

Article

Response of Community Characteristics and Assembly Mechanisms of *cbbL*-Carrier Carbon-Fixing Microorganisms to Precipitation Changes in Alpine Lakeshore Wetland

Ni Zhang ^{1,2,3} , Siyu Wang ^{2,3}, Shijia Zhou ^{1,2,3}, Desheng Qi ^{1,2,3}, Jing Ma ^{1,2,3} and Kelong Chen ^{1,2,3,*}

- ¹ Qinghai Province Key Laboratory of Physical Geography and Environmental Process, College of Geographical Science, Qinghai Normal University, Xining 810008, China; zhangni0224@163.com (N.Z.); ruyel125@163.com (S.Z.); 2018116@qhnu.edu.cn (D.Q.); qhmdmaging@163.com (J.M.)
- ² Key Laboratory of Tibetan Plateau Land Surface Processes and Ecological Conservation, Ministry of Education, Qinghai Normal University, Xining 810008, China; 15229584811@163.com
- ³ National Positioning Observation and Research Station of Qinghai Lake Wetland Ecosystem in Qinghai, National Forestry and Grassland Administration, Haibei 812300, China
- * Correspondence: ckl7813@163.com

Abstract: Precipitation change strongly influences soil microbial communities, and precipitation patterns have become a key factor affecting carbon and nitrogen cycling processes in wetland ecosystems. The *cbbL* gene is a key gene in the fixation of carbon dioxide during the Calvin cycle. However, the response of *cbbL*-carrier carbon-fixing microorganisms in the lakeshore wetland to precipitation change remains unclear. To this end, we established 25% and 50% increased and decreased precipitation treatments, along with a natural control, and used high-throughput sequencing to investigate the response of the *cbbL*-carrier carbon-fixing microbial community in a lakeshore wetland of Qinghai Lake in response to precipitation change. The results showed that a 25% reduced precipitation treatment significantly increased the relative abundance of Chlorophyta and *Bradyrhizobium*. pH was found to be the most important factor influencing the carbon-fixing microbial community, with a significant positive correlation with *Ferrithrix*. A 25% increased precipitation treatment significantly increased the relative abundance of aerobic chemoheterotrophy and chemoheterotrophy, while a 25% reduced precipitation treatment significantly increased the relative abundance of nitrogen fixation. The increased precipitation and 50% reduced precipitation treatments shift the community assembly process of *cbbL*-carrier carbon-fixing microorganisms from randomness to determinism. Co-occurrence network analysis showed that the network complexity and connectivity between species of *cbbL*-carrier carbon-fixing microorganisms initially decreased and then increased with increasing precipitation. In summary, precipitation change tended to reduce the carbon sequestration potential of the lakeshore wetland, while a 25% reduced precipitation treatment favored the nitrogen fixation process in these wetlands.

Keywords: Qinghai–Tibetan Plateau; Qinghai Lake; climate change; extreme precipitation; carbon and nitrogen cycling



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1. Introduction

Wetlands not only effectively regulate hydrological processes and protect biodiversity but also serve as important carbon sinks, playing a key role in the carbon cycle between the atmosphere and aquatic systems [1,2]. As a vital ecosystem, the lakeshore wetland has significant ecological functions and environmental regulation roles in the context of

global climate change [3,4]. Especially in plateau regions, lakeshore wetlands, due to their unique climate, soil, and hydrological conditions, have become important sites for carbon sequestration processes [5]. However, changes in precipitation patterns driven by climate change, particularly fluctuations in both the amount and distribution of precipitation, may have profound impacts on the carbon cycling processes in wetland ecosystems [6–8]. In the carbon sequestration mechanisms of the lakeshore wetland, microbial communities, especially carbon-fixing microorganisms, play a key role. Carbon-fixing microorganisms convert atmospheric carbon dioxide into organic matter through processes such as photosynthesis, chemoautotrophy, and carbon dioxide reduction, thereby effectively reducing the carbon concentration in the atmosphere [9]. The *cbbL* gene encodes a key enzyme in the carbon fixation pathway—ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). This gene is widely used to study the structure and function of bacterial communities involved in carbon dioxide fixation [10]. However, variations in hydrological conditions, temperature changes, and precipitation fluctuations may all affect the structure and function of the carbon-fixing microbial communities represented by the *cbbL* gene, thereby influencing the carbon fixation capacity of wetlands [11–13].

Community assembly is the process through which microbial biodiversity and function are formed [14,15]. Currently, two main ecological processes, namely determinism and stochasticity, govern microbial community assembly [16]. Environmental selection determines the structure of stable communities in the environment and is a major driver of deterministic processes [17]. Stochastic processes suggest that many species share similar or strong ecological niches, and competition does not lead to mutual exclusion [18]. Therefore, the trade-off between deterministic and stochastic microbial community processes is inherently linked to biogeochemical cycling. Soil function is typically determined by the structure, biomass, and interactions of soil microbial species or groups [19]. Co-occurrence network analysis is an important tool for understanding microbial community structure and interactions [20], as they play a key role in shaping community structure and driving ecosystem functions [21,22]. In recent years, with the intensification of climate change, precipitation patterns in the Tibetan Plateau region have undergone significant alterations. The frequent occurrence of extreme precipitation events and fluctuations in seasonal precipitation may have important impacts on the carbon-fixing microbial communities in the lakeshore wetland [23,24]. Qinghai Lake, located in the northeastern part of the Tibetan Plateau, is one of the largest inland lakes in China. The surrounding lakeshore wetland ecosystem provides a unique natural experimental platform for studying carbon cycling in plateau wetlands [25,26]. However, it remains unclear how *cbbL*-carrier carbon-fixing microorganisms respond to precipitation changes in the lakeshore wetlands under the unique environmental conditions of plateau wetlands, and how precipitation variations regulate their community characteristics, community assembly processes, and microbial interaction networks.

Therefore, this study focuses on the Qinghai Lake Basin in the Northeastern Tibetan Plateau, selecting the lakeshore wetlands of the Qinghai Lake Basin as the study area. Precipitation treatments of +25%, −25%, +50%, and −50% were applied, along with a natural control. The aim is to systematically investigate the response characteristics of the *cbbL*-carrier carbon-fixing microbial community, community assembly processes, and microbial interaction networks in the lakeshore wetlands of Qinghai Lake under different precipitation scenarios, using high-throughput genomic techniques. Soil properties were also measured. This study aims to address the following: (1) Investigate the response patterns of the *cbbL*-carrier carbon-fixing microbial community structure and functional characteristics in alpine lakeshore wetlands to different precipitation gradients. (2) Assess the impact of precipitation-driven soil properties on the *cbbL*-carrier carbon-fixing micro-

bial community. (3) Analyze the community assembly processes and changes in microbial interaction networks of *cbbL*-carrier carbon-fixing microorganisms under different precipitation gradients in the Qinghai Lake Basin. The results of this study will provide new scientific insights into the carbon cycling mechanisms of Qinghai Lake's lakeshore wetlands and similar plateau wetland ecosystems and offer a theoretical basis for addressing climate change and wetland conservation.

2. Materials and Methods

2.1. Overview of Study Area

Qinghai Lake Bird Island (36°57'~37°04' N, 99°44'~99°54' E) is a typical lakeshore wetland, with an elevation ranging from 3194 to 3226 m. The area has an average annual temperature of $-0.7\text{ }^{\circ}\text{C}$ and an average annual precipitation of 322.7 mm. The dominant vegetation species include *Allium przewalskianum*, *Astragalus adsurgens* Pall., and *Poa annua* L. [11].

