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Seasonal Variation Characteristics of Bacteria and Fungi in $PM_{2.5}$ in Typical Basin Cities of Xi'an and Linfen, China

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Abstract: Microorganisms existing in airborne fine particulate matter ($PM_{2.5}$) have key implications in biogeochemical cycling and human health. In this study, $PM_{2.5}$ samples, collected in the typical basin cities of Xi'an and Linfen, China, were analyzed through high-throughput sequencing to understand microbial seasonal variation characteristics and ecological functions. For bacteria, the highest richness and diversity were identified in autumn. The bacterial phyla were dominated by Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes. Metabolism was the most abundant pathway, with the highest relative abundance found in autumn. Pathogenic bacteria (*Pseudomonas*, *Acinetobacter*, *Serratia*, and *Delftia*) were positively correlated with most disease-related pathways. Besides, C cycling dominated in spring and summer, while N cycling dominated in autumn and winter. The relative abundance of S cycling was highest during winter in Linfen. For fungi, the highest richness was found in summer. Basidiomycota and Ascomycota mainly constituted the fungal phyla. Moreover, temperature (T) and sulfur dioxide (SO_2) in Xi'an, and T, SO_2, and nitrogen dioxide (NO_2) in Linfen were the key factors affecting microbial community structures, which were associated with different pollution characteristics in Xi'an and Linfen. Overall, these results provide an important reference for the research into airborne microbial seasonal variations, along with their ecological functions and health impacts.

Keywords: $PM_{2.5}$; microorganisms; seasonal variation characteristics; microbial community structures; environmental factors; functions

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1. Introduction

Haze pollution has been recognized as a serious environmental issue threatening many cities in China. The airborne fine particulate matter ($PM_{2.5}$) plays a critical role in the formation of haze [1], constraining urban development and human health [2]. In recent years, proactive policies have been implemented by the Chinese government to stem air pollution, such as "Three-Year Action Plan to Fight Air Pollution" in 2018, with a special focus on Fenwei Plain (https://www.gov.cn/, accessed on 3 July 2018). As a region with the second-highest concentration of $PM_{2.5}$ and the highest concentration of sulfur dioxide (SO_2) in China, the Fenwei Plain has become the focal area of China's blue-sky protection campaign. Located in the Fenwei Plain, both Xi'an and Linfen have been hotspots of hazy events, with Linfen suffering from additional SO_2 pollution [3,4]. The special basin topography with static wind conditions renders the dissipation of air pollution difficult. Therefore, the typical basin cities of Xi'an and Linfen deserve attention from researchers.

Bacteria and fungi, the most important part of bioaerosols, can be adsorbed on $PM_{2.5}$ [5,6]. Through long-term exposure to pathogenic bacteria and fungi, humans might

suffer from respiratory diseases, infectious diseases, and cancers [7,8]. At present, researches of bacteria and fungi absorbed on $PM_{2.5}$ mainly focus on daily scale (i.e., day and night) [9], the period of special events (i.e., haze events [10–12], rainfall events [13], APEC summit periods [14], Asian dust events [15]), and limited seasons [16,17]. In short, these researches were usually conducted over a short timescale. Long-term researches such as seasonal dynamic variations of bacteria and fungi are being gradually explored [18–20]. Studies indicate that the bacterial and fungal communities are influenced by many factors, including the location of sampling sites, pollutant factors (i.e., $PM_{2.5}$, inhalable particles (PM_{10}), SO_2, nitrogen dioxide (NO_2), ozone (O_3), carbon monoxide (CO), and air quality index (AQI)), and meteorological factors (i.e., temperature (T), wind speed (WS), and relative humidity (RH)) [21,22]. Fan et al. demonstrated that heavy or severe pollution levels mainly influence bacterial diversity and composition [23], but through one-year systematic monitoring, Zhen et al. found that meteorological factors have a greater influence on the airborne bacterial community than air pollutants, such as air pressure in winter, T and RH in spring, RH in summer, and vapor pressure in autumn [24]. The pollutant and meteorological factors vary greatly among seasons and sites, and there are no consistent seasonal variation characteristics of microbial richness, diversity, compositions, and structures. In Hong Kong, China, the highest and lowest bacterial diversity of $PM_{2.5}$ were found in summer and winter, respectively [25]. Meanwhile, in Beijing, China, the highest bacterial richness and diversity of $PM_{2.5}$ were observed in winter, with summer showing lower richness and diversity [26], yet Zhang et al. indicated that the highest richness is observed in spring and the highest diversity in autumn [19]. Seasonal variations did not significantly affect the existence of dominant microbial phyla, while microbial community compositions at the genus level showed seasonal heterogeneity with different dominant genera across seasons [27,28]. Du et al. found that microbial community structures have no significant correlation with different pollution levels in Beijing, which are mainly influenced by seasonal changes [28]. Therefore, seasonal variation characteristics of airborne microbial communities deserve further study.

Airborne microbes play an important role in biogeochemical cycles such as carbon cycling, nitrification/denitrification, cell metabolism, and organic degradation [12,29]. The airborne bacterial function has been gaining research interest in recent years. The dominant bacterial genus *Pseudomonas* of autumn $PM_{2.5}$ in Beijing and Shanghai, China, has been significantly associated with disease infections, while *Ralstonia* and *Sphingomonas* showed a significant correlation with xenobiotics biodegradation and metabolism [30]. The bacterial community of summer $PM_{2.5}$ in Yucheng, China, has been related to some metabolism pathways, participating in carbon and nitrogen cycling [31]. Ji et al. demonstrated that membrane transport of environmental information processing is the most abundant bacterial function of $PM_{2.5}$ in Jinan, China, from August to December 2017 [32].

Up to now, studies of seasonal variations of airborne microbes in the basin cities of the Fenwei Plain were still limited, especially the comparisons between different cities. In this study, the $PM_{2.5}$ samples were collected during the four seasons in the typical basin cities of Xi'an (a large-sized city) and Linfen (a small-sized city) located in the Fenwei Plain. The microbial community compositions and structures were explored, environmental influences on microorganisms and samples were investigated, and bacterial functions were predicted. The results enhanced our understanding of the seasonal variation of airborne microbial communities, providing a reference for microbiology research of the typical basin cities in the Fenwei Plain.

2. Materials and Methods

2.1. Sampling Site and $PM_{2.5}$ Collection

Xi'an and Linfen are the typical basin cities in the Fenwei Plain, with the climate characterized by distinct seasonality: warm and dry spring, hot and rainy summer, cool and wet autumn, and cold and foggy winter. $PM_{2.5}$ sampling sites were set on the roof of the College of Urban and Environmental Sciences (34°9′ N, 108°52′48″ E, ~1.5 m above

the roof surface, ~36 m above the ground) at the Northwest University in Xi'an and a residential building (35°37′48″ N, 111°23′24″ E, ~1.5 m above the roof surface, ~39 m above the ground) in Linfen. These two sampling sites were surrounded by teaching and residential areas, without industrial pollution nearby. A high-volume particulate matter sampler (TH-1000CII, Wuhan, China) was used to collect $PM_{2.5}$ on a quartz fiber membrane filter (Whatman, UK) at a flow rate of 1.05 m^3/min for 23 h of continuous sampling (from 10:00 a.m. to 9:00 a.m. the next day). Before sampling, each filter was sterilized in a muffle furnace at 300 °C for 5 h. A total of 35 and 30 $PM_{2.5}$ samples were collected in Xi'an (XA) and Linfen (LF), respectively, from December 2017 to November 2018, including winter samples (W: WXA 1–14, WLF 1–7), spring samples (SP: SPXA 15–21, SPLF 8–16), summer samples (SU: SUXA 22–28, SULF 17–23), and autumn samples (A: AXA 29–35, ALF 24–30). In each season, a blank membrane filter without instrument operation was taken as a control sample. All collected filters were stored at –80 °C until microbiological analysis. The air quality data of $PM_{2.5}$, PM_{10}, SO_2, NO_2, O_3, CO, and AQI were recorded from the state-controlled air quality automatic monitoring stations adjacent to the two sampling sites. Meteorological factors, including T, WS, and RH, were obtained from air quality online monitoring and analysis platform (https://www.aqistudy.cn/, accessed on 1 January 2019). The pollutant and meteorological factors are listed in detail in Table S1.

