

## Article

# Microbial Community Structure and Co-Occurrence Patterns in Closed and Open Subsidence Lake Ecosystems

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**Abstract:** Different types of subsidence lakes formed by underground coal mining are severely polluted by mine-production wastewater, domestic sewage, and agricultural irrigation water. Microbial communities perform a crucial role in biogeochemical cycling processes and responses to natural and anthropogenic disturbances in lake waters. Therefore, it is important to investigate the characteristics of microbial community diversity in subsidence lakes for the utilization of water resources in mining areas. In this study, we collected water samples from open and closed subsidence lakes and explored the bacterial communities based on Illumina amplicon high-throughput sequencing. Results showed that bacterial diversity and community and functional composition in open and closed lakes differed greatly, and the bacterial diversity in open lakes was significantly higher than that in closed lakes ( $p < 0.001$ ). Canonical correspondence analysis found that pH explained 31.73% and 39.98% of the variation in the bacterial community and functional composition in closed lakes, while  $\text{NH}_4^+$  and COD accounted for 29.73% and 26.02% of the changes in the community and functional composition in open lakes, respectively. Modified stochasticity ratios based on null-model analysis demonstrated that stochastic processes were the main factor affecting bacterial community assembly in both closed (MST = 0.58) and open lakes (MST = 0.63). In the closed-lake network, *hgcI\_clade* was inferred to be a keystone species, while *Lactococcus*, *Acinetobacter*, *Psychrobacter*, and *Chryseobacterium* were detected as keystone species in the open-lake network. By way of discussion, we provide evidence regarding the bacterial community diversity, structure, co-occurrence patterns, and assembly processes in closed and open subsidence lakes. This study contributes to providing a reference for the utilization of subsidence water resources.

**Keywords:** subsidence lakes; aquatic bacterial community; community diversity; assembly process; co-occurrence pattern



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## 1. Introduction

As a primary resource in China's economy, the exploitation and utilization of coal on a large scale have brought enormous economic and social benefits and provided a fundamental guarantee for the development of the economy [1,2]. However, reliance on coal resources has created a number of environmental problems [3]. For example, long-term large-scale coal mining causes land subsidence, ground fissures, landslides, and soil erosion [4,5]. It is estimated that every 1000 tons of coal mined in China results in about 0.002–0.0033 km<sup>2</sup> of land subsidence and increases the subsidence area by about 700 km<sup>2</sup> each year [6]. Under the action of high phreatic water levels and atmospheric rainfall, different areas of ponding have been formed in coal-mining subsidence areas [7]. Coal-mining subsidence soil evolves into aquatic sediment in which heavy metals, nutrients, and microorganisms

in the soil will be released into the aquatic environment and become potential pollution sources [8]. At the same time, the area of some subsidence lakes influenced by human and mining activities has further increased. If these areas gradually connect with adjacent rivers, different types of subsidence lakes will form, such as open and closed lakes [9]. With the comprehensive promotion of geological environment management in mining areas, the subsidence lakes have been developed and utilized as different types of water bodies such as fish ponds, wetland parks, and plain reservoirs [10], and these water bodies play an important role in maintaining the ecological balance of mining areas and promoting local economic development [11]. Therefore, investigating the nutrient status and biodiversity characteristics of water bodies in coal-mining subsidence areas is important for the rational development, as well as use of water resources in mining areas.

In aquatic ecosystems, microbial communities participate in various ecosystem functions and services, including organic degradation, nutrient cycling, and regulation of greenhouse-gas emissions [12,13]. The structure and function of water-body microbial communities can respond rapidly to natural and anthropogenic disturbances. For example, microbial communities can have important broad environmental impacts by regulating the accumulation and transformation of organic matter and influencing water-body greenhouse-gas emissions and carbon and nutrient cycling [14]. Given the importance of microbial communities in water bodies to ecosystem functions and services, it is important to determine the patterns of microbial community response to changes in the water environment of mining area caused by mining activities. To date, many researches have focused on the influence of natural and human factors on microbial communities in eutrophic lakes [15,16], rivers [17,18], and oceans [19,20] and have observed changes in community composition and assembly processes. These studies have shown that microbial communities in water bodies under the influence of various environmental and anthropogenic factors have significant spatial and temporal heterogeneity and that community co-occurrence patterns and assembly processes are influenced by various environmental parameters, such as pH, dissolved oxygen, and salinity [21,22]. Previous studies have investigated the spatial and temporal succession characteristics of microbial community structure and co-occurrence patterns in open subsidence lakes [23,24], and microbial community diversity and stability has been found to be higher in summer than in winter in open subsidence lakes. Is the microbial community succession in closed subsidence lakes which do not exchange with external natural water bodies consistent with that in open lakes? The differences in microbial community structure and co-occurrence patterns between open and closed subsidence lakes under the influence of strong human activities have not been investigated.

Water microorganisms in natural ecosystems do not exist as isolated individuals but in complex interaction systems, which determine the composition of microbial communities [25]. These interactions and the resulting co-existence patterns of different microorganisms can be examined by ecological network analysis [26,27]. Although co-occurrence network analysis may not always represent real biological interactions, it can help comprehend the complexity of the microbiome. How does this complexity vary in response to environmental factors and how do microbial interactions affect ecosystem functions? [28,29]. In recent years, co-occurrence network analysis has been widely used to study the interactions of microbes in lakes [30,31], rivers [32,33], farmland [26,34], and other habitats, as well as their relationships with the environment. For example, a study found that microbial co-occurrence networks in eutrophic lakes undergo significant seasonal changes [35]. In addition, key taxa are species that perform a key role in the community. Regardless of their spatial and temporal abundance, they have a significant impact on the structure and function of the microbiome either alone or in a guild [36]. Research has shown that the extinction of key taxa might have a negative impact on the stability of the microbial community and cause great changes in the composition and function of the community [37]. Therefore, to better understand the structure and function of ecosystems, it is important to determine the co-occurrence patterns of microbial communities and the key groups in the

communities. However, the co-occurrence relationship of microbial communities between different types of subsidence lakes (open and closed) and the differences in key groups of microbial communities have not been studied in depth.

To address these knowledge gaps, we selected coal-mining subsidence lakes in Huainan as a model ecosystem (closed lakes vs. open lakes) and analysed the microbial community structure, diversity, co-occurrence patterns, and assembly processes based on 16S rRNA sequencing, network analysis, and null modelling. The purpose of this study was twofold. First, this study was designed to determine the overall differences in microbial community structure, diversity, co-occurrence relationships, and assembly mechanisms between open and closed subsidence lakes in Huainan, China. Secondly, the study was designed to investigate the environmental factors driving the variations in the species and functional composition of microbial communities. The results of this research provided basic data and a theoretical foundation for the management and utilization of water resources in coal-mining subsidence areas.

