 3. Rarefaction curves of microbial communities at the same interface with different levels of contamination.

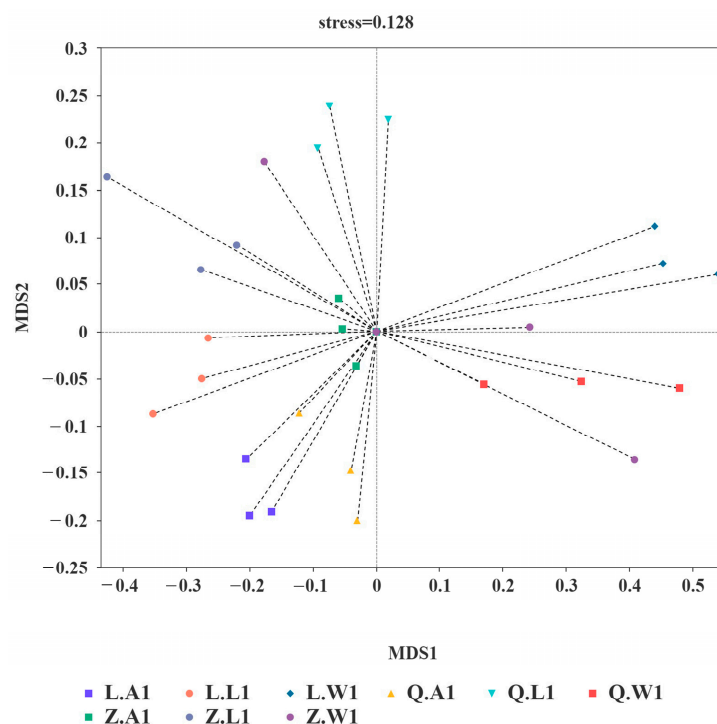
3.1.3. Beta Diversity of the Community

Typically, β -diversity refers to the degree of species variation substitution along an environmental gradient, or the dissimilarity in species composition between communities on different habitats due to the environmental gradient. MRPP is a parametric test based on Bray–Curtis distance and was used to analyze whether the differences in microbial community structure between groups were significant. Table 3 shows the results of the MRPP test between two samples. where an A value greater than 0 indicates that between-group differences are greater than within-group differences, and an A value lower than 0 indicates that within-group differences are greater than between-group differences. A smaller Observed-Delta value indicates a smaller intra-group variation, and a larger Expected-Delta value indicates a larger inter-group variation. The data showed a significantly larger between-group gap than within-group gap between samples. It indicates that there is an effect of the change in pollution concentration on the community structure of the samples.

Table 3. MRPP parameters among microbial communities.

Sample	A	Observed-Delta	Expected-Delta
L.A1-Q.A1	0.2605	0.3755	0.5078
Q.A1-Z.A1	0.1843	0.4679	0.5736
L.A1-Z.A1	0.2199	0.4693	0.6015
L.L1-Q.L1	0.1582	0.6207	0.7374
Q.L1-Z.L1	0.07057	0.73	0.7855
L.L1-Z.L1	0.05019	0.6796	0.7155
L.W1-Z.W1	0.278	0.5023	0.6956
L.W1-Q.W1	0.5456	0.2391	0.5261
Q.W1-Z.W1	0.09382	0.5298	0.5847

The Non-Metric Multi-Dimensional Scaling (NMDS, Non-Metric Multi-Dimensional Scaling) statistic is a ranking method applicable to ecological studies. NMDS is a nonlinear model based on Bray–Curtis distances for analysis, reflected as points on a two-dimensional plane based on the species information contained in the sample. It is designed to overcome the shortcomings of linear models and to better reflect the nonlinear structure of ecological data. Applying NMDS analysis, the information about the species contained in the samples is reflected in the form of points on a multidimensional space, while the degree of variation between different samples is reflected by the distance between points, which is able to reflect the inter- and intra-group differences in the samples, etc. Figure 4 shows the NMDS plot made based on the sample data of this experiment. The STRESS value is lower than 0.2, which indicates that the closer the data of the same color are to each other, the less different the communities of samples from the same inner surface will be under the same pollution conditions. The relatively long distance between different colors shows that the differences between sample communities at the same interface under different contamination conditions are much greater than those within the same interface under the same contamination conditions. The variability among the sample communities is shown visually and concretely in the figure.