2.2. DNA Extraction, PCR Amplification, and Illumina Sequencing

One-fourth membrane filter enriched with $PM_{2.5}$ was cut into small pieces and placed into the beaded tube of TIANamp soil DNA kit 107 (TIANGEN BIOTECH Inc., Beijing, China) to extract the total DNA. After DNA extraction, as primers of polymerase chain reactions (PCRs), B341F (5′-CCTACGGGNGGCWGCAG-3′) and B785R (5′-GACTACHVGGGTATCTAATCC-3′) were used to amplify the V3–V4 regions of 16S rRNA genes in bacteria, and EF4 (5′-GGAAGGGRTGTATTTATTAG-3′) and NS2 (5′-GGCTGCTGGCACCAGACTTGC-3′) were used to amplify the V1–V2 regions of 18S rRNA genes in fungi. PCRs were performed in a 25 μL PCR mixture consisting of 12.5 μL KAPAHiFi HotStart ReadyMix (2×), 10 ng DNA templates, 0.25 μL of each primer (25 μM), and PCR-grade water. The reaction process was as follows: initial denaturation at 95 °C for 3 min; 25 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s; and final elongation at 72 °C for 5 min. The PCR products were purified using QIAquick Gel Extraction Kit (Qiagen, Holland) and quantified with KAPA Library Quantification Kit (KAPA Biosystems, USA). Finally, the PCR products were sequenced on the Illumina MiSeq platform of the Beijing Yuanyi gene company (Beijing, China).

2.3. Sequence Analyses

At first, paired-end sequences of every sample were assembled by Flash [33], and then the inferior sequences were filtered according to the following criteria: sequences with an average mass score of ≤20, sequences containing nitrogen, sequences with a homomer of >10 bp, sequences with a length of ≤200 bp or ≥500 bp, and sequences including mismatched primers of ≥4 bp. After filtering the sequences and removing the chimera, valid sequences were clustered into operational taxonomic units (OTUs) at 97% similarity threshold by UPARSE [34]. Taking Silva database as a reference, the OTU sequences were systematically classified into kingdom, phylum, class, order, family, and genus using an RDP classifier [35,36], and the microorganisms were mainly analyzed at phylum and genus levels.

2.4. Statistical Analysis

The alpha diversity indexes Chao1 and ACE were employed to analyze microbial richness, while Shannon and Simpson were applied to analyze microbial diversity in all collected samples [37–39]. Significance analysis was carried out by the Wilcoxon rank-sum test. Principal coordinates analysis (PCoA) of beta diversity based on the Bray–Curtis distance was used to visualize the relationship among the microbial communities in dif-

ferent seasons. Functional prediction of the bacterial community was conducted through the Tax4Fun2 algorithm and functional annotation of prokaryotic taxa (FAPROTAX) analysis. Tax4Fun2 was based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The top 25 abundant functional groups of FAPROTAX analysis were shown by a bubble diagram, which was drawn by Matlab (2018 a). Column chart, Wilcoxon rank-sum test, PCoA, redundancy analysis (RDA), Spearman's correlation analysis, and heatmap were performed using OmicShare tools, an online platform for data analysis (http://www.omicshare.com/tools, accessed on 1 February 2021).

3. Results and Discussion

3.1. Microbial Richness and Diversity

As shown in Table 1, a total of 35,888–57,706 and 55,630–105,503 valid sequences for bacteria and fungi were obtained after quality control, respectively. After the final homology analysis and sequence clustering, 570–2684 and 87–183 OTUs of bacteria and fungi were acquired with a 97% similarity threshold, respectively (Table 1). The highest OTUs of bacteria were found in autumn.

Table 1. Comparison of species estimators of the bacterial and fungal communities in $PM_{2.5}$ in different seasons.

	Sample	Sequence Numbers	OTUs	Chao 1	ACE	Shannon	Simpson	Coverage
Bacteria	WXA	57706	676	759	732	4.26	0.937	99.88%
	SPXA	37522	1162	1336	1303	5.48	0.982	99.46%
	SUXA	38214	570	738	694	4.59	0.98	99.73%
	AXA	35888	2684	3126	3079	6.47	0.996	98.62%
	WLF	49533	592	675	641	4.50	0.957	99.90%
	SPLF	38228	701	824	802	4.58	0.961	99.68%
	SULF	38070	862	966	942	5.08	0.983	99.62%
	ALF	39175	2462	2789	2721	6.39	0.994	98.88%
Fungi	WXA	78090	126	160	163	2.71	0.899	99.97%
	SPXA	102636	141	161	162	2.60	0.858	99.98%
	SUXA	55630	183	204	201	2.62	0.848	99.96%
	AXA	96725	126	143	142	3.17	0.934	99.98%
	WLF	77206	87	101	111	0.98	0.301	99.98%
	SPLF	105503	130	144	144	2.55	0.849	99.98%
	SULF	100109	115	141	162	2.92	0.887	99.98%
	ALF	61915	110	132	127	2.84	0.869	99.97%

For bacteria (Table 1), the autumn samples in Xi'an (Chao 1 of 3126, ACE of 3079, Shannon of 6.47, and Simpson of 0.996) presented the highest species richness and diversity, which were significantly higher than those in winter and summer (Simpson exception) ($p < 0.05$), while the richness and diversity were lower in winter and summer. Similar to Xi'an, in Linfen the richness and diversity in autumn (Chao 1 of 2789, ACE of 2721, Shannon of 6.39, and Simpson of 0.994) were significantly higher than those in other seasons ($p < 0.01$), while winter had the lowest richness and diversity. Overall, the richness and diversity of bacteria were the highest in autumn, but lower in winter and summer.