## 2. Materials and Methods

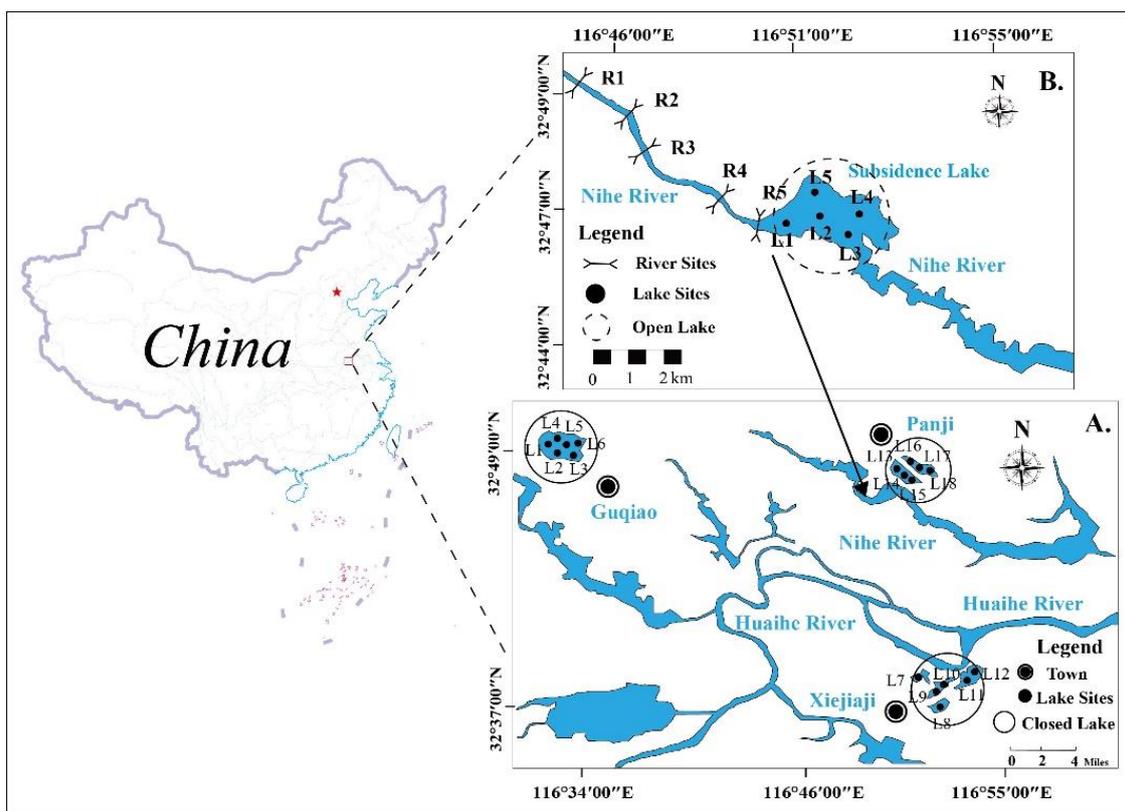
### 2.1. Study Area and Sample Collection

The subsidence lakes were located in Huainan, Anhui Province, China (Figure 1). The open subsidence lakes ( $116^{\circ}45'38''$ – $116^{\circ}55'7''$  E and  $32^{\circ}44'48''$ – $32^{\circ}49'54''$  N) were located in the subsidence water area of the Nihe River. The closed subsidence lakes ( $116^{\circ}33'49.35''$ – $116^{\circ}56'3.65''$  E and  $32^{\circ}32'47.99''$ – $32^{\circ}49'58.02''$  N) were located in the subsidence waters of Panji, Guqiao, and Xiejiaji. The open subsidence lakes were connected with the adjacent Nihe River and greatly affected by river-flow dynamics. The open subsidence lakes covered an area of 4.9 km<sup>2</sup>. The mine corresponding to the open subsidence lake was closed and stopped mining in 2015. Therefore, the mining process had no effect on the microbial community of the water body at the time of sampling in this study. Microbial communities are mainly affected by anthropogenic activities such as seine farming, agricultural irrigation, and photovoltaic power generation [23].

The closed subsidence lakes did not exchange water with other water bodies, and these lakes were mainly fed by rainfall, surface runoff, and groundwater. The areas of the closed subsidence lakes were 3.2 km<sup>2</sup>, 10.71 km<sup>2</sup>, and 4.5 km<sup>2</sup> [38]. The mine corresponding to the closed subsidence lake was closed and stopped mining in 2018. Therefore, the mining process also had no impact on the microbial community of the water body. The microbial community was mainly influenced by anthropogenic activities (agricultural irrigation) and the heavy metal content in the open and closed subsidence lakes was low after environmental treatment. For example, some studies have analyzed the content of heavy metals such as Cd, Cr, Pb, Ni, and Zn in the subsidence lakes and found that heavy metal content was lower than the class III standard of China's Environmental Quality Standards for Surface Water (GB3838-2002), and there was no heavy metal pollution [39,40].

To compare the differences of bacterial communities in the closed and open subsidence lakes, 10 sampling points were arranged in the open lakes, and 18 sampling points were arranged in the closed lakes. In the open subsidence lake, 5 sampling points were arranged based on the lake area. In order to explore the impact of adjacent rivers on the microbial community of the subsidence lakes, 5 sampling points were arranged in the rivers. In the closed subsidence lake, 6 sampling points were arranged in each lake based on the lake area. Due to the large number of closed lakes in the study area, three typical closed lakes (Panji, Guqiao, and Xiejiaji) were selected to compare whether there were differences between different lakes.

Water samples were collected in May 2021. 5 L water samples of lake surface (0.5 m from the surface) were obtained and stored in pre-rinsed sterile plastic bottles at 4 °C and then taken to the laboratory within 12 h. In the laboratory, a portion of the water samples was filtered through 0.22 µm pore pre-washed polycarbonate filters. The filters were stored at −80 °C for the next step of DNA extraction. The other part of the samples was immediately tested for the physical and chemical parameters of the water body.



**Figure 1.** The sampling sites in the closed and open subsidence lakes. (A) Sampling points for closed subsidence lakes. (B) Sampling points for open subsidence lakes. (B) is an enlarged view of the river indicated by the arrow in (A).

## 2.2. Physicochemical Analysis

At each sampling point, pH, temperature (T), dissolved oxygen (DO), oxidation-reduction potential (ORP), and electrical conductivity (EC) were measured using a multiparameter water quality sonde (YSI 6600 V2; Yellow Springs, OH, USA). Based on standard methods, total nitrogen (TN), total phosphorus (TP), nitrite ( $\text{NO}_2^-$ -N), ammonia nitrogen ( $\text{NH}_4^+$ -N), chemical oxygen demand (COD), chlorophyll-a (Chl-a), nitrate ( $\text{NO}_3^-$ -N), and biochemical oxygen demand (BOD) were measured [41].