**Figure 4.** NMDS plots of microbial communities at the same interface with different levels of contamination.

3.1.4. Comparison of Microbial Community Structure at Multiple Interfaces

LefSE analysis is commonly used to analyze species that differ between groups, i.e., to look for biomarkers that are statistically different. The study results include histograms of the distribution of LDA values as well as evolutionary branching plots. One of the bar charts shows the species with significantly different LDA scores greater than the preset value (the preset value of LDA in this paper is 4.0) for each taxonomic species from phylum to family. The longer bar graphs represent the greater degree of influence of significantly different species. The branching diagram represents the taxonomic level from phylum to genus from inside to outside, and the circle in the middle represents the boundary. The size of the diameter of the small circles represents the relative abundance size; species without significant differences are colored the same in yellow, and species with significant differences are colored following the group, i.e., red nodes indicate microbial taxa that play an important role in the red group and blue nodes indicate microbial taxa that play an important role in the blue group. Community names not indicated in the figure are shown in the legend to the right of the image, showing only the phylum to family of differential species. The following three sets of figures show the LefSE analysis of microbial communities in the atmosphere, water bodies and leaf surfaces at different pollution levels (Figure 5).

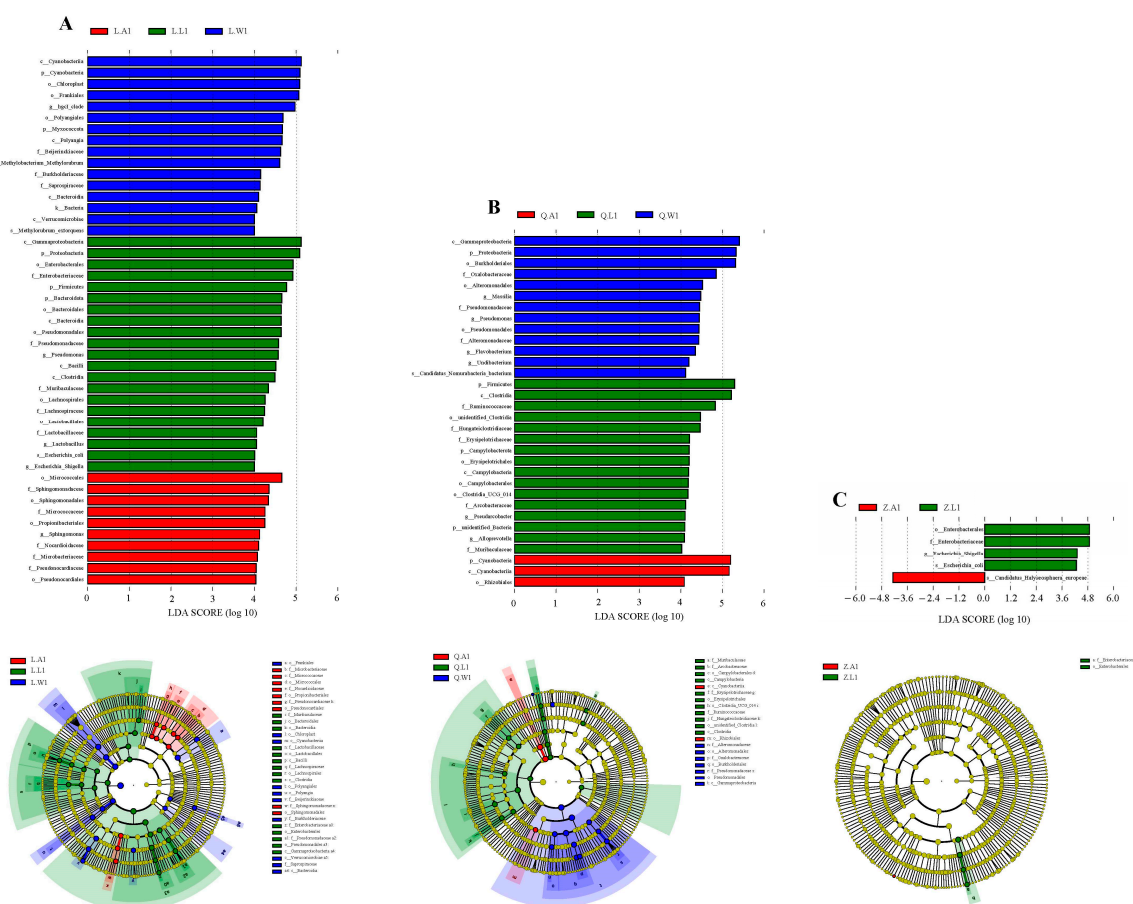


Figure 5. LefSE analysis of microbial communities at different interfaces. Group (A,B,C) plots represent the results of lefse analysis at different levels of contamination, respectively.