In autumn, suitable meteorological factors (temperature: Xi'an: 5–14 °C, Linfen: 9–13 °C; relative humidity: Xi'an: 43–94%, Linfen: 32–45%) and lower pollution levels (the concentrations of $PM_{2.5}$, PM_{10}, CO, NO_2, SO_2, and O_3 were far lower than the secondary control standards of China's ambient air quality standards (GB3095–2012)) were helpful for the survival, growth, and reproduction of bacteria [20,40]. Compared with other seasons, $PM_{2.5}$ pollution was the most severe in winter. Toxic and hazardous substances such as organic matter, heavy metal (i.e., Cr, Cu, Zn, As, and Pb), and water-soluble ions (i.e., SO_4^{2-} and NO_3^-) adsorbed on $PM_{2.5}$ exerted adverse effects on bacteria [41]. Moreover, low temperature in winter (Xi'an: −3–4 °C; Linfen: −1–2 °C) was not conducive to the activity of enzymes and metabolism of substances in cell membrane fluidity [42], thus inhibiting the growth and reproduction of bacteria. Since solar radiation has germicidal properties that

kill bacterial bioaerosol [43], the concentration of airborne bacteria is negatively correlated with solar radiation [44]. Thus, solar radiation in summer may reduce the richness and diversity of bacteria. Summer temperatures exceeded 24 °C in Xi'an (27–30 °C) and Linfen (25–30 °C), which inhibited the survival of bacteria [24,45]. Additionally, the high O_3 concentration in summer sampling days in Xi'an (avg, 203 ug/m^3) and Linfen (avg, 186 ug/m^3) exceeded secondary control standards (160 ug/m^3, GB3095–2012) and might kill or inhibit the growth of bacteria [46–48]. As a result, the richness and diversity of atmospheric bacteria were lower in winter [25] and summer [26].

The richness and diversity of the fungal community in Xi'an showed distinct seasonal variation characteristics (Table 1). Summer (Chao 1 of 204, ACE of 201) exhibited the highest richness, significantly higher than that in autumn (the lowest richness) ($p < 0.05$), while autumn showed the highest diversity (Shannon of 3.17, Simpson of 0.934), significantly higher than that in summer ($p < 0.05$), with spring and summer having lower diversity. Similar results were observed at the Qinling sampling site in Xi'an, with the highest richness in summer and highest diversity in autumn [20]. In Linfen, the richness and diversity were the highest in summer (Chao 1 of 141, ACE of 162, Shannon of 2.92, Simpson of 0.887) and lowest in winter.

Different characteristics of fungal richness and diversity were found in Xi'an and Linfen. Previous studies demonstrated that the seasonal variation characteristics of fungi were also dynamic in the same sampling site during different periods. For example, in Beijing, the richness and diversity of fungi were highest in winter, followed by autumn, spring, and summer from April 2014 to January 2015 [28], while the richness was the highest in spring, followed by winter, autumn, and summer, and diversity the highest in winter, followed by summer, spring, and autumn from December 2015 to October 2016 [49]. These results indicate that the seasonal variation characteristics of fungal richness and diversity are affected by sampling location, sampling time, and other environmental factors. Additionally, the richness and diversity of fungi are also related to their existence situation in the atmosphere. Fungi exist in the atmosphere in the form of individual spores, spores clusters, hyphae, and small fungal fragments [50]. During atmospheric circulation, large spore clusters may break, with only fungal fragments of less than 2.5 μm retained in $PM_{2.5}$, leading to an uncertainty of fungal quantities in $PM_{2.5}$ and random changes in fungal richness and diversity in $PM_{2.5}$ [49].

3.2. Community Compositions of Microorganisms

Community compositions of the samples were analyzed at different species classification levels, and the phyla and genera of microorganisms were mainly discussed. As shown in Figure 1, the top 20 dominant bacteria and fungi were identified according to the average relative abundance among all sequences in 65 collected $PM_{2.5}$ samples. If the similarity threshold was lower than 80%, OTU sequences were classified as unidentified, which only accounted for 0.02–0.76% (Xi'an) and 0.01–0.56% (Linfen) of bacteria, and 1.33–8.68% (Xi'an) and 1.26–11.53% (Linfen) of fungi at the phylum level. Other than the dominant and unidentified microorganisms, the rest were categorized as others.

At the phylum level (Figure 1a), the dominant bacteria of Xi'an were similar to those of Linfen. Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes were the top four abundant phyla, accounting for 86.62–93.49% (Xi'an) and 84.05–92.10% (Linfen). These four bacteria also predominated in previous studies of $PM_{2.5}$ in Beijing, China; Gwangju and Seoul, South Korea; and Nagasaki, Japan [27,51,52], suggesting adaptation to various atmospheric environments and wide distribution as primary bacteria in the atmosphere. Proteobacteria was the most abundant phylum (38.70–58.48% (Xi'an); 32.29–47.35% (Linfen)), containing a variety of pathogens and nitrogen oxidizing bacteria [53]. In Beijing, Proteobacteria was also the main component across seasons [49]. Most Actinobacteria and Firmicutes may produce spores that withstand extreme conditions such as heat, dehydration, and radiation [53,54]. Therefore, Actinobacteria and Firmicutes as dominant bacteria are widely distributed in a variety of environmental media, such as atmosphere [55],

water [56], soil [57], and plant [58]. The seasonal differences were found in the distribution of bacterial species. Note that the relative abundance of Actinobacteria in autumn (Xi'an: 23.55%; Linfen: 29.41%) and Firmicutes in summer (Xi'an: 23.44%; Linfen: 19.07%) was higher than that in other seasons (Actinobacteria: $p < 0.05$ (SPXA-AXA exception); Firmicutes: WXA-SUXA, $p < 0.01$). Many species of Actinobacteria participated in the decomposition of plant and animal debris and degradation of organic substances in soil [53]. In autumn, due to the fall and decay of plants, Actinobacteria is speculated to be active and migrate from soil to atmosphere to participate in the ecosystem cycle. In both Xi'an and Linfen, the highest relative abundance of Euryarchaeota, Saccharibacteria, Nitrospirae, SR1_Absconditabacteria, and Spirochaetae was observed in winter. Cyanobacteria was adapted to survive in spring, while the peak relative abundance of Chloroflexi and FBP occurred in autumn.