2.2. Sample Collection

The plot was established in 2018, and the actual situation of the sample land is shown in the figure (Figure S1). We reduced the natural precipitation by installing equidistant inclined diversion channels of different areas. The diversion channels collect rainwater into the horizontal tank in the concourse and increase the natural precipitation through the spray device. In June 2020 (early in the growing season), surface soil samples (0–10 cm) were collected using a 4.5 cm diameter soil auger following an 'S'-shaped curve and a five-point sampling method. To ensure data accuracy and reliability, a total of 15 soil samples were collected and divided into 5 treatment groups, ck (natural control), Za (50% increased precipitation), Zb (25% increased precipitation), Ja (50% reduced precipitation), and Jb (25% reduced precipitation), with three replicates for each treatment group. After collection, the soil samples were sieved through a 2 mm mesh to remove impurities, stored in ice bags, and immediately transported to the laboratory for further analysis. In the laboratory, a portion of the soil samples was air-dried for subsequent physicochemical analysis, while the other portion was stored at $-80\text{ }^{\circ}\text{C}$ for molecular analysis.

2.3. Determination of Soil Biogeochemical Properties

Soil moisture (0–10 cm) was monitored using a TDR-300 (Spectrum Technologies, Plainfield, IL, USA), and soil temperature (0–10 cm) was measured with a LI-8100 instrument (LI-COR, Lincoln, NE, USA). The soil-to-water ratio was homogenized at 1:2.5, and soil pH was measured using a pH meter (FE20-FiveEasy pH, Mettler Toledo, Switzerland, Germany). Total carbon (TC) and total nitrogen (TN) were determined using an elemental analyzer (Vario EL III, Elemental Analysis System GmbH, Frankfurt, Germany) [11].

2.4. DNA Extraction and Illumina HiSeq Sequencing

Soil DNA was extracted using the PowerSoil DNA Isolation Kit (Mio-bio, Carlsbad, CA, USA), and DNA concentration and purity were assessed using a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The *cbbL* gene fragment was amplified using standard primers for carbon-fixing microorganisms: (5'-GACTTCACCAAAGACGACGA-3') and (5'-TCGAACCTTGATTTCTTTCCA-3'). PCR was performed in a 25 μL volume system. The following PCR cycling conditions were used: initial denaturation at $98\text{ }^{\circ}\text{C}$ for 2 min, denaturation at $98\text{ }^{\circ}\text{C}$ for 15 s, annealing at $55\text{ }^{\circ}\text{C}$ for 30 s, and extension at $72\text{ }^{\circ}\text{C}$ for 30 s, 30 cycles [13]. The target bands were

recovered and purified using the Agarose Gel DNA Recovery Kit, followed by Illumina MiSeq high-throughput sequencing.

2.5. Statistical Analysis

To investigate the relationship between data, we calculated α diversity (ACE, Chao1, Simpson, Shannon index) using the 'get_alphaindex' function from the MicrobiotaProcess package (v1.18.0). The 'get_pca' function was used to perform Principal Component Analysis (PCA). The 'metaMDS' function from the Vegan package (v2.6.6.1) was used to calculate the NMDS value, and the 'adonis' function calculated the adonis value. The 'anosim' function was employed to calculate the anosim value to verify the validity of the grouping. The UpSetR package (v1.4.0) contained the 'upset' function to draw the OTU plot, and the 'rda' function was used to perform redundancy analysis (RDA). The 'chordDiagram' function of the circlize package (v0.4.16) was used to draw the circos diagram of functional groups, while the 'glmm.hp' function of the glmm.hp package (v0.1.7) calculated the specific effects of soil physicochemical factors on the composition and diversity of microorganisms. The 'pNST' function of the Picante package (v1.8.2) was used to calculate the betaNTI index, and the microeco package (v1.11.0) was used to calculate the Raup–Crick (RCbray) index. The 'corr.test' function of the psych package was used to calculate the correlation between microorganisms within the group, and the 'aov' function of the stats package (v4.4.1) was used to calculate the significance of the microbial community composition, community diversity, and soil physicochemistry among different groups. All of these analyses were performed in R software (v4.4.1). The Gephi software (v0.9.7) was used to map the collinear network of microorganisms within the group. The functional groups of microorganisms were predicted by FAPROTAX [27].

3. Results

3.1. Soil Properties Under Precipitation Changes

Soils of the lakeshore wetland showed unique physicochemical characteristics under different precipitation treatments (Figure 1). The highest total carbon content was observed under the 50% reduced precipitation treatment, ranging from 66.4 g/kg to 69.7 g/kg. Compared to the natural control, both the 25% and 50% increased precipitation treatments significantly increased the total carbon content ($p < 0.05$). The 25% reduced precipitation treatment did not significantly alter the total carbon content ($p > 0.05$), which was slightly higher than the natural control. Soil temperature significantly increased under reduced precipitation treatments ($p < 0.05$), with the 50% reduction resulting in higher temperatures than the 25% reduction, though the difference was not statistically significant. With precipitation changes, soil moisture in the lakeshore wetland ranged from 33.4% to 40.4%, pH ranged from 8.3 to 8.8, and total nitrogen content ranged from 1.82 g/kg to 2.53 g/kg, with no significant differences observed between the groups ($p > 0.05$).

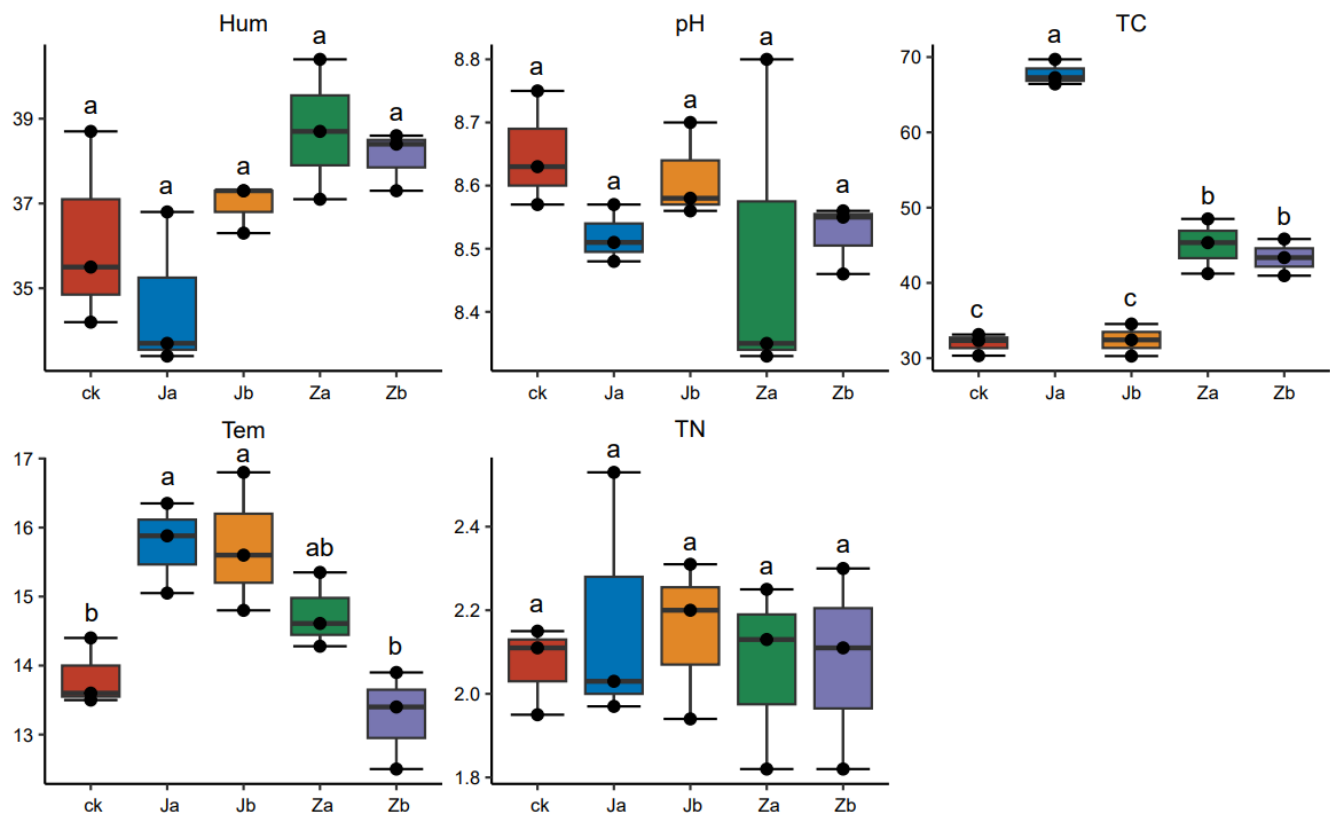


Figure 1. Physicochemical properties of soil in lakeshore wetlands under precipitation changes. Letters 'abc' indicate significance; identical letters denote no significant difference ($p > 0.05$), while different letters indicate significant differences ($p < 0.05$). Tem: soil temperature; Hum: soil humidity; TN: total nitrogen; TC: total carbon; pH: soil pH.