## 2.3. DNA Extraction, PCR Amplification, and Sequence Analyses

Using a Fast DNA Spin Kit for Soil (MP Biomedical, Irvine, CA, USA) to extract the total DNA of the bacterial community, based on manufacturer's protocols, the V3–V4 regions genes of bacterial community 16S rRNA were amplified using the universal primer pair of 338F/806R. The detailed procedure of PCR amplification is described in the study of Zhang et al. [42]. PCR products were pool-purified with equimolar solution and then sent to Personal Biotechnology Co., Ltd., (Shanghai, China) for sequencing on the MiSeq PE300 platform (Illumina, San Diego, CA, USA) and generation of paired-end reads ( $2 \times 300$  bp).

Based on the previous study, bioinformatic analysis, including quality control; merging of paired sequences; removal of chimeric sequences; and clustering of amplicon sequence variant (ASV) was conducted [43]. The ASVs from chloroplasts and mitochondria were excluded from downstream analysis.

## 2.4. Statistical Analyses

Chao1, Shannon, and phylogenetic diversity indices for each sample were calculated using normalized sequencing depth and the package "vegan". Principal coordinate analysis (PCoA) was used to calculate the beta-diversity of bacterial community based on the

Bray–Curtis distance. A comparison of microbial communities in different sampling types was performed by an `adonis()` function in the `vegan` package. Canonical correspondence analysis (CCA) was used to explore the relationships of bacterial community composition, functional composition, and significant environmental variables because detrended correspondence analysis (DCA) showed the length of the first axis  $> 4$  [44]. To avoid collinearity among significant factors, we calculated the variance inflation factors (VIFs), and removed factors with a VIF  $> 10$ . Finally, a mantel test with 999 permutations was performed to remove factors with insignificant ( $p > 0.05$ ) effects, and the contribution of different physicochemical parameters was assessed using the “`rdacca.hp`” package [45]. Functional annotation of the bacterial community was performed by using the package of FAPROTAX [46]. The difference in community diversity, community composition, functional composition, and physicochemical parameters between the closed and open lakes was determined by using the software of STAMP v2.1.3 [47]. The Kruskal–Wallis test was used to explore the differences in community diversity, community composition, functional composition, and physicochemical parameters between different closed subsidence lakes. The relationships of geographic distance, physicochemical parameters, and community similarity were calculated based on the mantel permutation test using “`vegan`” and “`linkET`” packages [48].

Modified stochasticity ratio (MST) based on the null model was calculated to explore the bacterial community assembly process. Bacterial community structure was influenced by stochastic process if  $MST > 0.5$ , while bacterial community structure was influenced by deterministic process if  $MST < 0.5$ . The value of MST was calculated based on Jaccard similarity metrics and MST package in R [49].

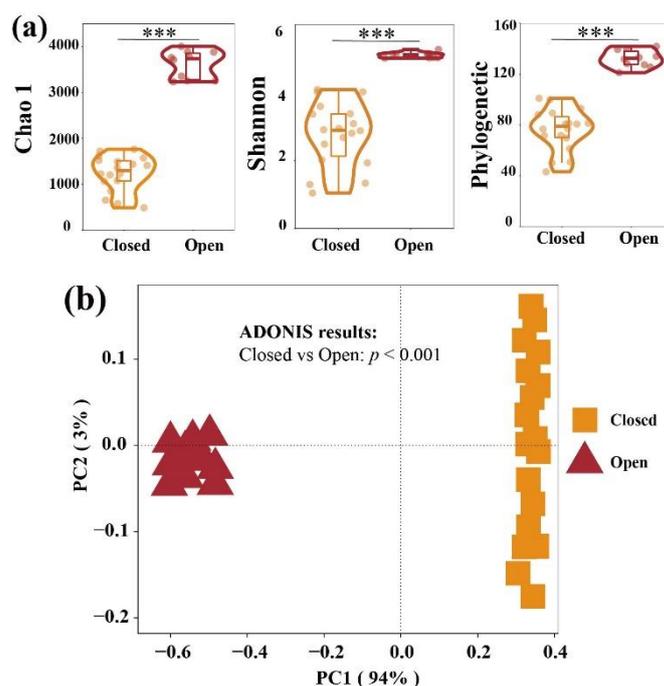
To compare the difference in bacterial community co-occurrence patterns, networks analyses was performed in R based on the “`psych`” package. Before calculating Spearman’s correlation, the relative abundance of ASVs  $> 0.05\%$  was selected to simplify the set. When constructing networks,  $p$ -values (adjusted)  $< 0.01$  and correlation coefficient  $|r| > 0.8$  was selected. The “`igraph`” package was used to calculate topological properties and random networks in R [50]. The greedy modularity optimization method was used for the modular division of ecological networks [22] and the software of Gephi was used to visualize networks. The nodes of the network were classified into connectors, peripheral nodes, module hubs, and network hubs based on the method of within-module connectivity ( $Z_i$ ) and among-module connectivity ( $P_i$ ) [51]. The network and module hubs and connectors are generally proposed as keystone module members [52].

### 3. Results

#### 3.1. Alpha and Beta Diversity of Bacterial Communities

A total of 45,360 ASVs were detected from 2,106,424 high-quality sequences of 28 water samples. Rarefaction curves approached an asymptote after 43,000 reads, and the good’s coverage for individual samples ranged from 97 to 99%, indicating that the sequencing depth was sufficient and recovered most of the local species (Figure S1; Table S1). Alpha diversity analysis suggested that the Chao 1, Shannon, and phylogenetic diversity indices in the open lakes ( $3609 \pm 303$ ,  $5.1 \pm 0.08$ , and  $132.1 \pm 6.8$ , respectively), were higher than those in the closed lakes ( $1234 \pm 388$ ,  $2.8 \pm 0.9$ , and  $101 \pm 19.2$ ) (Figure 2a; Wilcoxon test,  $p < 0.001$ ).

The PCoA was performed to reveal the spatial differences in the bacterial community. The results showed that bacterial communities in the open lakes were distinctly different from those in the closed lakes ( $p < 0.001$ ). Each group formed a distinct cluster among all samples, and samples from open lakes were separated from closed lakes (Figure 2b). Overall, the alpha diversity indices and the difference in bacterial community composition between the open lakes and closed lakes were statistically significant.