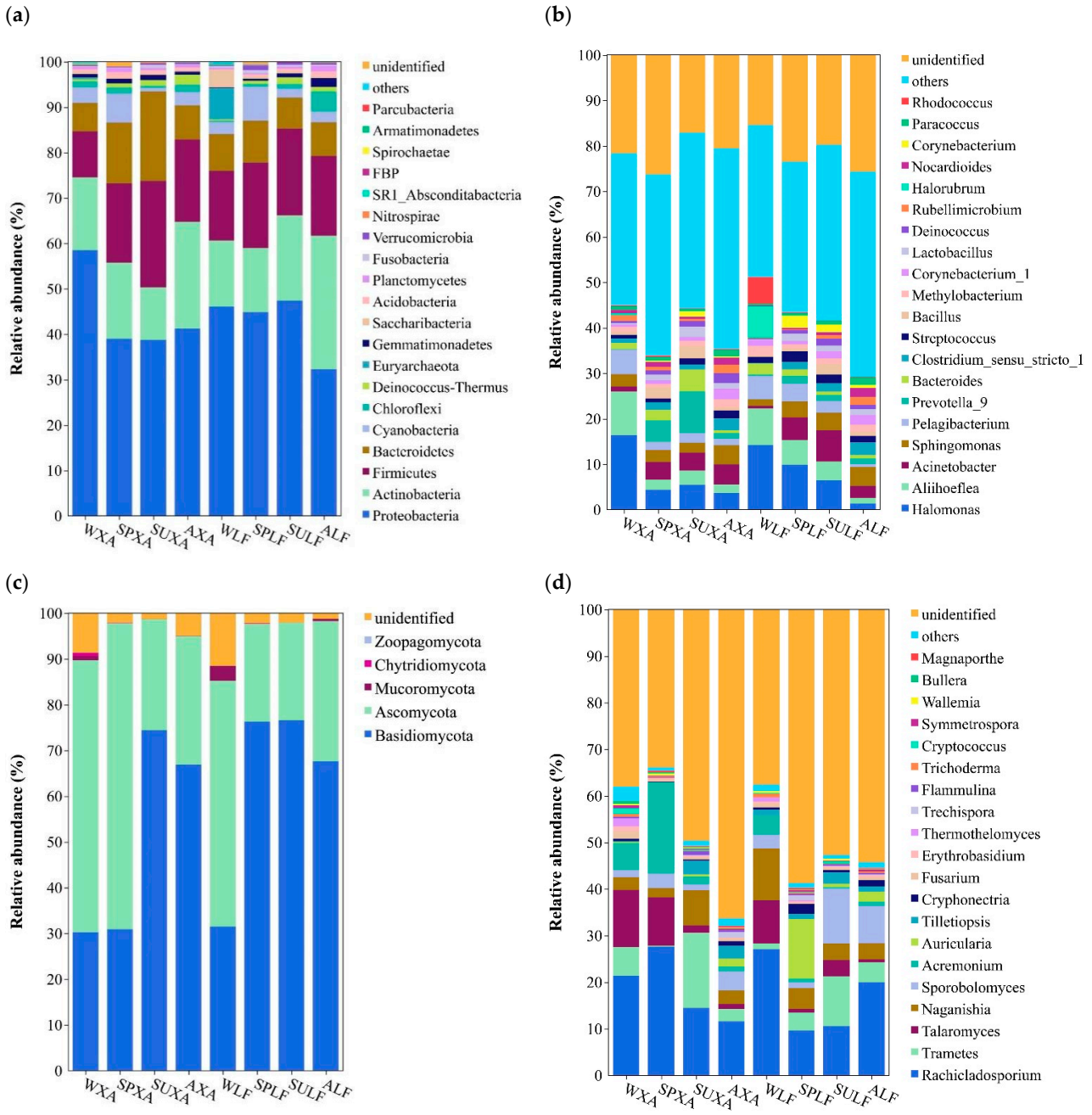

Figure 1. Microbial community compositions of the top 20 abundant OTUs across all $PM_{2.5}$ samples in Xi'an and Linfen. (**a**) Bacterial phyla; (**b**) bacterial genera; (**c**) fungal phyla; (**d**) fungal genera.

For the bacterial genus compositions (Figure 1b), *Halomonas*, *Aliihoeflea*, *Acinetobacter*, *Sphingomonas*, and *Pelagibacterium* were the primary genera in Xi'an and Linfen, accounting for 14.91–35.21% (Xi'an) and 9.96–29.44% (Linfen), respectively. *Sphingomonas*, a Gram-negative bacteria, was widely distributed in aquatic and terrestrial environments [59]. *Sphingomonas*, *Methylobacterium*, *Bacillus*, and *Clostridium_sensu_stricto* were the top 10 dominant genera examined in our and others' research [14]. The compositions of bacteria in Xi'an were similar to those in Linfen, but the major bacterial genera varied across seasons. The most abundant genus in winter, spring, summer, and autumn were *Halomonas* (16.40%), *Prevotella_9* (4.70%), *Prevotella_9* (9.22%), *Acinetobacter* (4.38%) in Xi'an, respectively, while *Halomonas* (14.23%), *Halomonas* (9.89%), *Acinetobacter* (6.90%), *Sphingomonas* (4.25%) in Linfen, respectively, which differed from a previous study in Beijing (*Kocuria*, *Streptophyta*, *Kocuria*, *Sphingomonas*, respectively) [28]. The highest proportion of each genus appeared in different seasons. The peak relative abundance of *Halomonas*, *Aliihoeflea*, *Pelagibacterium*, *Halorubrum*, and *Rhodococcus* were observed in winter, among which *Halorubrum* was only detected in winter (Xi'an: 0.39%; Linfen: 6.92%), indicating that these five genera were well adapted to winter environment. The relative abundance of *Rhodococcus* in winter in Linfen (5.87%) was significantly higher than that in the other three seasons in Xi'an ($p < 0.01$), which may be related to the severe pollution of SO_2 in winter in Linfen (avg, 161 ug/m^3), and *Rhodococcus* could participate in the biodesulfurization [60]. Compared with other seasons, *Clostridium_sensu_stricto_1*, *Corynebacterium_1*, *Rubellimicrobium*, *Nocardioides*, and *Paracoccus* were more suitable to survive in autumn. *Bacillus* had the highest relative abundance in summer (Xi'an: 2.74%; Linfen: 2.28%), which was consistent with the microbial study in the four-season $PM_{2.5}$ samples in Beijing [26].

The dominant fungi at the phylum level in Xi'an were the same as in Linfen (Figure 1c). *Basidiomycota* and *Ascomycota* were dominant across all samples, with the total relative abundance exceeding 85% and rising up to 98.65% (summer in Xi'an). The proportion of the remaining known phyla only accounted for 0.02–1.64% (Xi'an) and 0.01–3.25% (Linfen). In previous studies, *Basidiomycota* and *Ascomycota* were also the top two predominant fungi in $PM_{2.5}$ samples (i.e., in Beijing and Harbin, China) [61,62]. *Basidiomycota* and *Ascomycota* reproduced actively by releasing spores into the atmosphere [53]. In an unsuitable external environment, the spores can be dormant and live for a long time, resisting extreme conditions [63]. Therefore, *Basidiomycota* and *Ascomycota* as dominant fungi exist stably in the atmosphere. Fröhlich-Nowoisky et al. explored the global distribution patterns of *Basidiomycota* and *Ascomycota* and found that the relative abundance of *Basidiomycota* species (61–68%) in all continental samples (Austria, Arizona, Brazil, and Germany) is about two times higher than that of *Ascomycota* species (30–39%) [64]. In this study, the proportion of *Basidiomycota* was higher than that of *Ascomycota* in summer and autumn in Xi'an, as well as in spring, summer, and autumn in Linfen.

The top 20 fungal genera were identified in almost each season, but their relative abundance varied greatly, with the inconsistent representative genus in each season (Figure 1d). The fungal genus in Xi'an was dominated by *Rachicladosporium*, ranging from 11.55% to 27.61% across seasons. In Linfen, the absolutely dominant genus was concentrated in *Rachicladosporium* in winter (27.07%) and autumn (19.96%), *Auricularia* in spring (12.81%), and *Sporobolomyces* in summer (11.84%). *Auricularia* contains polysaccharide, such as d-glucosinol, which has anticoagulant effect in plasma, and was the third-most abundant bacterium in spring in Qinling of Xi'an [20]. Kuo and Li regarded *Aspergillus*, *Penicillium*, and *Cladosporium* as dominant airborne fungi in spring, summer, and autumn, and *Penicillium* and *yeast* during winter from May 1992 to April 1993 in Taipei [65], but these were not competitive genera in Xi'an and Linfen, which may be attributed to the differences in sampling time and location. Some dominant fungi showed different seasonal variation characteristics. The proportion of *Acremonium* in spring in Xi'an (19.53%) was significantly higher than in other seasons ($p < 0.05$), as well as all seasons in Linfen ($p < 0.05$). The highest relative abundance of *Trametes*, *Tilletiopsis*, and *Flammulina* was observed in summer in Xi'an and Linfen, and *Trametes* was also the dominant genera in summer in

Qinling [20]. The relative abundance of *Talaromyces*, *Fusarium*, *Thermothelomyces*, *Trichoderma*, and *Bullera* all peaked in winter in Xi'an and Linfen. *Talaromyces marneffei* was an opportunistic pathogen related to acquired immune deficiency syndrome (AIDS), which forced lymphatic system pathological changes [66].