3.2. Response of Community Diversity of *cbbL*-Carrier Carbon Sequestration Microorganisms to Precipitation Changes

Precipitation changes did not significantly alter the alpha diversity of *cbbL*-carrier carbon-fixing microorganisms in a lakeshore wetland ($p > 0.05$), although microbial richness and evenness responded differently to various precipitation gradients (Figure 2a). Under both increased and decreased precipitation treatments, species richness (ACE and Chao1 indices) of *cbbL*-carrier carbon-fixing microorganisms decreased, while species evenness (Shannon and Simpson indices) increased, reaching the highest values under the Za treatment (Figure 2a). The first axis of the PCA (Principal Component Analysis) explained 65.35% of the variation, and the second axis explained 15.4%, with the total explanation accounting for over 80% (Figure 2b). The heterogeneity was greatest under the Za treatment. Further analysis using ANOSIM confirmed the validity of the groupings, revealing significant differences ($R = 0.28$, $p = 0.027$) (Figure 2c).

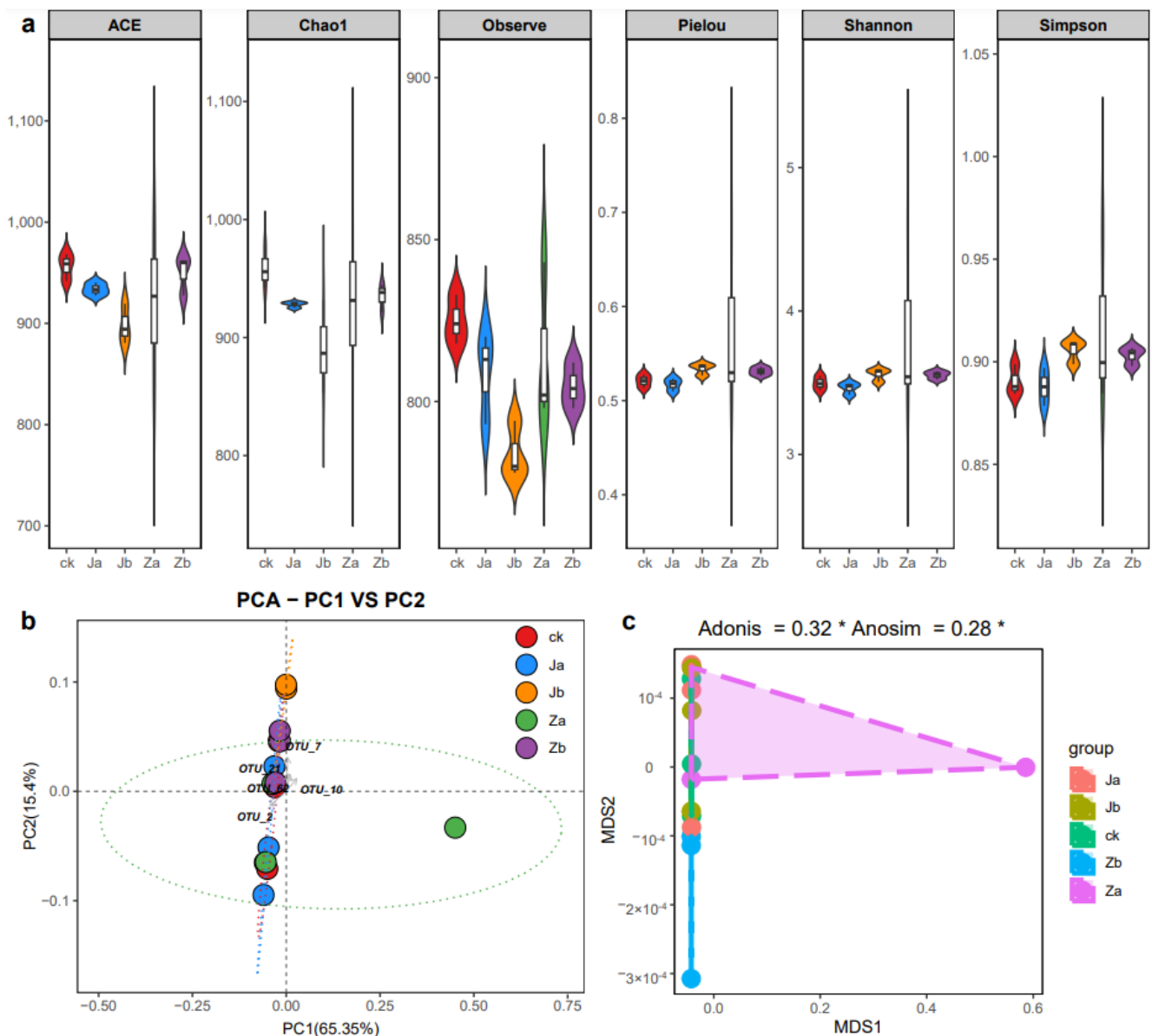


Figure 2. Diversity characteristics of cbbL-carrier carbon-fixing microbial communities in lakeshore wetland under precipitation changes. (a) Alpha diversity indices; (b) PCA (Principal Component Analysis); (c) grouping validity verification. * indicates $p < 0.05$.

3.3. Response of Community Structure of cbbL-Carrier Carbon Sequestration Microorganisms Under Precipitation Changes

The dominant phyla of cbbL-carrier carbon-fixing microorganisms (Figure 3a) in the lakeshore wetland were Proteobacteria (63.02%), Actinobacteria (19.77%), Cyanobacteria (6.67%), and Chlorophyta (6.60%), which together accounted for over 96% of the total sequences. Among these, Chlorophyta significantly responded to precipitation change, with its relative abundance increasing significantly under the Jb treatment ($p < 0.05$) (Figure 3c). At the genus level, the highest proportion of unclassified taxa was 34.83%. The eight known dominant genera (Figure 3b) were *Thioflexothrix* (22.49%), *Ferrithrix* (19.76%), *Bradyrhizobium* (5.61%), *Rhodospirillum* (5.23%), *Thiohalomonas* (2.44%), *Thiobacillus* (2.11%), *Sulfuricaulis* (1.77%), and *Phormidium* (1.28%). *Bradyrhizobium* was the only genus-level dominant microbial group that significantly responded to precipitation change ($p < 0.05$) (Figure 3d), showing a relative abundance trend similar to that of Chlorophyta. The relative abundance of *Bradyrhizobium* significantly increased under the Jb treatment, with no significant increase

observed under the other treatments. Additionally, the number of sequences obtained from the carbon-fixing microbial communities across the 15 samples ranged from 79,137 to 117,263. A total of 794 OTUs were shared across all treatments, while the total OTU numbers for Za, Zb, ck, Ja, and Jb were 1075, 996, 1025, 1006, and 980, respectively (Figure 3e). The number of unique OTUs for these treatments was 48, 10, 11, 14, and 11, respectively, with Za exhibiting the highest total and unique OTU counts (Figure 3e).