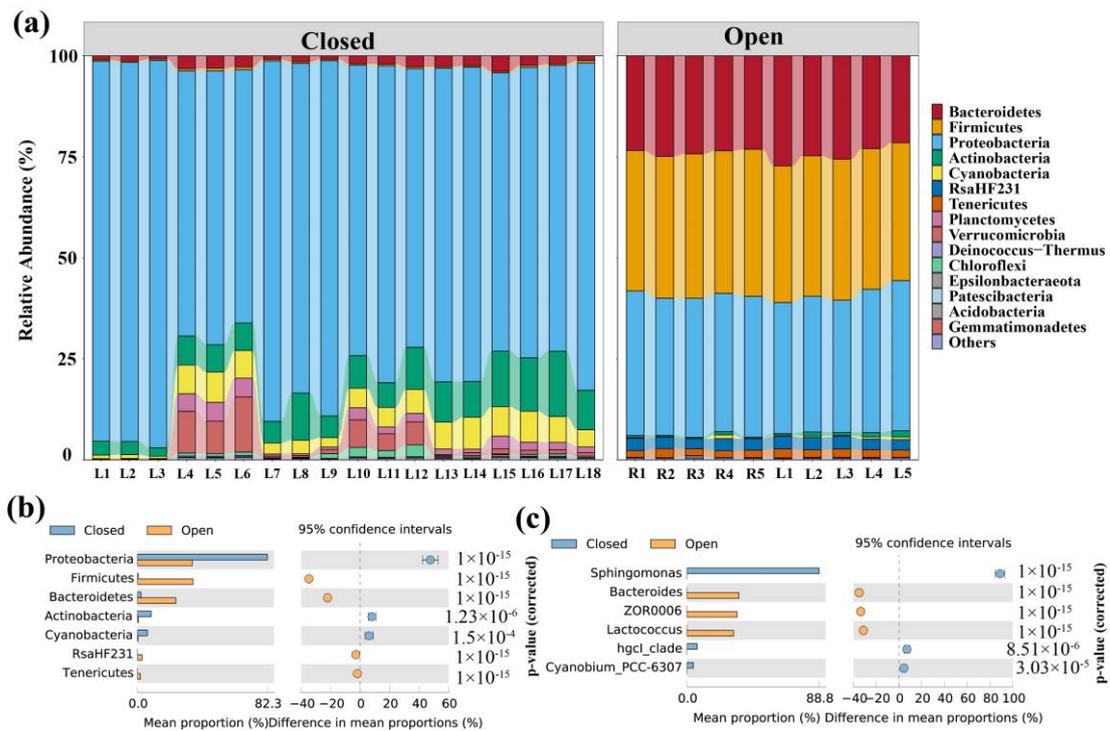


**Figure 2.** (a) Bacterial community alpha diversity indices between the open lakes and closed lakes. (b) Principal coordinate analysis of bacterial community structures between the open lakes and closed lakes. \*\*\*  $p < 0.001$ .

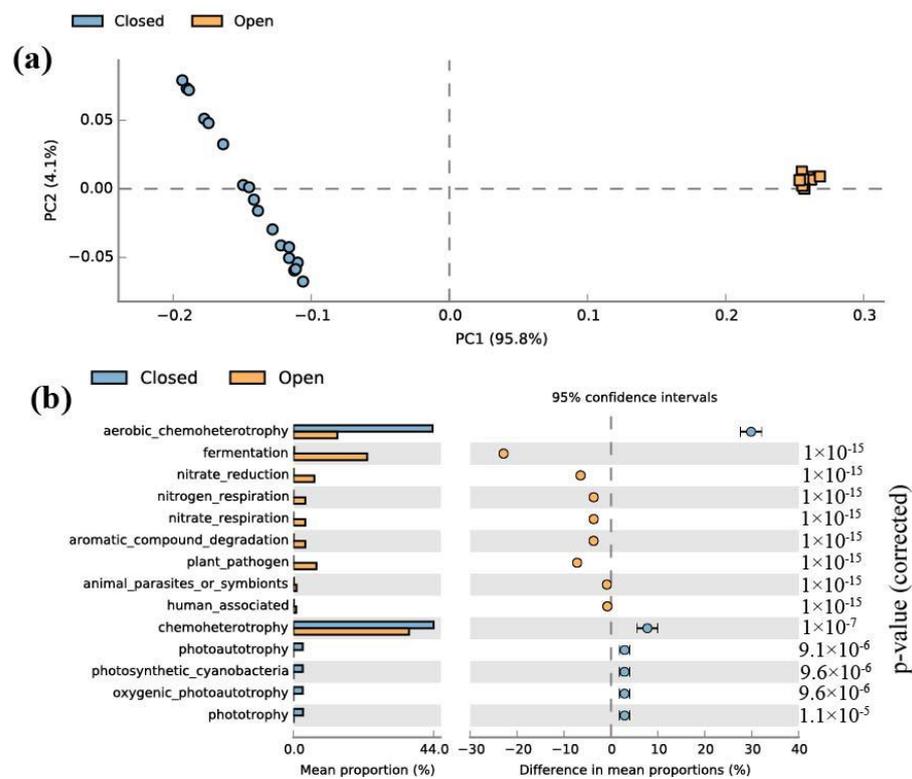
### 3.2. Composition of Bacterial Communities

The results also showed that bacterial community composition in different types of subsidence lakes was different (Figure 3a). At the phylum level, bacterial communities were dominated by Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, and Cyanobacteria. Dominant species in different sampling types of lakes show significant differences (Figure 3b). The closed lakes were significantly enriched in Proteobacteria, Actinobacteria, and Cyanobacteria. Conversely, Firmicutes and Bacteroidetes were significantly more abundant in the open lakes (Figure 3b, Wilcoxon test,  $p < 0.001$ ). At the genus level, *Sphingomonas*, *Bacteroides*, *ZOR0006*, *Lactococcus*, *hgcI\_clade*, and *Cyanobium\_PCC-6307* were the most abundant genera in the open and closed lakes' water samples (Figure S2). *Sphingomonas*, *hgcI\_clade*, and *Cyanobium\_PCC-6307* were significantly enriched in the closed lakes. Conversely, *Bacteroides*, *ZOR0006*, and *Lactococcus* were significantly higher in the open lakes than that in closed lakes (Figure 3c, Wilcoxon test,  $p < 0.001$ ).

The predicted metabolic functional groups were further revealed using FAPROTAX (Figure S3). A total of 63 functional groups were obtained in open lakes and closed lakes, which involved the nitrogen cycle, sulfur cycle, carbon cycle, energy source, and other functions. PCoA of functional groups suggested a clear separation between open lakes and closed lakes, and the functional variation in closed lakes was much higher than that in open lakes (Figure 4a). Among the predicted functions, aerobic chemoheterotrophy, chemoheterotrophy, photoautotrophy, photosynthetic cyanobacteria, oxygenic photoautotrophy, and phototrophy were the most abundant groups in closed lakes. However, the communities in open lakes had a high percentage of functional groups with fermentation, nitrate reduction, nitrate respiration, nitrogen respiration, and plant pathogens (Figure 4b).