As can be seen in Figure 5A, the number of species that differed significantly between interfaces when the pollution level was good was as many as 45. There were 15 species with significant differences in the microbial community of the water body, among which the differences in the *p-Cyanobacteria* had a greater impact. There were 20 species with

significant differences in microbial communities on the leaf surface, with a greater impact of differences in *c-Gammaproteobacteria*. There were 10 species with significant differences in atmospheric microbial communities, among which the differences in the *o-Micrococcales* had a greater impact.

As can be seen in Figure 5B, the number of species with significant differences between interfaces amounted to 33 when the pollution level was mild. There were 13 species with significant differences in the microbial community of the water body, among which the differences in the *c-Gammaproteobacteria* had a greater impact. There were 16 species with significant differences in the microbial communities on the leaf surface, with a greater impact of the differences on the *p-Firmicutes*. There were four species with significant differences in atmospheric microbial communities, among which *c-Cyanobacteria* had a greater impact on the differences.

As can be seen in Figure 5C, the species that differed significantly among interfaces at moderate pollution levels were concentrated only in the leaf surface microbial communities. There were four species with significant differences, with a greater effect of differences in the *o-Enterobacterales*. At an LDA score value of 4, there were no species with significant differences between the atmospheric and water column microbial communities.

3.2. Changes in Microbial Community Function

Based on the annotation results of the database, the top 20 functional information values in terms of maximum abundance were selected to generate a functional clustering heat map (Figure 6). The horizontal coordinates in the figure are the sample information and the vertical coordinates are the functional annotation information. The left side of the image is the functional abundance clustering tree, and the top side of the image is the sample clustering tree. The legend indicates the magnitude of functional abundance.

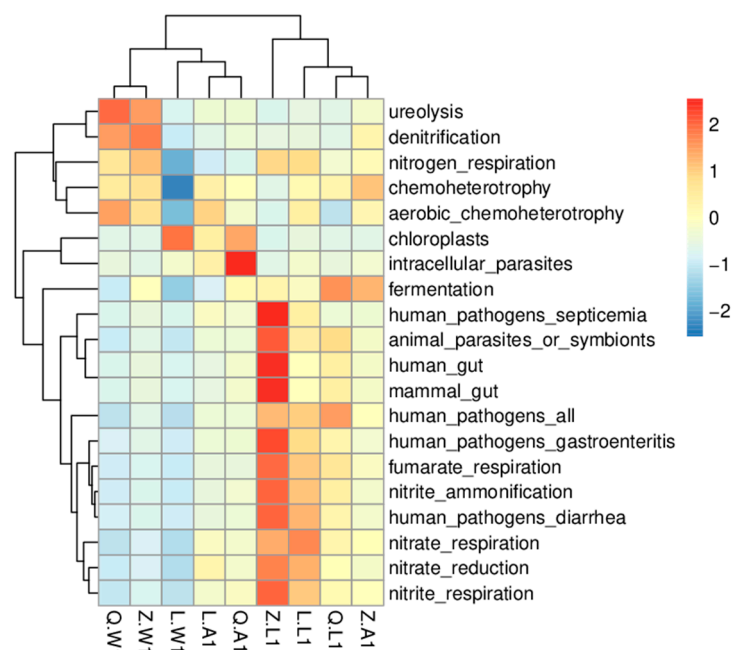


Figure 6. Species functional clustering heat map.

As can be seen from Figure 6, the first eight categories of species functions are mostly concentrated in water samples and atmospheric samples. These eight categories of functions are the main metabolic functions of microorganisms, indicating a strong metabolic function of the microbial community in the water samples. Intracellular_parasites are also highly functional in lightly polluted atmospheres. This also indicates that the metabolic

function of the microbial community is relatively active in the water column and that the microorganisms living in that environment are well nourished. As shown in the figure, the microbial functions that can produce pathogenic hazards are mostly concentrated in the leaf surface microbial community. In the case of moderate pollution, the atmospheric microbial community is more pathogenic and dangerous than the aqueous microorganisms. The function of microorganisms harmful to humans in the microbial community of the moderately contaminated leaf surface was the highest among the nine samples. And in the body of water, the pollution situation is always harmful to human microbial function.