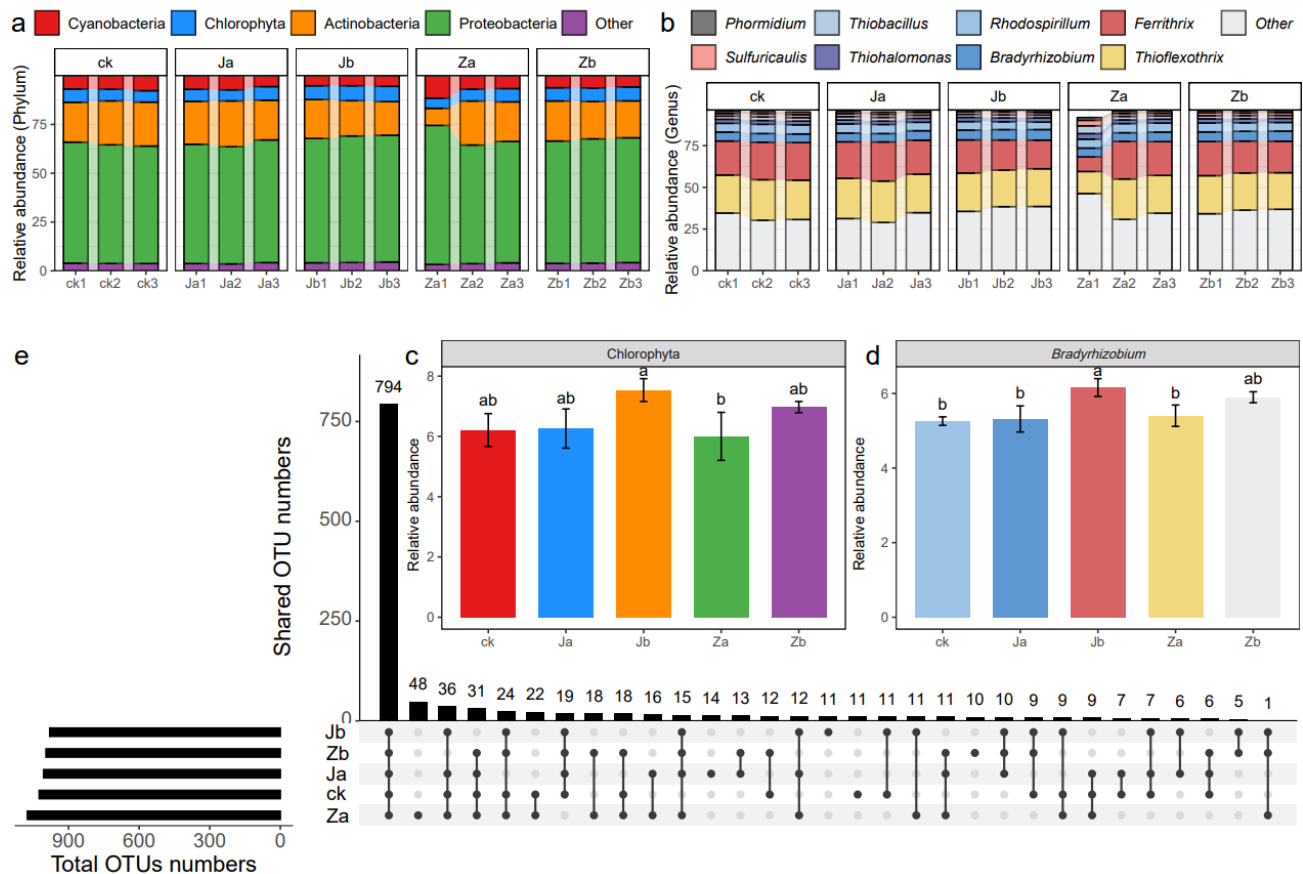


Figure 3. Composition of cbbL-carrier carbon-fixing microbial communities in lakeshore wetland under precipitation changes. (a) Dominant phyla; (b) dominant genera; (c) differential phyla; (d) differential genera; (e) ASV distribution across groups. Letters 'ab' indicate significance; identical letters denote no significant difference ($p > 0.05$), while different letters indicate significant differences ($p < 0.05$).

3.4. Response of Functional Groups of cbbL-Carrier Carbon Sequestration Microbial Communities Under Precipitation Changes

FAPROTAX predicted the functional guilds of the carbon-fixing microbial community in the lakeshore wetland, identifying 22 functional guilds (relative abundance > 1%) among the cbbL-carrier carbon-fixing microorganisms. The functions of these microorganisms were primarily associated with dark iron oxidation (14.70%), phototrophy (10.67%), chemoheterotrophy (8.17%), aerobic chemoheterotrophy (8.16%), photoautotrophy (6.81%), nitrogen fixation (4.33%), dark oxidation of sulfur compounds (4.18%), oxygenic photoautotrophy (4.17%), photosynthetic cyanobacteria (4.17%), and photoheterotrophy (4.06%), which together accounted for more than 69% of the total sequences (Figure 4a). Statistical analysis revealed that precipitation changes significantly altered the relative abundances of aerobic chemoheterotrophy, chemoheterotrophy, and nitrogen fixation (Figure 4b). The response trends of aerobic chemoheterotrophy and chemoheterotrophy to precipitation change were consistent, with both showing a significant increase in relative abundance

under the Zb treatment ($p < 0.05$) (Figure 4b). Meanwhile, nitrogen fixation showed the highest relative abundance under the Jb treatment. The analysis of the functional groups with reversed localization revealed that most microbial communities possessed the functions of aerobic chemoheterotrophy and chemoheterotrophy (Figure 4c). Nitrogen fixation was primarily observed in *Calothrix* and *Nostoc* of the phylum Cyanobacteria, as well as in *Bradyrhizobium* and *Rhodopseudomonas* of the phylum Proteobacteria (Figure 4c).



Figure 4. cbbL-carrier microbial functional groups under precipitation change in lakeshore wetland. (a) Top 10 major functional groups; (b) differential functional groups between treatments; (c) microbial taxa corresponding to differential functional groups. Letters 'ab' indicate significance; identical letters denote no significant difference ($p > 0.05$), while different letters indicate significant differences ($p < 0.05$).

3.5. Response of Environmental Factors to Precipitation Change in Lakeshore Wetland

Redundancy analysis (RDA) revealed the relationship between the top 10 cbbL-carrier carbon-fixing microbial genera and soil environmental factors (Figure 5a). The results showed that different environmental factors had varying effects on the carbon-fixing microbial community, with pH being the most important factor. Further correlation heatmap analysis (Figure 5b) indicated that pH and total nitrogen (TN) were significantly positively correlated with *Ferrithrix*, while total carbon (TC) was significantly negatively correlated with *Bradyrhizobium* but positively correlated with *Rhodospirillum*. To further examine

the specific effects of environmental factors on the *cbbL*-carrier carbon-fixing microbial community, a stratified analysis method was adopted. The results showed that pH was the most important explanatory factor for changes in the community structure and diversity of carbon-fixing microorganisms (Figure 5c,d), explaining 23.78% and 15.1% of the variation, respectively, which supports the findings from the redundancy analysis. Other environmental factors had a lesser negative impact on the carbon-fixing microbial community (Figure 5c,d).