3.3. Comparative Analysis of Bacterial and Fungal Community Structures

PCoA was further performed to explore microbial community structures of the four seasons in Xi'an and Linfen at the OTU level. PCoA showed the similarities and differences of microbial community structures through the distance between coordinates. The bacterial or fungal communities from the same sampling site showed seasonal similarities (Figure 2), and similar results of the bacterial communities were obtained seasonally in suburban Beijing [49]. Regarding the bacterial community (Figure 2a), the samples from the same season in Xi'an and Linfen occupied adjacent coordinates, suggesting that Xi'an and Linfen had similar seasonal distribution. The samples from spring, summer, and autumn were clustered, yet separated from those in winter, indicating a similar seasonal distribution of the bacterial community structures in spring, summer, and autumn. The significant differences observed in winter likely arose from serious pollution. In terms of the fungal community (Figure 2b), summer, autumn, and winter samples in the coordinate system in Xi'an were close to those of Linfen, respectively, while in spring, the samples of Xi'an were separated from Linfen, indicating that both seasonal and regional similarities were found in summer, autumn, and winter, but regional differences existed in spring. Besides, the fungal samples of winter and spring in Xi'an were clustered together and separated from those of summer and autumn. Similar fungal sample clustering characteristics were observed in Beijing [23]. Therefore, the fungal community structures were affected by both sampling sites and seasons.

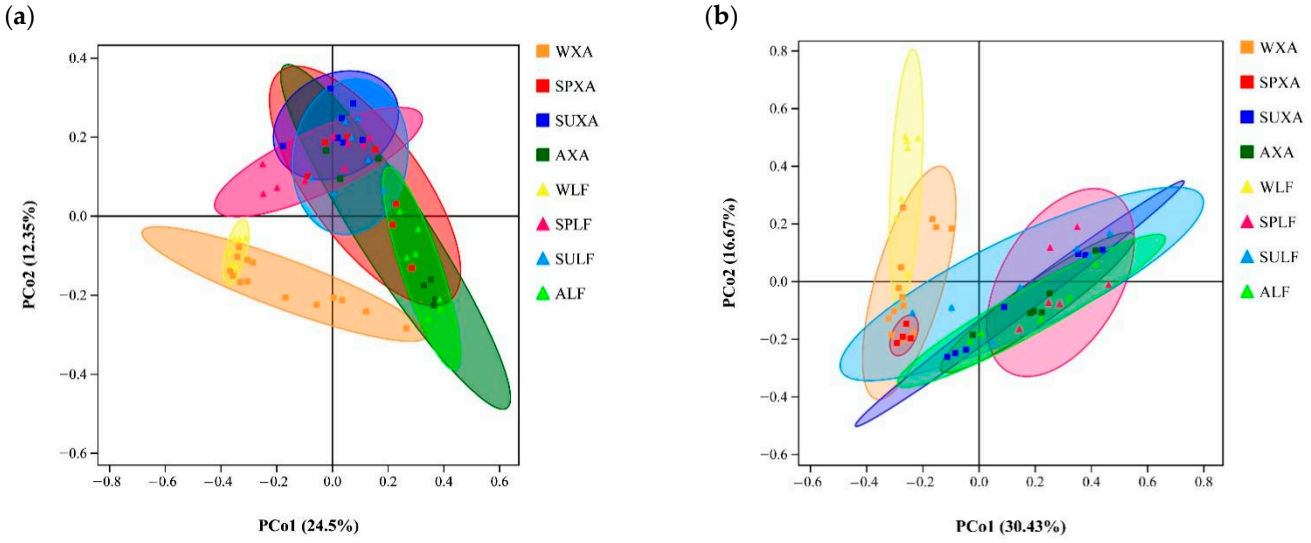

Figure 2. Comparing the bacterial (**a**) and fungal (**b**) communities in Xi'an and Linfen in the four seasons using PCoA at the OTU level. Samples of Xi'an and Linfen are represented by squares and triangles, respectively. Color ellipses (winter, yellow; spring, red; summer, blue; autumn, green) based on 95% confidence interval demonstrate seasonal changes of microbial communities.

3.4. Environmental Influences on Microbial Communities

Pollutant factors ($PM_{2.5}$, PM_{10}, CO, NO_2, SO_2, O_3, and AQI) and meteorological factors (T, RH, and WS) significantly affect the characteristics of atmospheric microbial communities [67,68]. Linear model RDA was used to explore the effects of these 10 factors on the top 10 bacterial and top 10 fungal genera. The lengths of the maximum axis (Xi'an: 2.11; Linfen: 1.91) being less than 4, the results of RDA were appropriate, with the first two

axes explaining 69.08% (Figure 3a) and 43.09% (Figure 3b) of the accumulated variance, respectively.