3.3. Influence of Atmospheric Particulate Matter Particle Size on Microbial Communities

In this study, we found that the structure and function of multi-interface microbial communities in urban wetland ecosystems changed under different pollution concentrations. Using environmental factor correlation analysis methods, we further analyzed the relationship between the particle size of atmospheric particulate matter and the structure and function of microbial communities within urban wetland ecosystems (Figure 7). The community structure was represented by the top 10 phyla in terms of relative abundance of the samples, and the community function was represented by the six types of biometabolic pathways in KEGG PATHWAY. A, B and C represent the correlation between the structure and function of microbial communities in the atmospheric interface, the leaf interface and the water interface, respectively (the upper part is the community structure, in which the color close to red is a positive correlation, and the color close to green is a negative correlation; the lower part is the community function, and the darker the color, the stronger the correlation), and the particle size of atmospheric particulates.

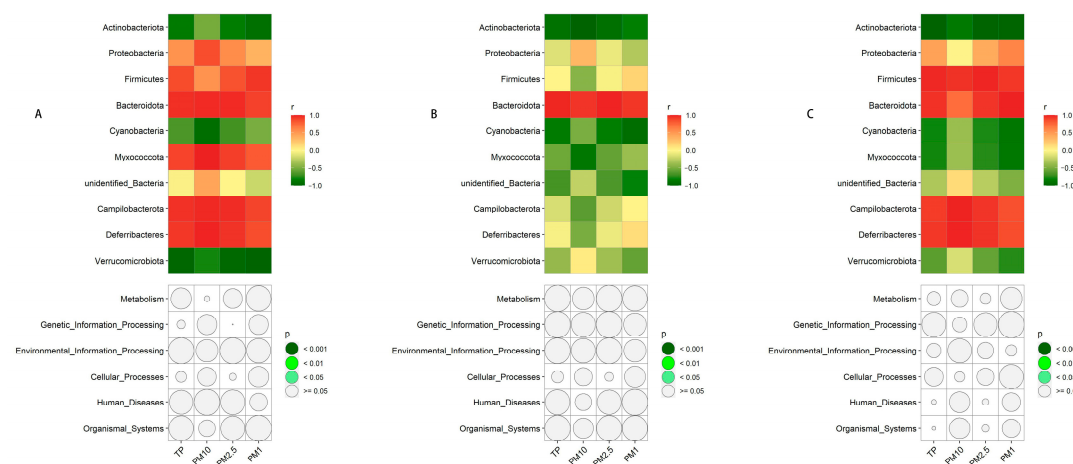


Figure 7. Correlation between particle size of atmospheric particulate matter and the structure and function of microbial communities within urban wetland ecosystems.

From Figure 7A, it can be seen that among the atmospheric microbial communities, *Actinobacteriota*, *Cyanobacteria* and *Verrucomicrobiota* were strongly negatively correlated with TSP, PM10, PM2.5, and PM1. The correlation with TSP, PM10, PM2.5 and PM1 was called weak for *unidentified_Bacteria*. The remaining six phyla were positively correlated with PM10, PM2.5 and PM1. From Figure 7B, it can be seen that the only microbial community on the leaf surface that was strongly positively correlated with TSP, PM10, PM2.5 and PM1 was *Bacteroidota*. Those negatively correlated with TSP, PM10, PM2.5 and PM1 were *Cyanobacteria*, *Myxococcota*. Species of the other seven phyla were weakly correlated with PM10, PM2.5 and PM1 to varying degrees. From Figure 7C, it can be seen that the microbial communities in the water column were strongly and positively correlated with TSP, PM10, PM2.5 and PM1 by *Firmicutes*, *Bacteroidota*, *Campilobacterota*,

and *Deferribacteres*. Those negatively correlated with TSP, PM₁₀, PM_{2.5} and PM₁ were *Actinobacteriota*, *Cyanobacteria* and *Myxococcota*. The species of the other three phyla were weakly correlated with PM₁₀, PM_{2.5} and PM₁. There was no significant correlation between the functions of the three interfacial microorganisms and the particle size of atmospheric particles.