**Figure 3.** (a) Bacterial community composition between the open lakes and closed lakes. (b) Significantly difference of bacterial community at phylum (b) and genus (c) level between the open lakes and closed lakes.



**Figure 4.** Predicted metabolic function groups of bacterial communities in open lakes and closed lakes. (a) Principal coordinate analysis of bacterial function composition. (b) The difference of functional groups in the open lakes and closed lakes.

### 3.3. Relationship between Environmental Factors and the Bacterial Community

Physicochemical properties in open lakes and closed lakes indicated strong spatial variations (Table S3). Overall, the EC values and contents of  $\text{NO}_2^-$ , DO, COD, BOD, TP, and Chla in open lakes were higher (Wilcoxon test,  $p < 0.05$ ), but T and ORP were significantly higher in closed lakes (Wilcoxon test,  $p < 0.05$ ). However, there was no significant difference in physical and chemical indicators between open subsidence lakes and adjacent rivers (Wilcoxon test,  $p > 0.05$ ), as well as between different closed subsidence lakes (Kruskal–Wallis test,  $p > 0.05$ ).

The results of canonical correspondence analysis (CCA) showed that the variations in the bacterial community and function were related to six environmental factors in closed lakes: pH,  $\text{NO}_3^-$ , ORP, TN,  $\text{NH}_4^+$ , and EC (Figure 5a,b), which together explained 68.48% and 98.14% of the total bacterial community and functional variation, respectively. Among them, pH was the most crucial factor driving bacterial community and function. In open lakes, the bacterial community was influenced by five factors: TN,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ , and COD (Figure 5c), which together explained 94.12% of the total community change.  $\text{NH}_4^+$  was the most crucial environmental factor. In contrast, COD, TN, Chla, and  $\text{NO}_2^-$  together explained 57.68% of the community function variation in open lakes (Figure 5d). Among them, COD was the most crucial factor shaping bacterial community function.

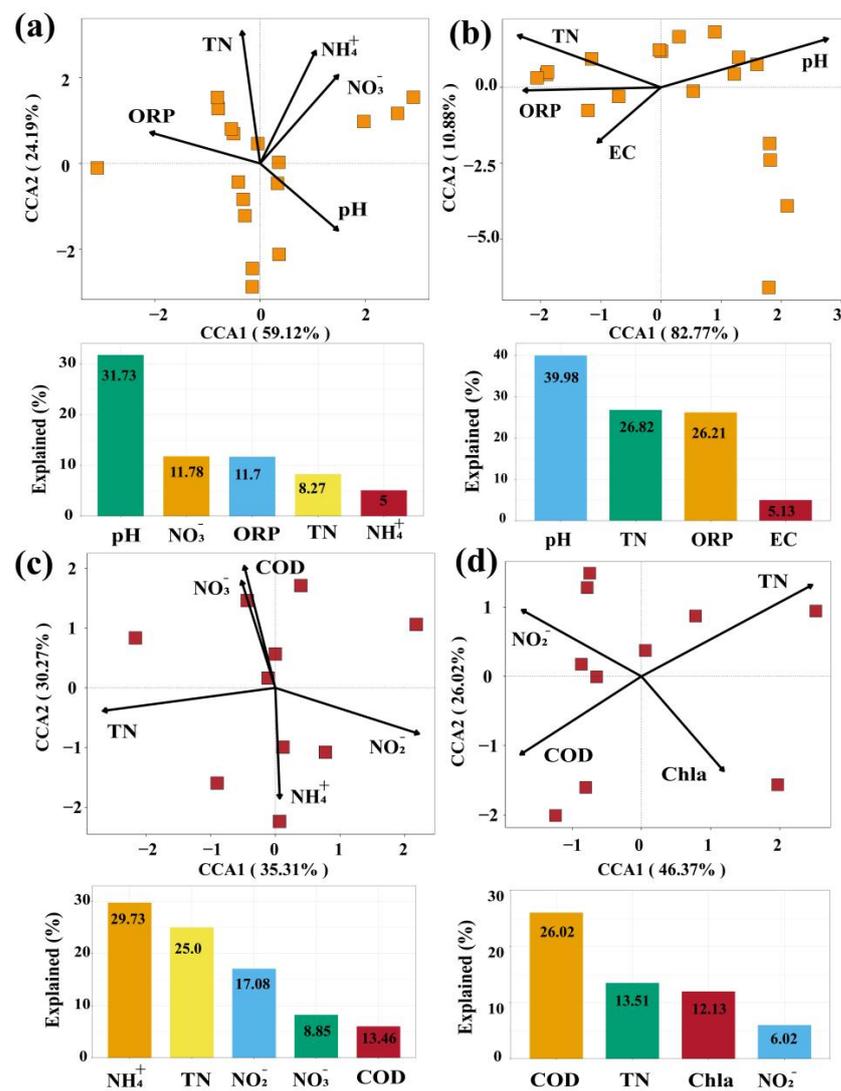
The Mantel test showed that the bacterial community similarity was significantly correlated with geographic factors ( $r = 0.34$ ,  $p = 0.002$ ) and physicochemical parameters ( $r = 0.21$ ,  $p = 0.033$ ) in closed lakes, suggesting that there was a strong interaction of the bacterial community with spatial and environmental variables (Figure S4a,b). In contrast, the bacterial community showed slight negative correlations with geographic factors ( $r = -0.18$ ,  $p = 0.76$ ) and physicochemical parameters ( $r = -0.19$ ,  $p = 0.76$ ) in open lakes (Figure S4c,d).

### 3.4. Assembly Processes of Bacterial Community

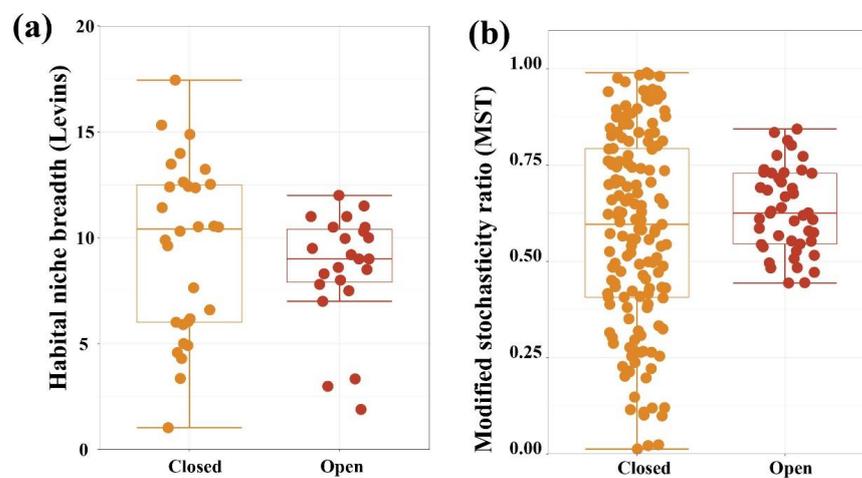
MST results showed that the bacterial community in both closed (MST =  $0.58 \pm 0.25$ ) and open lakes (MST =  $0.63 \pm 0.1$ ) was governed by stochastic processes (MST > 0.5), and the influence of stochastic processes in open lakes was higher than in closed lakes (Figure 6). Niche analysis showed that the community niche width in closed lakes ( $9.5 \pm 4.1$ ) was higher than in open lakes ( $8.8 \pm 2.3$ ).