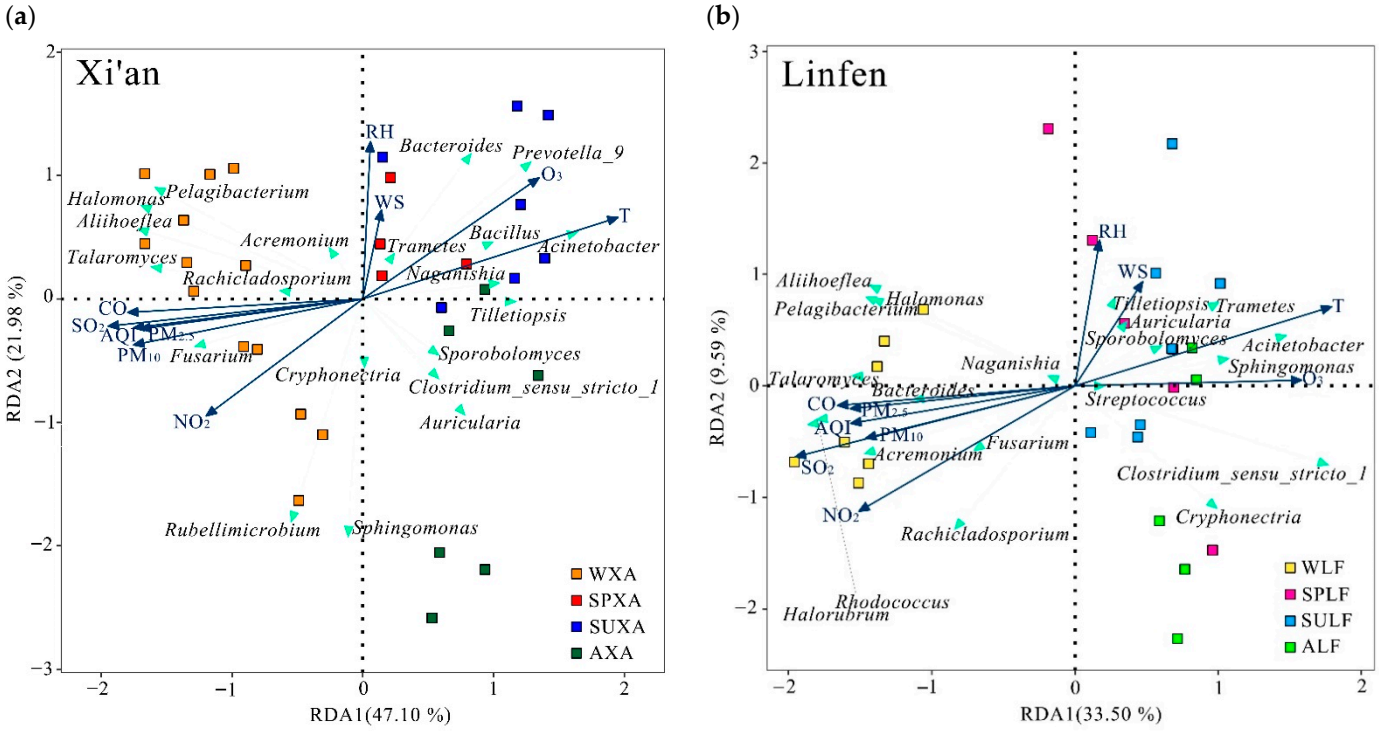

Figure 3. Redundancy analyses of the relationships between environmental factors and dominant microbial community structures in Xi'an (**a**) and Linfen (**b**). Arrows represent pollutant and meteorological factors, triangles represent dominant microorganisms, and squares represent samples in different seasons.

$PM_{2.5}$, PM_{10}, CO, NO_2, SO_2, O_3, AQI, and T exerted a stronger effect on microbial communities than WS and RH did (Figure 3). $PM_{2.5}$, PM_{10}, CO, NO_2, SO_2, and AQI were positively correlated with each other and had a close relationship with winter samples (Figure 3). Pollutant factors ($PM_{2.5}$, PM_{10}, CO, NO_2, SO_2, and AQI) were negatively related to WS. Xi'an and Linfen were the typical northern basin cities with low WS, air stagnation, and temperature inversion in winter, which inhibited the horizontal movement of the atmosphere and aggravated the accumulation of pollutants [69,70]. T and O_3 were close to summer samples and had an important influence on microbial communities (Figure 3), mainly because the T and O_3 concentrations in summer were higher than those in the other three seasons. T and SO_2 in Xi'an (Figure 3a) and T, SO_2, and NO_2 in Linfen (Figure 3b) were the most important factors affecting the microbial communities. The same key factors were reported in previous studies, such as T, O_3, NO_2, and SO_2 in Xi'an [54] and T, RH, SO_2, NO_2, O_3, and WS in Shanghai [71]. Research showed that T is the best indicator of fungal concentrations [72]. T affected the microbial communities by promoting the release, dispersal, and growth of microorganisms [13,27,73] and the viability of the bacteria by evaporating their cell water [74]. In Linfen, SO_2 and NO_2 were also major factors affecting the microbial communities and had a close relationship with winter samples (Figure 3b), which was caused by the high concentrations of SO_2 and NO_2 (around half samples exceeding secondary control standards (GB3095-2012)).

Different pollution factors exert different effects on the same microbial species, so do the same pollution factor on different microbial species. Bacteria or fungi were negatively correlated with most of $PM_{2.5}$, PM_{10}, CO, NO_2, SO_2, and AQI, except for *Talaromyces*, *Halomonas*, *Pelagibacterium*, *Aliihoeflea*, *Sphingomonas*, *Rubellimicrobium*, *Rachicladosporium*, *Acremonium*, and *Fusarium* in Xi'an (Figure 3a) and *Bacteroides*, *Halorubrum*, *Rhodococcus*, *Pelagibacterium*, *Aliihoeflea*, *Halomonas*, *Talaromyces*, *Rachicladosporium*, *Naganishia*, *Acremo-*

nium, and Fusarium in Linfen (Figure 3b), indicating that these six pollution factors inhibit the growth and propagation of microorganisms under severe air pollution [75]. Similar results were observed in previous studies, where *Acinetobacter* in atmospheric cloud water was negatively correlated with SO_2, CO, and $PM_{2.5}$ [76], and *Clostridium* in $PM_{2.5}$ with SO_2 [23]. *Rhodococcus* showed a positive correlation with SO_2 in Linfen (Figure 3b) due to its active involvement in biological desulfurization [60], especially under severe SO_2 pollution in winter. T affected microbial species differently; it positively correlated with some microorganisms (i.e., *Acinetobacter* and *Trametes*) and negatively correlated with others (i.e., *Halomonas* and *Pelagibacterium*) (Figure 3). Similar results have been reported in previous research: T was positively correlated with *Aspergillus* and *Alternaria* and negatively correlated with *Penicillium* and *Cladosporium* in air in Helwan district, Egypt [77]; T showed a positive correlation with *Pyronema* and *Podosphaera* and a negative correlation with *Fusicolla* in $PM_{2.5}$ in Nanchang, China [78].

3.5. Ecological Function Analysis of Microorganisms

3.5.1. Predicted Functional Analysis of Bacterial Community through Tax4Fun2

Although the structures, species compositions, abundance, and sources of bacteria in $PM_{2.5}$ have been explored, the understanding of bacterial functions is still limited. Functional prediction analysis of bacterial OTU sequences was carried out by Tax4Fun2 based on the KEGG database, and the predicted functional categories of Tax4Fun2 were grouped into three levels.

At KEGG level 1 (Figure S1a), in both Xi'an and Linfen, metabolism was the most abundant pathway (Xi'an: 73.89–75.45%; Linfen: 73.70–76.07%), followed by environmental information processing (Xi'an: 10.18–11.31%; Linfen: 9.96–11.30%) and cellular processes (Xi'an: 6.32–7.05%; Linfen: 6.06–6.90%). Metabolism was also dominant in predicting bacterial functions in $PM_{2.5}$ in Jinan, China [32], and Antarctic bioaerosols [79]. The highest relative abundance of metabolism was observed in autumn, indicating that environmental condition in autumn is conducive to the metabolic activities of bacteria, so bacterial richness and diversity are also the highest in this season.

A total of 45 pathways were acquired at level 2. The top 20 pathways were presented in Figure S1b. The relative abundance of every functional pathway in the four seasons remained stable in both Xi'an and Linfen, indicating that varying seasons have little influences on the functional pathways of bacteria. The top three pathways, global and overview maps (Xi'an: 36.36–37.20%; Linfen: 36.48–37.31%), carbohydrate metabolism (Xi'an: 9.24–9.67%; Linfen: 9.44–9.62%), and amino acid metabolism (Xi'an: 7.67–8.08%; Linfen: 7.54–7.97%), all belong to metabolism. Pathways such as membrane transport (environmental information processing), cellular community prokaryotes (cellular processes), and signal transduction (environmental information processing) also exhibited high relative abundance (>4%). Previous studies showed that high representation of dominant functional genes is closely associated with metabolism and human disease functions [30,80]. Therefore, we mainly focused on metabolism and human disease subsystems.