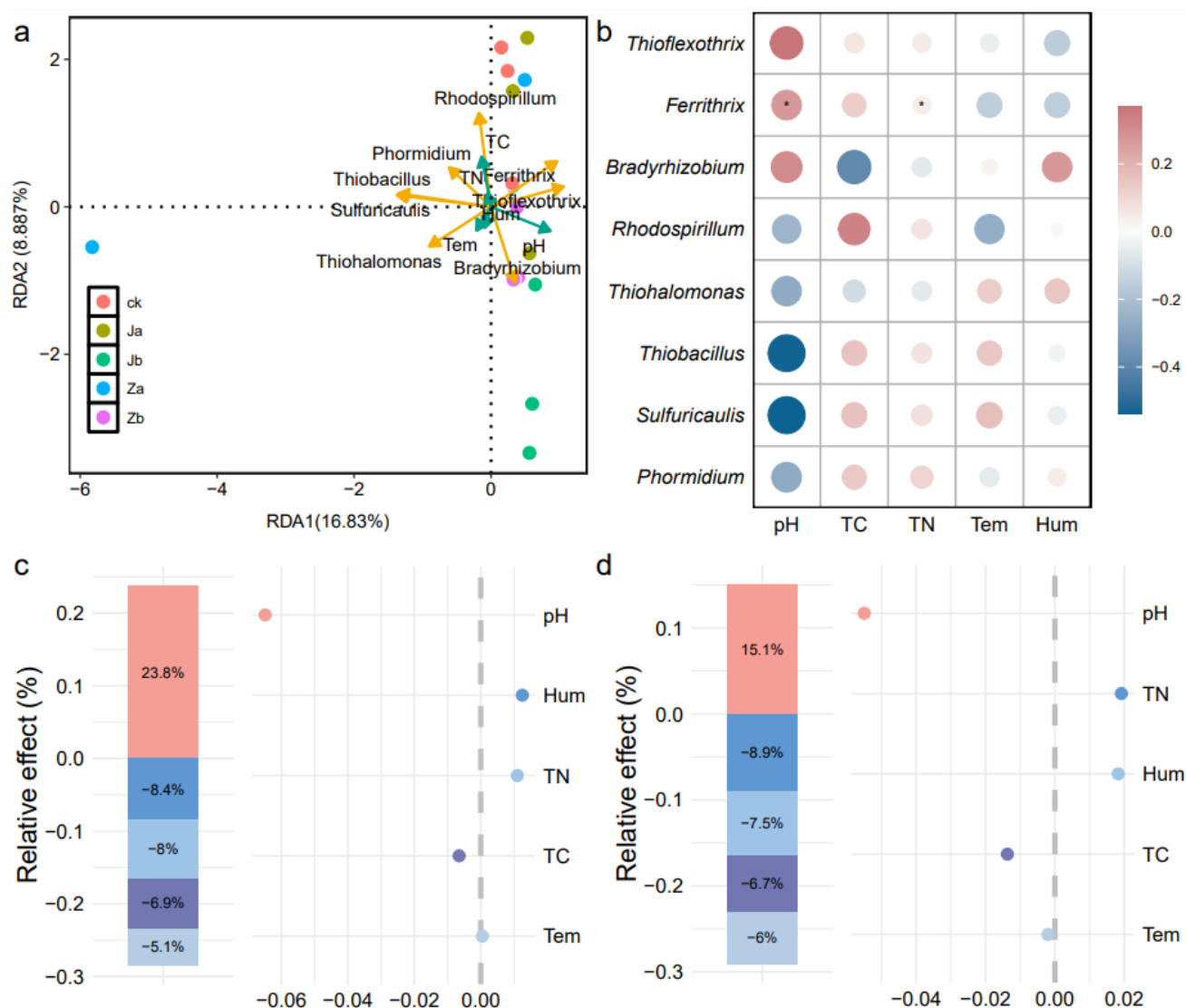


Figure 5. Correlation analysis between *cbbL*-carrier carbon-fixing microorganisms and environmental factors in lakeshore wetland. (a) Redundancy analysis of dominant genera and physicochemical factors; (b) correlation heatmap of dominant genera and physicochemical factors; (c) hierarchical partitioning analysis of factors influencing microbial community structure; (d) hierarchical partitioning analysis of factors influencing diversity of denitrifying microbial communities. * indicates $p < 0.05$. Tem: soil temperature; Hum: soil humidity; TN: total nitrogen; TC: total carbon; pH: soil pH.

3.6. Community Assembly Process of *cbbL*-Carrier Carbon-Fixing Microorganisms Under Precipitation Changes

Both deterministic and stochastic processes jointly drove the assembly of *cbbL*-carrier carbon-fixing microbial communities (Figure 6). The β NTI values for community assembly calculations indicated that, at the regional scale, deterministic processes dominated community assembly of carbon-fixing microorganisms under different precipitation treatments

($|\beta NTI| > 2$) (Figure 6a). Further calculation of RCbray was used to distinguish the relative contributions of diffusion limitation, drift, homogenizing dispersal, and selection to community changes (Figure 6b). The results indicated that, during microbial community assembly in the Qinghai Lake wetland, the relative contributions of deterministic and stochastic processes changed under different precipitation treatments. Heterogeneous selection dominated the community assembly of carbon-fixing microorganisms under the Ja, Za, and Zb treatments, while diffusion limitation dominated the assembly under the ck and Jb treatments. Additionally, drift was observed in the community assembly processes under the ck, Ja, and Zb treatments, each accounting for 33.3%. Under the Za treatment, the carbon-fixing microbial community exhibited some homogenizing dispersal, also accounting for 33.3%. Overall, except for the Jb treatment, all other precipitation treatments shifted the assembly of carbon-fixing microbial communities from a stochastic to a deterministic process.

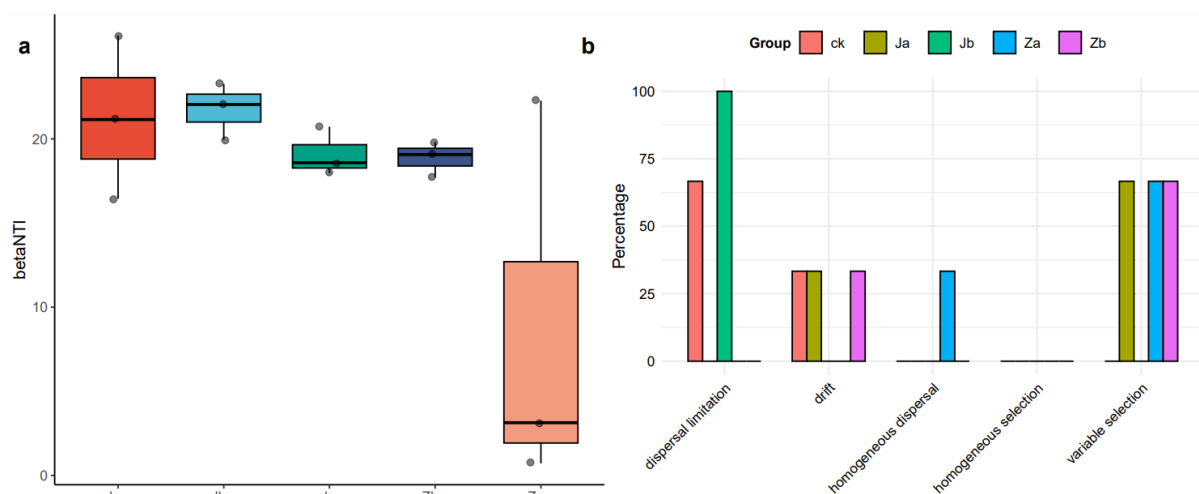


Figure 6. Community assembly of *cbbL*-carrier carbon-fixing microorganisms in lakeshore wetland. (a) Distribution of βNTI index; (b) distribution of carbon-fixing microbial community assembly processes.

3.7. Network Analysis of *cbbL*-Carrier Carbon Sequestration Microbial Communities Under Precipitation Changes

The network analysis of carbon-fixing microorganisms under different precipitation treatments revealed differences in the trends of topological properties (Figure 7, Table 1). The network complexity and connectivity between species of *cbbL*-carrier carbon-fixing microorganisms initially decreased and then increased with increasing precipitation. The number and average degree of positive and negative correlations exhibited a similar trend. Compared to the natural control, the total number of edges in the microbial network increased under both the 50% precipitation increase and 25% precipitation reduction treatments, while the total number of edges decreased under the 25% precipitation increase treatment. Additionally, the modularity of the network initially increased and then decreased with increased precipitation. Precipitation treatments reduced the modularity of the microbial network. In the *cbbL*-carrier carbon-fixing microbial network, the proportion of positive correlations decreased from 68.29% (Ja) to 53.73% (Jb) and then increased to 60.53% (Za) with increasing precipitation (Table 1). Compared to the natural control, the proportion of positive correlations only decreased under the Jb treatment, while it increased under all other treatments (Figure 7, Table 1).