### 3.5. Network Analysis

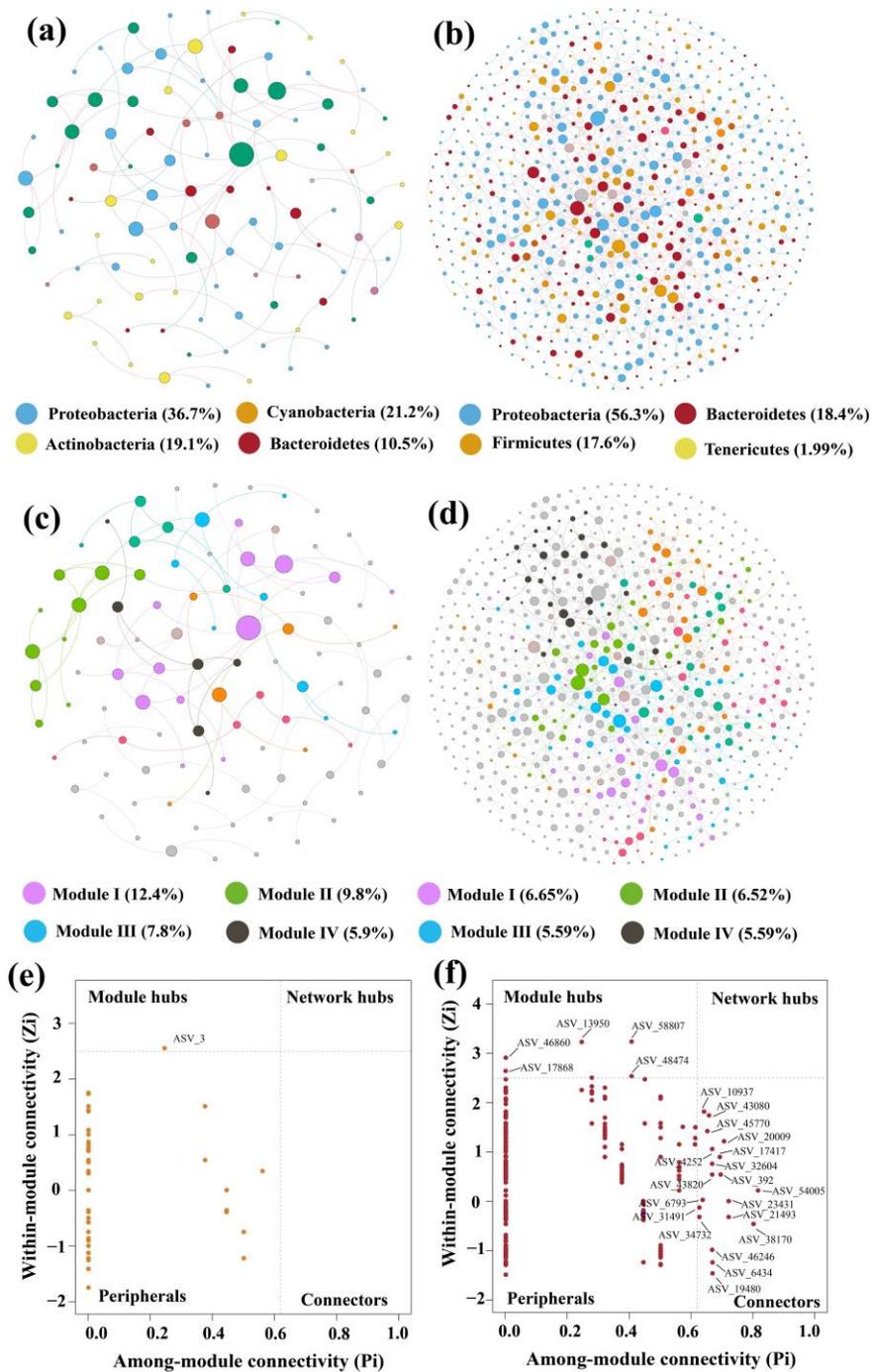
Co-occurrence networks of the bacterial communities in closed and open lakes were conducted based on correlation analysis. Bacterial communities in closed and open lakes displayed distinctly different co-occurrence patterns (Figure 7). The resulting network was composed of 102 nodes with 95 edges in closed lakes, and 752 nodes with 900 edges in open lakes (Table S4). In addition, we recorded a higher proportion of negative edges and a lower modularity in the open-lake network (70.4% and 0.83, respectively), than in the closed-lake network (33.7% and 0.85, respectively). Moreover, the number of keystone species in open lakes was higher than in closed lakes (Figure 7e,f). These discoveries indicated that the bacterial communities in open lakes were more stable than those in closed lakes. In the closed-lake network, *hgcI\_clade* (*Sporichthyaceae*) was inferred to be keystone species. In the open-lake network, *Lactococcus* (*Streptococcaceae*), *Acinetobacter* (*Moraxellaceae*), *Psychrobacter* (*Moraxellaceae*), *Chryseobacterium* (*Weeksellaceae*), *Myroides* (*Flavobacteriaceae*), *Cetobacterium* (*Fusobacteriaceae*), *Reyranella* (*Reyranellaceae*), *Aeromonas* (*Aeromonadaceae*), *Bacteroides* (*Bacteroidaceae*), *Sediminibacterium* (*Chitinophagaceae*), and *Vibrio* (*Vibrionaceae*) were inferred to be keystone species (Figure 7e,f).



**Figure 5.** Canonical correspondence analysis (CCA) showing the physicochemical parameters influence the bacterial community composition (a,c) and bacterial functional composition (b,d) in closed lakes (a,b) and open lakes (c,d).



**Figure 6.** Community niche width (a) and community assembly processes (b) based on modified stochasticity ratio (MST).



**Figure 7.** Co-occurrence networks of microorganism colored by (a,b) phylum and (c,d) modularity in closed lakes (a,c) and open lakes (b,d). Identified keystone species in closed lakes (e) and open lakes (f). The edge suggests a strong ( $|r| > 0.8$ ) and significant ( $p < 0.01$ ) correlation. The size of node is the proportion to the number of connections (i.e., degree).