The correlations between functional bacteria and pathways of metabolism and human disease were explored by Spearman's correlation analysis (Figure 4). Bacteria are widely involved in the metabolism and degradation of substances in the natural environment. *Massilia*, *Micrococcus*, *Clostridium_sensu_stricto_1*, *Corynebacterium_1*, and *Bacillus* showed a positive correlation with most metabolism-related pathways, especially microbial growth pathways (i.e., carbohydrate metabolism, glycan biosynthesis and metabolism, and metabolism of cofactors and vitamins). *Bacillus* is a kind of Gram-positive bacteria that can produce resistant endospores and maintain metabolic activity [81]. *Methylobacterium*, *Sphingomonas*, *Massilia*, *Enterococcus*, and *Rhodococcus* were significantly positively associated with xenobiotics biodegradation and metabolism ($p < 0.05$). In previous studies, these five bacteria were proved to degrade and metabolize xenobiotic organic compounds [60,82–85]. *Methylobacterium populi VP2* was capable of degrading xenobiotic organic compounds such as polycyclic aromatic hydrocarbons (PAHs) [84]. *Sphingomonas genus* degraded a

wide variety of spontaneously and xenobiotically produced complex organic compounds, such as tetracyclic and pentacyclic PAHs [82,85]. *Massilia sp. WG5* contained 200 genes related to the biodegradation and metabolism of xenobiotics [83]. *Rhodococcus sp. RHA1* was characterized by a strong ability to transform polychlorinated biphenyls (PCBs) [60]. Overall, bacteria with xenobiotics metabolism and degradation function impacted the fate of organic compounds.

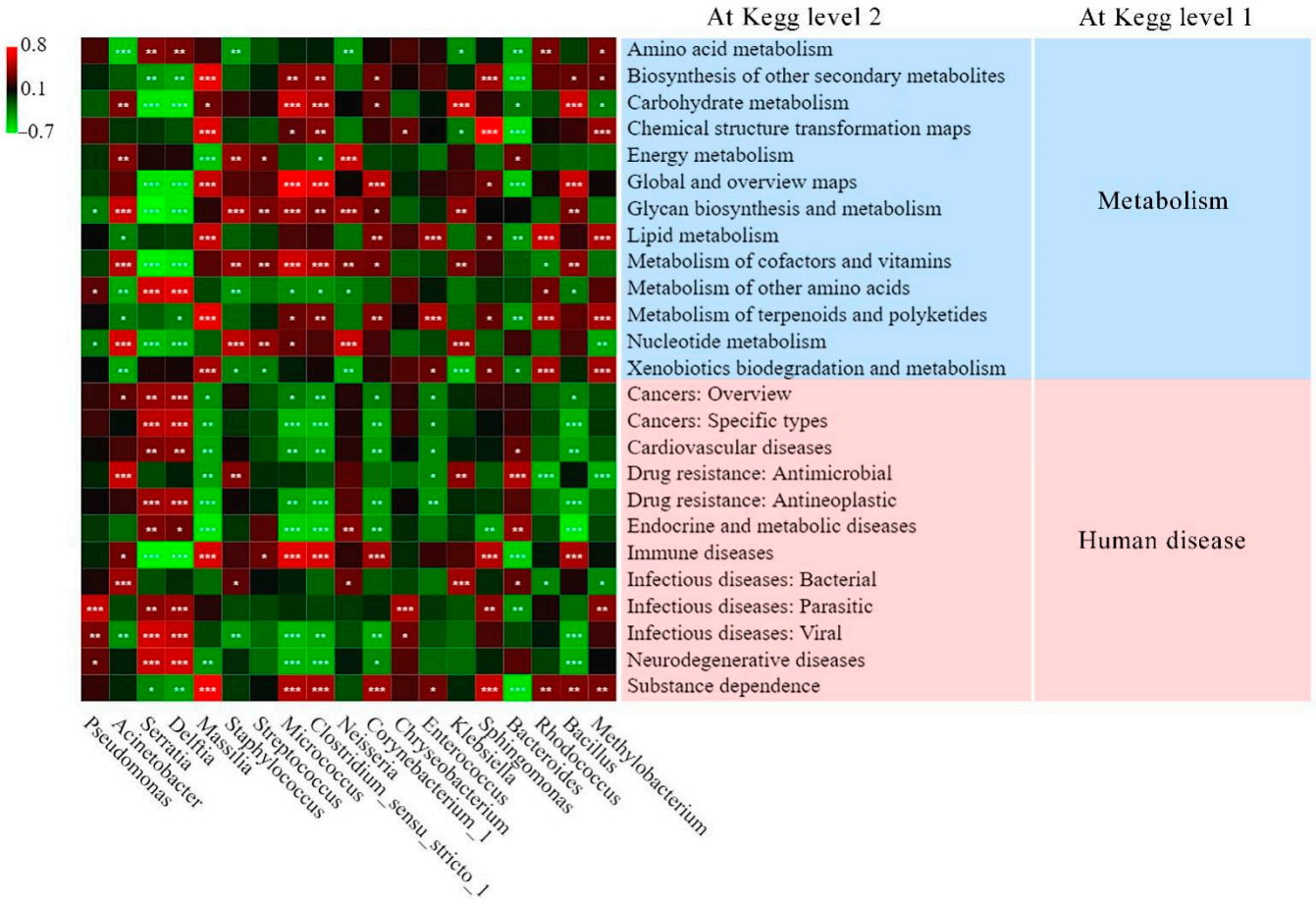

Figure 4. Spearman's correlation coefficients were calculated for analyzing functional bacteria genus and KEGG pathways (metabolism and human disease). Red showed positive correlation and blue represented negative correlation. Significant correlation levels were indicated with * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Most functional bacteria showed a positive correlation with immune diseases and substance dependence. Pathogenic bacteria (*Pseudomonas*, *Acinetobacter*, *Serratia*, and *Delftia*) were positively correlated with most disease-related pathways, which were also detected in $PM_{2.5}$ in Urumqi [39]. *Pseudomonas* is a common pathogenic bacterium that renders cystic fibrosis patients susceptible to chronic respiratory infections [86]. *Acinetobacter baumannii* has become a major source of nosocomial infections, which can easily lead to infections of the respiratory tract, surgical site, and urinary tract [87]. *Serratia marcescens* is prone to cause urinary tract infection, genital tract infection, and septicemia [88]. *Delftia acidovorans* was associated with extremely rare infectious keratitis [89]. *Streptococcus* was related to neonatal sepsis and meningitis [90], and *Chryseobacterium indologenes* could easily cause infections of the biliary tract and surgical wound [91]. *Corynebacterium diphtheria* could cause serious respiratory diseases such as diphtheria [92]. *Clostridium difficile* might lead to diarrhea [93]. Adsorbed on the surface of $PM_{2.5}$, pathogenic bacteria can be suspended in the air for a long time, posing a threat to humans [76]. Therefore, the public health risk of pathogenic bacteria in $PM_{2.5}$ deserves further attention.

3.5.2. Predicted Functional Analysis of Bacterial Communities Using FAPROTAX

The ecological function of airborne bacteria was manifested in promoting the atmospheric biogeochemical cycling processes. According to the classification annotation results of the 16S rDNA sequence, 69 known ecological functional groups of the bacterial community were acquired after FAPROTAX analysis. Chemoheterotrophy (Xi'an: 9.40–27.34%; Linfen: 11.92–26.82%) and aerobic chemoheterotrophy (Xi'an: 16.39–22.16%; Linfen: 4.34–22.13%) attributed to C cycling were the most abundant functions (Figure 5). The following main functional groups were fermentation, animal parasites or symbionts, nitrate reduction, aromatic compound degradation, human pathogens, and ureolysis (the proportion of each > 2%), which were consistent with the study of $PM_{2.5}$ in Jinan [32]. These functional groups of bacteria were mainly related to C cycling (Table S2), N cycling (Table S3), human pathogens (Table S4), and S cycling (Table S5). Note that C cycling dominated in spring and summer, and N cycling in autumn and winter (Figure S2a,b). The relative abundance of S cycling was highest in winter in Linfen (Figure S2a,b). Within the S cycling, respiration of sulfur compounds and sulfate respiration were dominant, and the highest relative abundance of these two functional groups was found in the winter of Linfen (Table S4), which responded to the highest SO_2 pollution.