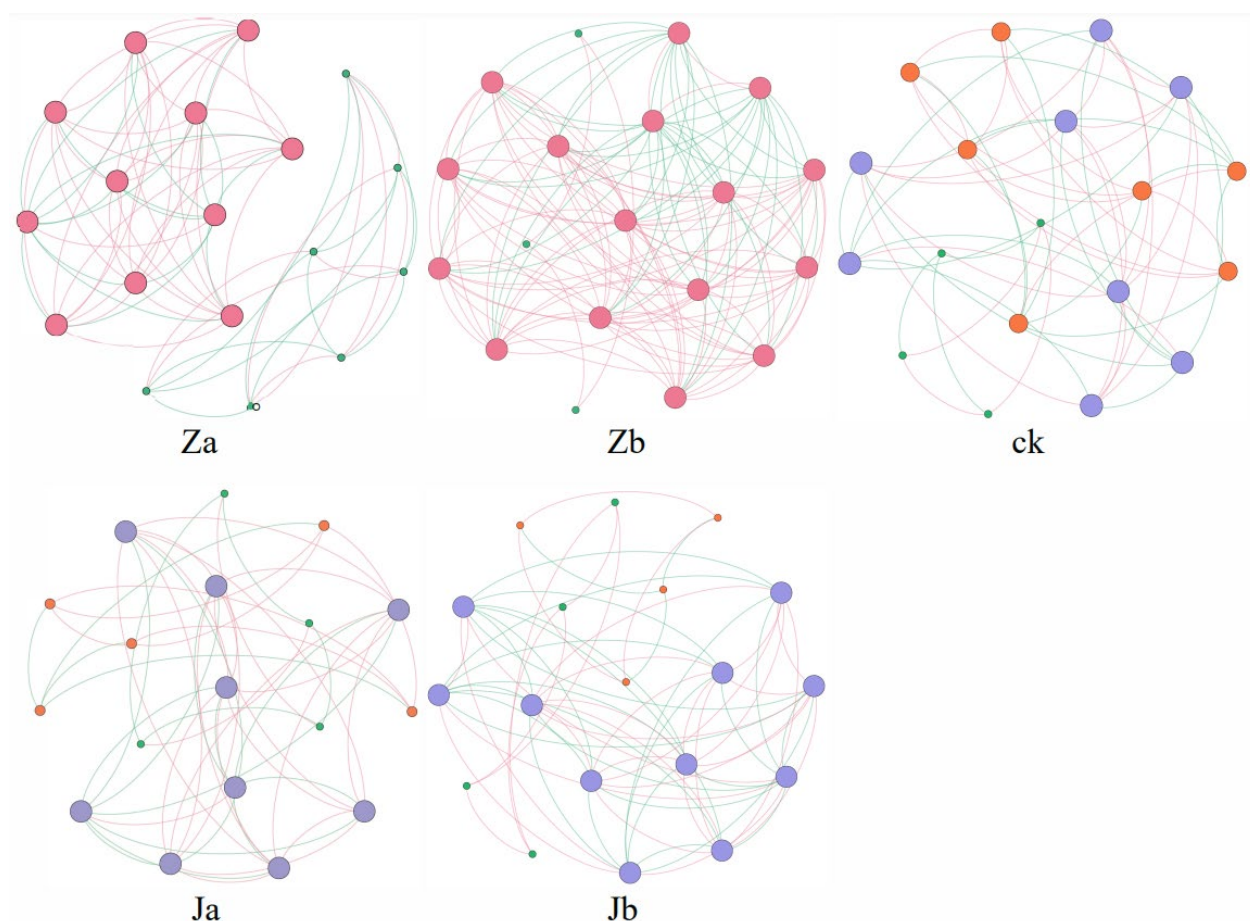


Figure 7. The network patterns of the cbbL-carrier carbon-fixing microorganisms under precipitation changes in the lakeshore wetland. The size of the nodes represents their degree; the node colors indicate different modules; the edge colors represent positive or negative correlations, with red indicating positive correlations and green indicating negative correlations.

Table 1. Topological parameters of cbbL-carrier carbon-fixing microbial network under precipitation changes in lakeshore wetland.

Group	Average Degree	Modularization	Edge	Negative Correlation	Positive Correlation	Point
Ja	6.474	0.048	123	39	84	19
Jb	3.526	0.310	67	31	36	19
ck	2.895	0.583	55	25	30	19
Zb	2.889	0.470	52	22	30	18
Za	4.222	0.400	76	30	46	18

4. Discussion

4.1. Response of cbbL-Carrier Carbon Sequestration Microbial Communities to Precipitation Change in Lakeshore Wetland

Precipitation change typically has a significant impact on the α -diversity of cbbL-carrier microbial communities [13,28]. In this study, however, precipitation change did not significantly alter the α -diversity of cbbL-carrier carbon-fixing microbes in the lakeshore wetland, which is inconsistent with previous findings. This discrepancy may be due to the fact that the soil moisture under precipitation change in the lakeshore wetland was much lower than the moisture threshold required for the optimal growth of carbon-fixing microbes, leading to minimal changes in α -diversity [29]. Under the increased and de-

creased precipitation treatments, the species richness of cbbL-carrier carbon-fixing microbes (measured by ACE and Chao1 indices) decreased, indicating that precipitation change tends to reduce the carbon sequestration potential of the lakeshore wetland [30–32]. In contrast, species evenness (measured by Shannon and Simpson indices) increased with higher precipitation. This conclusion is consistent with the findings of Maestre et al. [33], who demonstrated that reduced precipitation generally weakens microbial diversity. However, a study by Li et al. [13] on cbbL-carrier microbes in natural grassland ecosystems found that the Shannon index decreased with increasing precipitation. This is likely due to increased microbial mortality under water stress following precipitation increases, which leads to a decline in α -diversity [34].

The most dominant phylum of cbbL-carrier carbon-fixing microbes in the lakeshore wetland was Proteobacteria. Previous studies have also indicated that Proteobacteria is the most abundant phylum of carbon-fixing bacteria in soil [13,35]. This is closely related to the strong environmental adaptability and rapid growth of this microbial group [36,37]. In the lakeshore wetland, the dominant genera of cbbL-carrier carbon-fixing microbes were *Thioflexothrix* (22.49%) and *Ferrithrix* (19.76%), which differs from previous findings. For example, Wang et al. [38] found *Bradyrhizobium*, *Hydrogenophaga*, *Mesorhizobium*, *Methylibium*, and *Sulfuricaulis* to be the dominant genera in a karst wetland. Zhu et al. [39] identified *Cupriavidus*, *Thiobacillus*, and *Thioalkalivibrio* as the dominant genera in rice fields. The reason for this discrepancy was explained by Tolli et al. [40], who found that the dominant populations of CO₂-fixing microbes varied greatly across different study regions, with these differences ultimately determined by soil properties and genetic characteristics. We also found that Chlorophyta and *Bradyrhizobium* were highly sensitive to precipitation change in the lakeshore wetland, with both showing similar trends. The relative abundance of these groups was significantly increased under the 25% reduced precipitation treatment. This may be due to the fact that insufficient precipitation affects microbial activity [41], while excessive precipitation reduces oxygen levels, which can be detrimental to the growth and reproduction of aerobic microbes. Moreover, precipitation change significantly altered the relative abundances of aerobic chemoheterotrophy, chemoheterotrophy, and nitrogen fixation, with the 25% reduced precipitation treatment benefiting the nitrogen fixation process in the lakeshore wetland. Na et al. [42] also demonstrated that changes in precipitation can disrupt the ecological functions of soil microbial communities, which supports our findings.