The closed- and open-lake networks could be divided into four major modules. Module I accounted for 12.4% of the network and module II accounted for 9.8% of the closed-lake network, and module I and module II accounted for 6.65% and 6.52%, respectively, of the open-lake network (Figure 7c,d). In the closed-lake network, Actinobacteria, Proteobacteria, Cyanobacteria, and Bacteroidetes had a higher abundance in Module I, while Firmicutes and Proteobacteria were the dominant species in Module I in the open-lake network.

## 4. Discussion

### 4.1. Community and Functional Diversity of Bacteria in Closed and Open Lakes

Analysis of community properties showed that bacterial communities in open and closed lakes exhibited significant differences in community diversity and structure. This is consistent with the finding that microbial biodiversity is distributed heterogeneously among different habitat types [35]. Habitat heterogeneity is considered one of the main determinants affecting biodiversity [53]. The  $\alpha$ -diversity of bacterial communities in open lakes was higher than those in closed lakes (Figure 2). A similar study reported on the phytoplankton community in closed and semi-closed lakes in the northern area of Wenzhou City [54]. Compared with open lakes, closed lakes exhibited lower nutrient contents but higher oxidation potential, indicating an oligotrophic and distinctive hydrochemistry environment. The results also found a significant positive correlation between diversity index and pH and ORP in closed lakes (Table S2). A previous study found that nitrogen and phosphorus were important environmental factors affecting the growth of the microbial community [24]. The low nutrient content in the closed lakes may have limited the growth of other low-abundance bacterial communities, forming a community structure with *Proteobacteria* as the dominant bacteria (77.4%), and thus reducing the community diversity. In contrast, as open lakes can exchange water with rivers, upstream rivers import large amounts of nutrients to lakes. The open lakes are used for fish-farming, and artificial baiting will also increase the content of nutrients in the water body. Adequate nutrients provide a good environment for the growth of bacterial communities in open lakes, and some rare species are able to proliferate, which increases species diversity. This is consistent with the finding that the diversity index was significantly positively correlated with COD and  $\text{NH}_4^+$  in this study (Table S2). Furthermore, terrestrial soils are known to typically contain a high diversity of microorganisms that can enter rivers in response to surface runoff [55]. The exotic species in rivers are further imported into lakes through water exchange, which also increases community diversity in open lakes.

Functional annotation indicated enrichment of metabolic functional groups in bacterial communities in closed and open lakes, which showed spatial heterogeneity (Figure 4). In closed lakes, the metabolic functional groups of aerobic chemoheterotrophy, chemoheterotrophy, and photoautotrophy (contributed by the genera *Sphingomonas* and *Cyanobium\_PCC-6307*) were enriched. In comparison, the metabolic functional groups of nitrate reduction, nitrate respiration, and nitrogen respiration (contributed by the genus of *Shewanella*) were enriched in open lakes (Figure 4b), and the CCA results showed that TN and  $\text{NO}_2^-$  explained 13.51% and 6.02% of the functional changes in bacterial communities, respectively (Figure 5d). A significant enrichment of genes associated with nitrogen metabolism was found in open lakes, which may have been due to the higher content of  $\text{NO}_2^-$ . This result is in line with previous studies, which demonstrated that genes related to nitrate reduction had a higher abundance when nitrate was enriched [56]. However, the lack of various nutrients for bacterial growth in closed lakes means that bacteria can only maintain normal growth through intercommunity heterotrophy and autotrophy. Therefore, the expression of aerobic chemoheterotrophy, chemoheterotrophy, and photoautotrophy genes was increased in the closed lakes. This was also confirmed by the results that Cyanobacteria was the dominant species in the closed lakes (5.8%), while it was not observed in open lakes.

### 4.2. Differences in Bacterial Community Composition and Assembly Processes

In this study, we found that stochastic processes (i.e., ecological drift, random speciation, death, and dispersal events) are essential mechanisms impacting community variation in closed and open lakes (Figure 6). In open lakes, distance-decay patterns were observed in the bacterial community, suggesting dispersal limitation (Figure S4). Since the water flow was directional, microorganisms in subsidence waters in the midstream and downstream rivers were difficult to transfer back upstream, resulting in limited dispersion [57]. Higher nutrient levels in open lakes promote microbial colonization, and large proliferation

of microbial communities may enhance stochastic processes (e.g., births, deaths), which in turn lead to stochastic changes in the abundance of microbial communities [58]. The large proliferation of microbial communities will further increase the community diversity, which is supported by the fact that the diversity of bacterial communities in open lakes is higher than that in closed lakes. In closed lakes, while stochastic processes were the main influencing mechanism, we found that deterministic processes partly influenced the community variation (Figure 6). This finding is consistent with the result that community similarity was significantly correlated with physicochemical parameters ( $r = 0.21$ ,  $p = 0.033$ ) in closed lakes. pH may be a major deterministic process affecting bacterial communities in closed lakes, which explained 31.73% of the bacterial community structure variation (Figure 5). Studies showed that changes in pH in the environment affected the properties of microbial nucleic acids, extracellular polymerases, and other substances, and thus their activity [59].

Proteobacteria (77.4%), specifically *Alphaproteobacteria* and *Gammaproteobacteria*, were the dominant bacteria in the closed lakes. This is consistent with the finding that Proteobacteria was the dominant bacteria in other shallow lakes [24]. It supports that *Alphaproteobacteria* are widespread in aquatic ecosystems and participate in the biogeochemical cycles of C, N, and S [60]. *Gammaproteobacteria* was found to be widely distributed in oligotrophic lake waters, and its community structure was influenced by pH and nutrient concentration [61]. *Sphingomonas* (71.6%) (*Alphaproteobacteria*) was the dominant genus in the closed lakes. *Sphingomonas* is a heterotrophic Gram-negative bacterium that was previously found to have good degradation capacity for a variety of aromatic organic compounds in water [62]. The high relative abundance of members of *Proteobacteria* in closed lakes may be due to the low nutrient content. *Sphingomonas*, for example, provides for its own growth by degrading aromatic compounds and microcystins into small-molecule organic matter. Moreover, we found Cyanobacteria in closed lakes, while no observations were made in open lakes (Figure 3). Previous research found that the gas vesicles contained in some cyanobacterial cells can provide buoyancy for cells in water environments, and gives them advantages in the competition for light and carbon dioxide [63]. Consequently, cyanobacterial blooms usually occur in lakes and reservoirs with stagnant water bodies, little wind-mixing, and low water-level fluctuations [64]. Closed lakes are unable to exchange water with the outside environment, which may lead to the appearance of cyanobacteria in closed lakes.