4. Discussion

4.1. Changes in Microbial Community Structure Under Different Pollution Levels

Through this study, we found structural and functional changes in the microbial communities at different interfaces under different levels of contamination. The first thing that is affected by atmospheric particulate pollution is the microbial community present in the atmosphere. In this study, we found that the number of OTUs in the atmospheric microbial community gradually increased with the deepening of the pollution level, and the alpha diversity of the community changed, and both the homogeneity and the richness were significantly higher within the community. The β -diversity of the atmospheric microbial community also showed significant variation when samples at different pollution levels were compared two-by-two, with the differences between samples being significantly stronger than those within samples. However, this study assessed the total concentration and viability of microorganisms in the atmosphere and the correlation of microorganisms with meteorological factors and atmospheric pollutants, concluding a weaker correlation between total atmospheric microbial concentrations and air pollutants. This is contrary to the conclusion of [37]. The article suggested that the reasons for such results may be due to differences between studies, different sampling and analysis methods, and inadequate sample sizes. Nevertheless, most studies have shown a strong correlation between microbial communities and atmospheric pollution. Chen et al. in 2020 noted that moderate concentrations of particulate matter (PM) can lead to increased microbial concentrations [38]. This condition occurs mainly because atmospheric particulate matter is the main living environment for atmospheric microorganisms. When the concentration of particulate matter in the atmospheric environment increases, the abundance and homogeneity of microbial communities within the atmosphere must also increase significantly [39,40], but some studies have found that this trend breaks down under conditions of severe pollution. As the accumulated concentration of particulate matter in the air increases, the total number of microorganisms in the air increases first, while under conditions of severe pollution, the haze concentration increases considerably and the number of microorganisms in the air begins to decrease. The reason for this phenomenon is due to the fact that air pollutants reduce the abundance of airborne microorganisms by inhibiting the growth of *bacteria* [41]. It has also been suggested that competition between the toxic effects of chemical contaminants attached to particulate matter and microbial-growth-promoting components may contribute to this result. Studies have shown that bioaerosol concentrations rise for a time after the onset of haze and then decline with the enrichment of chemicals in particulate matter in the later stages of the haze. In conclusion, the effect of atmospheric pollution on the community of atmospheric microorganisms is significant.

Some studies have found that the epiphytic microbiota appears to be more diverse and abundant and has a different composition compared to the endophytic microbiota of plant leaves [32,38,42]. In this study, we explored the changes in the microbial community of leaf surface samples at different levels of contamination. It was found that the number of OTUs showed a trend of increasing and then decreasing with the increase in pollution level, and the α -diversity of microbial communities on the leaf surface gradually decreased. A two-by-two comparison of samples at different contamination levels revealed significant differences in beta diversity between samples. There are very few studies on the effects of

climate, especially atmospheric pollution, on microorganisms on the surface of plant leaves. However, the effect of rainfall, a climatic variable, on the structure of microbial communities on the leaf surface has been investigated and confirmed that climatic variables are predictors of foliar bacterial abundance [24]. And the changes in atmospheric pollution that are the focus of this paper do have a corresponding effect on phyllosphere microorganisms. This is not difficult to deduce: the plant leaf surface environment is the main living space for phyllosphere microorganisms, and it has been found that the habitat condition on the leaf surface significantly affects the diversity of phyllosphere bacteria [43]. In turn, the settling and adhesion of atmospheric particulate matter will reduce leaf stomatal conductivity, causing spots to appear on the plant leaf surface, altering the environment on the leaf surface and thus having an impact on the community composition of phyllosphere microorganisms.

Within the water microbial community, the number of OTUs showed a gradual increase with the deepening of pollution, and the α -diversity and β -diversity of the community structure also showed significant changes. The increase in microbial community richness in the water samples was significant, as demonstrated by the dilution curves. The indices characterizing β -diversity also indicate that sample differences at different levels of contamination are much greater than within-group differences within the same sample. Water microorganisms are an essential part of the river ecosystem and are important for the ecological stability of the river as a whole. When a water body is polluted, the structure as well as the function of the microbial community of the water body change significantly. Atta Rasoo et al. found variability in the distribution of microorganisms with tolerance to metal-contaminated areas by studying the composition of microbial communities in Ti-contaminated river sediments [35]. Atmospheric pollution affects water bodies, and thus the degree of atmospheric pollution increases the microbial community in the water body accordingly. A study in 2016 confirmed that an increase in particulate matter within atmospheric aerosols leads to changes in the surface microbial communities of water bodies. The authors observed that phytoplankton biomass showed a trend of first increasing and then decreasing by adding aerosol particulate matter, leading to an increase in nitrate in still water bodies, revealing the effect of aerosol particulate matter on microorganisms in marine surface waters by alleviating nitrogen limitation in organisms. This is consistent with the conclusions drawn in this paper.