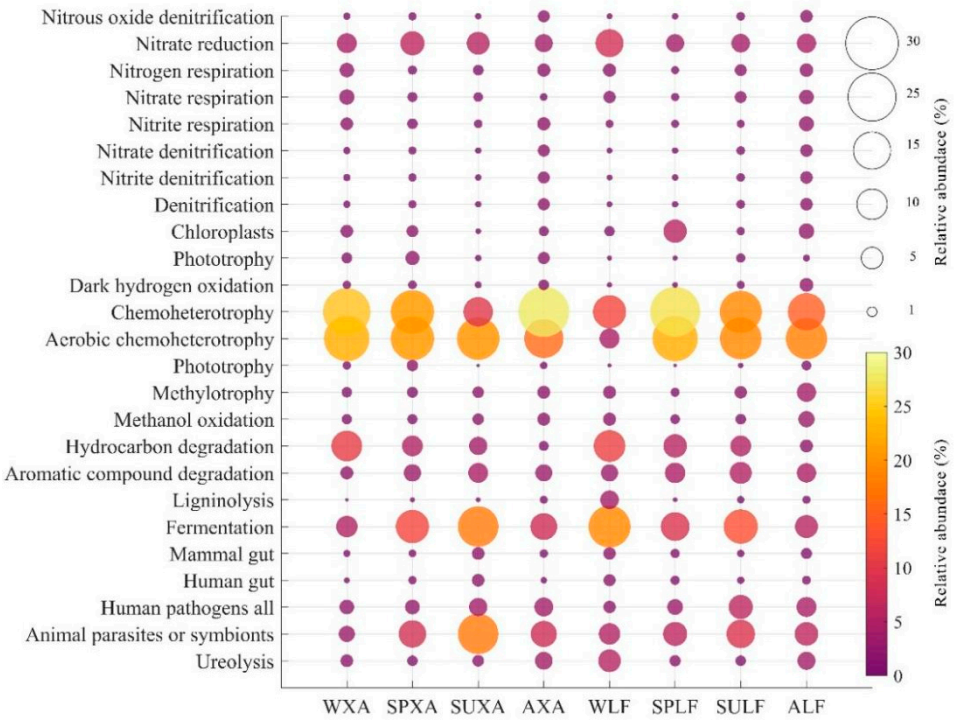

Figure 5. Relative abundance of top 25 functional groups in the four seasonal samples were based on the FAPROTAX database. The size and the color of the balloons represent the relative abundance (%) of bacterial community function.

3.5.3. Analysis of Pathogenic and Functional Fungi

For fungi, pathogenic fungi pose a great threat to living organisms [94]. Therefore, we mainly focused on the analysis of representative pathogenic fungi at the genus level in $PM_{2.5}$. Pathogenic fungi showed no distinct seasonal or regional clustering characteristics (Figure S3). In both Xi'an and Linfen, *Talaromyces* was the most abundant pathogenic fungi, its relative abundance varying greatly across sites and seasons, with its peak relative abundance in winter (Xi'an: 12.25%; Linfen: 9.26%). The relative abundance of *Cryptococcus* was moderate (Xi'an: 0.05–1.28%; Linfen: 0.05–0.53%). The same result was found in the Qinling sampling site (about 0.8%, mountainous region) in Xi'an, while it was lower than that in the Yanta sampling site (3.2%, urban region) in Xi'an [20]. *Talaromyces marneffei* and *Cryptococcus neoformans* were opportunistic pathogens, causing infections in immunosup-

pressed patients, especially patients suffering from acquired immune deficiency syndrome (AIDS) [66,95,96]. *Fusarium*, *Phakopsora*, *Magnaporthe*, and *Sarocladium* have been considered to be plant-related pathogens, which might bring about a variety of diseases and result in crop yield reduction [97–99]. Fortunately, the total relative abundance of these four genera associated with plant pathogenicity in Xi'an (0.73–1.91%) and Linfen (0.54–1.12%) are not very high. Although the relative abundance of most pathogenic fungi is low, the potential exposure risk of pathogenic fungi should not be ignored.

4. Conclusions

This study demonstrated the seasonal variation of bacterial and fungal community compositions and structures in $PM_{2.5}$ in the typical basin cities of Xi'an and Linfen. Microbial correlations with environmental factors and their biochemical functions were analyzed. The results showed that for bacteria, the highest richness and diversity are observed in autumn, while for fungi in summer (diversity in Xi'an exception). The species compositions of microorganisms were relatively stable at the phylum level across seasons, and seasonal dynamic variations were presented at the genus level. Bacterial community structures were similar in spring, summer, and autumn, contrasting with those in winter. Microbial community structures were mainly affected by the environmental factors T and SO_2 in Xi'an, and T, SO_2, and NO_2 in Linfen. Ecological function analysis of bacteria indicated that metabolism was dominant in predicting bacterial function, and *Massilia*, *Micrococcus*, *Clostridium_sensu_stricto_1*, *Corynebacterium_1*, and *Bacillus* showed a positive correlation with most metabolism-related pathways, especially the microbial growth pathways. Most functional bacteria and disease-related pathways (immune diseases and substance dependence) were positive, and particularly pathogenic bacteria (*Pseudomonas*, *Acinetobacter*, *Serratia*, and *Delftia*) were positively correlated with most disease-related pathways. The highest relative abundance of *Rhodococcus* and S cycling was found in the winter of Linfen, where the dominant groups (respiration of sulfur compounds and sulfate respiration) were also highest, which responded to the serious SO_2 pollution in Linfen. This study enhanced our understanding of microbial community structures and functions during different seasons. In future research, the relationship between the chemical composition (metals and organic matters) of $PM_{2.5}$ and microorganisms should be further investigated.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/atmos12070809/s1: Figure S1: Bacterial community functions were predicted using Tax4Fun2 algorithm during different seasons. (a) At level 1. (b) Top 20 KEGG subsystems at level 2; Figure S2: The relative abundance of carbon cycling, nitrogen cycling, sulfur cycling, and human pathogens in Xi'an and Linfen; Figure S3: Heatmap based on relative abundance (%) of pathogenic fungi in all $PM_{2.5}$ samples; Table S1: Pollution and meteorological data in sampling period of Xi'an and Linfen; Table S2: Functional groups related to the carbon cycling; Table S3: Functional groups related to the nitrogen cycling; Table S4: Functional groups related to the human pathogens; Table S5: Functional groups related to the sulfur cycling.

Author Contributions: Conceptualization, S.W., W.L. and Y.Q.; collecting samples, S.W., Y.Q., L.D., H.M. and J.L. (Jiao Li); data curation, S.W., W.L. and Y.Q.; methodology, S.W., W.L., J.L. (Jun Li), and L.D.; formal analysis, S.W., W.L., H.M. and J.L. (Jiao Li); writing—original draft preparation, S.W., W.L. and J.L. (Jun Li); writing—review and editing, S.W., W.L. and H.S.; supervision, S.W.; funding acquisition, S.W. All authors have read and agreed to the published version of the manuscript.

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