In open lakes, Bacteroidetes (*Bacteroides*), Firmicutes (*ZOR0006*, *Lactococcus*), and their corresponding genera were significantly enriched (Figure 3). Bacteroidetes and Firmicutes were considered indicator organisms for assessing faecal pollution and exogenous pollution input in water bodies [65,66]. *Bacteroides* were a group of pathogenic bacteria that were commonly abundant in humans and animals and participated in a complex cycle of carbon and protein-rich substances [67]. *ZOR0006* and *Lactococcus* were also dominant species in fish intestines [68,69]. These genera were significantly enriched in the open lakes which may be attributed to the fact that part of the open lakes was being exploited for seine farming and the other part was being exploited for photovoltaic power generation. In addition, the terrestrial ecosystem was transformed into a composite water–land ecosystem during the formation of subsidence waters. Large amounts of plant and animal residues and soil organic matter enter water bodies, together with exogenous substances imported from rivers, so *Bacteroidetes* may be enriched in open lakes.

However, the results found no significant differences in microbial community structure and physicochemical factors between the open lakes and the adjacent river (Figure 3; Table S3). This is probably due to the higher flow and velocity of the rivers in summer, combined with the fact that open lakes and rivers allow for frequent water exchange, resulting in homogenization of water bodies between rivers and lakes. Therefore, there is no significant difference in microbial community structure between rivers and open lakes. The results indicate that there were also no significant differences in microbial community structure between the different closed lakes (Figure 3). This is due to the similar type of use

of the closed lakes, mainly for agricultural irrigation. Similar utilization types in closed lakes lead to similar microbial community structures [70].

#### 4.3. Differences in Co-Occurrence Patterns of Bacterial Communities in Closed and Open Lakes

Network analysis provides deep insight into the complex interactions of bacterial communities in different types of subsidence lakes. The open-lake network in this study exhibited more species (nodes) and ecological connections (links) than the closed-lake network, indicating more densely connected and complex network patterns (Figure 7). This may have been due to the fact that open lakes receive more input from external substances, and the abundant nutrients in the lakes promote competition and cooperation among species, thus forming a complex ecological network. The closed-lake network was mainly positively correlated (66.3%), indicating that there was an ecological reciprocity or cooperation relationship between the microbial communities in the closed lakes, while the proportion of negative correlations in the open lakes was high (70.4%), indicating that there was a competitive effect in the open-lake microbial communities. The high proportion of positive correlations in the closed lakes may have been due to the low content of nutrients in the water body, as bacterial communities use limited resources through efficient cooperation [26]. Moreover, the ecological network of closed lakes exhibited lower mean distances, suggesting a more efficient transfer of information, energy, and material between species [71]. The nutrient content in the closed lakes was low, which is conducive to the symbiosis of species using limited resources.

Keystone species represent highly correlated microorganisms that perform a crucial role in the structure and function of microbial communities and serve as indicator species of environmental change [28]. Based on the Pi–Zi scatter plots, in closed lakes, *hgcI\_clade* was inferred to be a keystone species. This is in line with previous research, which observed that the *hgcI\_clade* may be a persistent keystone species in global oligotrophic aquatic ecosystems [72]. The *hgcI\_clade* can dissolve organic carbon and matches genes associated with globally important nitrogen-cycling pathways, including denitrification and nitrogen fixation [73]. The *hgcI\_clade* in closed lakes increases carbon and nitrogen sources in the water body through break-down organic carbon and nitrogen fixation, providing nutrients for the growth of bacterial communities in oligotrophic lakes. By comparison, *Lactococcus*, *Acinetobacter*, *Psychrobacter*, *Chryseobacterium*, *Myroides*, *Cetobacterium*, *Reyranella*, *Aeromonas*, *Bacteroides*, *Sediminibacterium*, and *Vibrio* were identified as keystone species in the open-lake network. Keystone species in open lakes are mainly associated with pathogenic bacteria in fish guts. For example, it was found that *Lactococcus*, *Acinetobacter*, and *Cetobacterium* were usually significantly enriched in fish intestines and were associated with pathogenicity in fish [74–76]. In addition, *Chryseobacterium* and *Myroides* were the potential opportunistic pathogen bacteria in water bodies [77,78]. These results indicate that anthropogenic activities such as seine farming and photovoltaic power generation in the open lakes are causing serious impacts on lake water bodies. Therefore, the keystone species in the open-lake network can be used as indicator species reflecting the impact of anthropogenic activities on the lakes. Moreover, *Bacteroides* and *Sediminibacterium* were found to be involved in the degradation of organic compounds such as aromatic compounds and perform a crucial role in the carbon cycle of open lakes [67,79]. This is consistent with the finding that the COD content in open lakes was significantly higher than that in closed lakes in this study.

## 5. Conclusions

This study reveals a distinct spatial heterogeneity of microbial community and the underlying community assembly mechanisms in closed and open subsidence lakes, providing potential insight into understanding how microbial diversity is generated and maintained in different types of subsidence lakes. We found that the diversity of the bacterial community in open lakes was significantly higher than that in closed lakes. The function of bacterial communities in closed lakes was mainly related to energy sources

(chemoheterotrophy and photoautotrophy, etc.), while bacterial communities in open lakes were mainly involved in the nitrogen cycle. Stochastic processes were the main factor affecting bacterial community assembly in closed and open lakes. In the closed-lake network, the *hgcI* clade was inferred to be a keystone species and may have been the key taxa for increasing the sources of carbon and nitrogen in the water bodies. In the open-lake network, *Lactococcus*, *Acinetobacter*, *Psychrobacter*, and *Chryseobacterium* were determined to be keystone species and could serve as indicators of the impact of human activities on the lake. In future research, it is necessary to further explore the spatiotemporal succession patterns of microbial communities in different types of subsidence lakes over a long time series. In addition, the relative contributions of heterogeneous selection, dispersal limitation, homogenizing dispersal, and drift in the microbial community assembly in different types of subsidence lakes need to be further explored.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w15101829/s1>. Figure S1: Rarefaction curves of richness of samples in closed and open lakes. (a) Closed lake; (b) open lake; Figure S2: Distribution of bacterial communities at genus levels. (a) Closed lake; (b) open lake; Figure S3: Heatmap representing the abundance of predicted functions based on FAPROTAX. (a) Closed lake; (b) open lake; Figure S4: Mantel test between the Bray–Curtis similarity of bacterial communities and geographical distance (a,c), physicochemical parameters (b,d) in closed lakes (a,b) and open lakes (c,d); Table S1: Diversity index and amplicon sequence variants (ASVs) of samples in closed and open lakes; Table S2: Correlation analysis between diversity index and significant environmental variables in closed and open lakes; Table S3: Physicochemical parameters of samples in closed and open lakes; Table S4: Topological properties of bacterial co-occurrence networks in closed and open lakes.

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