4.2. Influencing Factors of cbbL-Carrier Carbon Sequestration Microbial Community in Lakeshore Wetland and Their Relationship

Soil pH is commonly regarded as a key factor driving soil community development [43] and significantly influences the cbbL-carrier carbon-fixing microbial community [44]. Our study similarly showed that pH is the most important explanatory factor for changes in the structure and diversity of the cbbL-carrier carbon-fixing microbial community in the lakeshore wetland. Furthermore, Bahram et al. [45] suggested that relatively low local soil pH ranges may not significantly affect the overall soil autotrophic bacterial community. This may explain why the alpha diversity of carbon-fixing microbes did not show significant changes in our study. We also found that both deterministic and stochastic processes jointly drive the assembly of the cbbL-carrier carbon-fixing microbial community in the lakeshore wetland. The increased precipitation treatment and the 50% reduced precipitation treatment shifted the assembly process of the cbbL-carrier carbon-fixing microbial community from stochastic to deterministic, enhancing the environmental selection effect on the community. Xiao B. [46] studied microbial communities in alpine grasslands under climate change and found that reduced precipitation increased the proportion of deterministic processes in bacterial community assembly, while increased precipitation led

to a higher proportion of stochastic processes. In a study in deserts, Du et al. [47] showed that with increasing precipitation, the assembly process of the community shifted from stochastic to deterministic, and then back to a stochastic process. These differing results may be closely related to how ecosystem differences amplify the selective force of biological interactions [48].

Network analysis further unraveled the complex relationships between biological groups [49]. The network complexity and connectivity between species of the cbbL-carrier carbon-fixing microorganisms in the lakeshore wetland showed a trend of initially decreasing and then increasing with increased precipitation, and the number and average degree of both positive and negative correlations followed a similar trend. Li et al. [50] also indicated that reduced precipitation enhanced positive interactions between microbial species, likely due to niche overlap within microbial communities under drought and reduced soil moisture conditions [51]. Additionally, Wang et al. [52] found that higher precipitation strengthened interactions among soil microorganisms. Li et al. [53] conducted a precipitation gradient experiment in Inner Mongolia and showed that the robustness of microbial networks increased with higher precipitation. Hu et al. [54] also observed that the communities under increased precipitation treatment exhibited tighter interspecies connections. Therefore, a 50% increase in precipitation likely enhanced the synergistic or competitive interactions between different bacterial species [55].

5. Conclusions

Compared to the natural control, precipitation variations tend to reduce the carbon sequestration potential of the lakeshore wetlands, while the −25% precipitation treatment promotes nitrogen fixation processes in the wetlands. The increased precipitation and −50% precipitation treatments shifted the community assembly process of cbbL-carrier carbon-fixing microorganisms from stochasticity to determinism, enhancing the role of environmental selection in these microorganisms. In addition, the +50% precipitation treatment enhanced the synergistic or competitive interactions between different carbon-fixing microbial communities. These findings reflect the interactions between the carbon-fixing microbial community and soil factors, with pH being the most significant influencing factor for the cbbL-carrier carbon-fixing microbial community in the lakeshore wetlands. These findings significantly advance our understanding of the potential mechanisms underlying carbon storage in alpine lakeshore wetland microorganisms. Understanding these relationships is crucial for studying the carbon cycling and greenhouse gas emissions in lakeshore wetlands. They may also serve as reliable parameters for modeling alpine wetland ecosystems, contributing to the promotion of effective ecosystem management.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture15040379/s1>, Figure S1: Sample plot map.

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References

1. Costanza, R. Nature: Ecosystems without commodifying them. *Nature* **2006**, *443*, 749–750. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Taufik, M.; Setiawan, B.I.; Lanen, H.A.J.V. Modification of a fire drought index for tropical wetland ecosystems by including water table depth. *Agric. For. Meteorol.* **2015**, *203*, 1–10. [\[CrossRef\]](#)
3. Ye, C.; Li, C.H.; Deng, T.T. On the structure and ecological function of lakeside zone. *Environ. Sci. Res.* **2015**, *28*, 171–181. [\[CrossRef\]](#)
4. Chen, Y.; Li, S.; Zhang, Y.; Zhang, Q. Assessing soil heavy metal pollution in the water-level-fluctuation zone of the Three Gorges Reservoir, China. *J. Hazard. Mater.* **2011**, *191*, 366–372. [\[CrossRef\]](#)
5. Jyytinen, S.; Alm, J.; Larmola, T.; Huttunen, J.T.; Morero, M.; Saarnio, S.; Martikainen, P. Methane (CH₄) release from littoral wetlands of Boreal lakes during an extended flooding period. *Glob. Change Biol.* **2003**, *9*, 413–424. [\[CrossRef\]](#)
6. Sohail, T.; Zika, J.D.; Irving, D.B.; Church, J.A. Observed poleward freshwater transport since 1970. *Nature* **2022**, *602*, 617–622. [\[CrossRef\]](#)
7. Osland, M.J.; Enwright, N.M.; Day, R.H.; Gabler, C.A.; Stagg, C.L.; Grace, J.B. Beyond just sea-level rise: Considering macroclimatic drivers within coastal wetland vulnerability assessments to climate change. *Glob. Change Biol.* **2016**, *22*, 1–11. [\[CrossRef\]](#)
8. Saintilan, N.; Rogers, K.; Kelleway, J.J.; Ens, E.; Sloane, D.R. Climate Change Impacts on the Coastal Wetlands of Australia. *Wetlands* **2019**, *39*, 1145–1154. [\[CrossRef\]](#)
9. Lynn, T.M.; Ge, T.; Yuan, H.; Wei, X.; Wu, X.; Xiao, K.; Kumaresan, D.; Yu, S.S.; Wu, J.; Whiteley, A.S. Soil carbon-fixation rates and associated bacterial diversity and abundance in three natural ecosystems. *Microb. Ecol.* **2017**, *73*, 645–657. [\[CrossRef\]](#)
10. Qin, J.; Li, M.; Zhang, H.; Liu, H.; Zhao, J.; Yang, D. Nitrogen deposition reduces the diversity and abundance of *cbbL* gene-containing CO₂-fixing microorganisms in the soil of the stipa baicalensis steppe. *Front. Microbiol.* **2021**, *12*, 570908. [\[CrossRef\]](#)
11. Zhang, N.; Chen, K.; Wang, S.; Qi, D.; Zhou, Z.; Xie, C.; Liu, X. Dynamic Response of the *cbbL* Carbon Sequestration Microbial Community to Wetland Type in Qinghai Lake. *Biology* **2023**, *12*, 1503. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Zhang, N.; Chen, K.L.; Yang, Z.W.; Yang, Y.L.; Li, Y.; Wang, M.Y. Response of *cbbL* carbon sequestration microorganisms to simulated warming in marsh wetland of Qinghai Lake. *J. Grassl. Sci.* **2024**, 1–17.
13. Li, N.; Wang, B.; Huang, Y.; Huang, Q.; Jiao, F.; An, S. Response of *cbbL*-harboring microorganisms to precipitation changes in a naturally-restored grassland. *Sci. Total Environ.* **2022**, *838*, 156191. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Nemergut, D.R.; Anderson, S.P.; Cleveland, C.C.; Martin, A.P.; Miller, A.E.; Seimon, A.; Schmidt, S.K. Microbial community succession in an unvegetated, recently deglaciated soil. *Microb. Ecol.* **2007**, *53*, 110–122. [\[CrossRef\]](#)
15. Meng, X.T.; Liao, H.K.; Fan, H.X.; Zhang, X.C.; Li, Y.Y.; Yao, H.Y.; Razavi, B.S. The geographical scale dependence of diazotroph assembly and activity: Effect of a decade fertilization. *Geoderma* **2021**, *386*, 114923. [\[CrossRef\]](#)
16. Stegen, J.C.; Lin, X.; Konopka, A.E.; Fredrickson, J.K. Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J.* **2012**, *6*, 1653–1664. [\[CrossRef\]](#)
17. Cao, Q.; Li, X.Z.; Xie, Z.J.; Li, C.N.; Huang, S.Y.; Zhu, B.J.; Li, D.; Liu, X.F. Compartmentation of microbial communities in structure and function for methane oxidation coupled to nitrification-denitrification. *Bioresour. Technol.* **2021**, *341*, 125761. [\[CrossRef\]](#)
18. Jiang, Y.M.; Huang, H.Y.; Tian, Y.R.; Yu, X.; Li, X.K. Stochasticity versus determinism: Microbial community assembly patterns under specific conditions in petrochemical activated sludge. *J. Hazard. Mater.* **2021**, *407*, 124372. [\[CrossRef\]](#)
19. Zhou, Z.; Wang, C.; Luo, Y. Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. *Nat. Commun.* **2020**, *11*, 3072. [\[CrossRef\]](#)
20. Barberan, A.; Bates, S.T.; Casamayor, E.O.; Fierer, N. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J.* **2012**, *6*, 343–351. [\[CrossRef\]](#)
21. Banerjee, S.; Schlaeppi, K.; van der Heijden, M.G.A. Keystone taxa as drivers of microbiome structure and functioning. *Nat. Rev. Microbiol.* **2018**, *16*, 567–576. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Mikhailov, I.S.; Zakharova, Y.R.; Bukin, Y.S.; Galachyants, Y.P.; Petrova, D.P.; Sakirko, M.V. Co-Occurrence networks among bacteria and microbial eukaryotes of lake baikal during a spring phytoplankton Bloom. *Microb. Ecol.* **2019**, *77*, 96–109. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Liang, S.; Deng, J.; Jiang, Y.; Wu, S.; Zhou, Y.; Zhu, W. Functional distribution of bacterial community under different land use patterns based on faprotax function prediction. *Pol. J. Environ. Stud.* **2020**, *29*, 1245–1261. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Gao, Q.Z.; Li, Y.; Xu, H.M.; Wan, Y.F.; Jiangcun, W.Z. Adaptation strategies of climate variability impacts on alpine grassland ecosystems in Tibetan Plateau. *Mitig. Adapt. Strateg. Glob. Change* **2014**, *19*, 199–209. [\[CrossRef\]](#)
25. Jansson, J.K.; Hofmockel, K.S. Soil microbiomes and climate change. *Nat. Rev. Microbiol.* **2020**, *18*, 35–46. [\[CrossRef\]](#)
26. Qi, Y.F. Study on Wetland Changes in Qinghai Lake Basin in the Past 20 Years. Master's Thesis, Qinghai Normal University, Xining, China, 2012. [\[CrossRef\]](#)