We know that each environment is suitable for the growth of relatively fixed microbial species, which is related to the substrates available in the environment for microbial metabolism or growth [44]. Analysis of the number of OTUs revealed that the water and atmospheric environments are relatively stable even under altered pollution levels, but the leaf surface environment is more susceptible to altered pollution. This may be because microbial growth is also related to the temperature, pH, moisture, salinity and other conditions provided by the environment. The environment on the leaf surface is more unstable and easily disturbed compared to the atmosphere and water bodies; so, the microbial community on the leaf surface is more susceptible to alteration. The effect of environmental factors on microorganisms has been the focus of microbial ecology, and researchers have attempted to find a clear relationship between the two through various research methods, but it is still difficult to identify the mechanisms that produce this effect with the current technology. In this study, we focused on the correlation between atmospheric particulate matter particle size and microbial communities at different interfaces under different pollution levels. We found a significant correlation between the top 10 gates of species abundance in different interfaces and atmospheric particulate matter particle size. Previous studies have shown that *Proteobacteria* are highly resistant to low temperatures and widely distributed [44], and the relative abundance of *Proteobacteria* and *Cyanobacteria* in the atmosphere showed a negative correlation, which is consistent with the results of the present study. Overall,

Proteobacteria had a positive correlation with different particle sizes, while *Cyanobacteria* had a negative correlation with different particle sizes. In addition to this, it has been found that *Actinobacteria* are more likely to adhere to PM10 samples. From this paper, it is evident that the negative correlation between *Actinobacteriota* and PM10 is the weakest in the atmospheric samples, which is consistent with previous studies. *Bacteroidota* and *Firmicutes* belong to the same intestinal microflora, from which *Bacteroidota* are Gram-negative bacteria and *Firmicutes* are Gram-positive bacteria. In some cases, *Bacteroidota* may become pathogenic and synthesize certain active substances [45,46]. There was a strong positive correlation between *Bacteroidota* and particulate matter of different particle sizes in the microbial community on the leaf surface. However, the correlation between *Firmicutes* as well as other gates with different particle sizes was not strong. The harsh environment of the leaf surface may have contributed to this phenomenon of microbial communities on the leaf surface [47,48]. The effect of atmospheric particulate matter of different particle sizes on the microbial community on the leaf surface may be much lower than other environmental factors. On the whole, microorganisms in water bodies and in the atmosphere show the same correlation with particles of different particle size intervals. It has been noted that nitrate nitrogen in water inhibits *Proteobacteria* [49], which could explain the weak positive correlation found in this study between *Proteobacteria* and atmospheric particles of different particle sizes in water samples than between *Proteobacteria* and atmospheric particles of different particle sizes in atmospheric samples. The finding that *Actinobacteria* are more likely to adhere to PM10 samples also holds true for the aqueous microbial community. The negative correlation between *Actinobacteriota* and PM10 in the water samples remained the weakest among the different particle sizes. This also indicates that the microbial community structure in the water samples is similar to that in the atmospheric samples.

4.2. Changes in Microbial Community Functions Under Different Pollution Levels

In addition to structural changes in the microbial community, functional changes are also of interest. In this study, it was found that microbial community functions at different interfaces changed slightly with increasing atmospheric pollution levels, but this change was not significant for the whole microbial community. Also, the effect of environmental factors on microbial community function was not significant. Why did the apparent change in structure not produce a significant functional change? A number of studies have pointed out that the cause of this phenomenon is the synergistic effect within the microbial community. The microbial community itself, as a complex community, contains hundreds of microbial species, and the cultivable microbial species are no more than 1% of all species. In addition to a certain competitive relationship between these microorganisms within a complex community, they also have a synergistic relationship with each other, and this synergy maintains the stability of the microbial community function. So, usually, when no major ecosystem changes occur, the microbial community is able to quickly coordinate its internal functions and maintain stability. This is the main reason why microorganisms have existed on earth for a longer period of time and are more widely distributed compared to humans.

By comparing the microbial community functions at different interfaces, we found that the pathogenicity function of microbial communities on the surface of water bodies was always the weakest, while the pathogenicity function of microbial communities on the surface of leaves was prominent as the pollution level deepened, while *Bacteroidota*, which can be pathogenic bacteria, also formed a strong positive correlation between leaf samples and atmospheric particles of different particle sizes. The reason for this phenomenon may be due to the retention effect of forests on atmospheric pollution itself. Trees trap damaging microorganisms on the surface of their own leaves, resulting in a more pronounced

pathogenic function of the microbial community on the leaf surface [50,51] (Lee et al., 2020; Nele et al., 2015). Therefore, for humans, it is more beneficial for the body to move around polluted water than in polluted woods.

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