27. Duan, X.N.; Wang, X.K.; Yin, H.; Chen, L. Research progress on carbon sequestration potential of wetland ecosystems. *Ecol. Environ.* **2006**, *15*, 1091–1095. [\[CrossRef\]](#)
28. Teixeira, R.G.; Belimov, A.A.; Dodd, I.C. Microbial inoculum development for ameliorating crop drought stress: A case study of *Variovorax paradoxus* 5C–2. *New Biotechnol.* **2020**, *56*, 103–113. [\[CrossRef\]](#)
29. He, J.; Wang, W.; Liu, T.; Yan, W.; Wu, X.; Lei, J.; Xu, Y.; Chen, Y.; Yao, Y.; Jiang, W.; et al. Midseason drying increases soil dissolved organic carbon and rice yield via soil cbbL bacteria. *J. Environ. Manag.* **2024**, *371*, 123131. [\[CrossRef\]](#)
30. Li, Z.; Tong, D.; Nie, X.; Xiao, H.; Jiao, P.; Jiang, J. New insight into soil C fixation rate: The intensive co-occurrence network of autotrophic bacteria increases the C fixation rate in depositional sites. *Agric. Ecosyst. Environ.* **2021**, *320*, 107579. [\[CrossRef\]](#)
31. Yuan, H.; Ge, T.; Chen, C.; O'Donnell, A.G.; Wu, J. Significant role for microbial autotrophy in the sequestration of soil Carbon. *Appl. Environ. Microbiol.* **2012**, *78*, 2328–2336. [\[CrossRef\]](#)
32. Yuan, H.; Ge, T.; Wu, X.; Liu, S.; Tong, C.; Qin, H. Long-term field fertilization alters the diversity of autotrophic bacteria based on the ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) large-subunit genes in paddy soil. *Appl. Microbiol. Biotechnol.* **2012**, *95*, 1061–1071. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Maestre, F.T.; Delgado-Baquerizo, M.; Jeffries, T.C.; Eldridge, D.J.; Ochoa, V.; Gozalo, B. Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 15684–15689. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Schimel, J.; Balser, T.C.; Wallenstein, M. Microbial stress-response physiology and its implications for ecosystem function. *Ecology* **2007**, *88*, 1386–1394. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Li, C.; Li, X.; Shi, Y.; Yang, Y.; Li, H. Effects of nitrogen addition on soil carbon-fixing microbial diversity on different slopes in a degraded alpine meadow. *Front. Plant Sci.* **2022**, *13*, 921278. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Salcher, M.M.; Pernthaler, J.; Zeder, M.; Psenner, R.; Posch, T. Spatio-temporal niche separation of planktonic Betaproteobacteria in an oligo mesotrophic lake. *Environ. Microbiol.* **2010**, *10*, 2074–2086. [\[CrossRef\]](#)
37. Simek, K.; Kasalicky, V.; Zapomelova, E.; Hornak, K. Alga-derived substrates select for distinct Betaproteobacterial lineages and contribute to niche separation in Limnolobus strains. *Appl. Environ. Microbiol.* **2011**, *77*, 7307–7315. [\[CrossRef\]](#)
38. Wang, X.Y.; Li, W.; Xiao, Y.T.; Cheng, A.Q.; Shen, T.M.; Zhu, M.; Yu, L.J. Abundance and diversity of carbon-fixing bacterial communities in karst wetland soil ecosystems. *CATENA* **2021**, *204*, 105418. [\[CrossRef\]](#)
39. Zhu, Y.; Shao, T.; Zhou, Y.; Zhang, X.; Rengel, Z. Periphyton improves soil conditions and offers a suitable environment for rice growth in coastal saline alkali soil. *Land Degrad. Dev.* **2021**, *32*, 2775–2788. [\[CrossRef\]](#)
40. Tolli, J.; King, G.M. Diversity and structure of bacterial chemolithotrophic communities in pine forest and agroecosystem soils. *Appl. Environ. Microbiol.* **2005**, *71*, 8411–8418. [\[CrossRef\]](#)
41. Henry, H.A. Soil extracellular enzyme dynamics in a changing climate. *Soil Biol. Biochem.* **2013**, *56*, 53–59. [\[CrossRef\]](#)
42. Na, X.; Yu, H.; Wang, P.; Zhu, W.W.; Niu, Y.B.; Huang, J.Y. Vegetation biomass and soil moisture coregulate bacterial community succession under altered precipitation regimes in a desert steppe in northwestern China. *Soil Biol. Biochem.* **2019**, *136*, 107520. [\[CrossRef\]](#)
43. Li, P.P.; Chen, W.J.; Han, Y.L.; Wang, D.C.; Zhang, Y.T.; Wu, C.F. Effects of straw and its biochar applications on the abundance and community structure of CO₂-fixing bacteria in a sandy agricultural soil. *J. Soils Sediments* **2020**, *20*, 2225–2235. [\[CrossRef\]](#)
44. Liao, H.; Hao, X.; Qin, F.; Delgado-Baquerizo, M.; Liu, Y.; Zhou, J. Microbial autotrophy explains large-scale soil CO₂ fixation. *Glob. Change Biol.* **2022**, *29*, 231–242. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Bahram, M.; Hildebrand, F.; Forslund, S.K.; Anderson, J.L.; Soudzilovskaia, N.A.; Bodegom, P.M. Structure and function of the global topsoil microbiome. *Nature* **2018**, *560*, 233–237. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Xiao, B.W. Changes of Soil Microbial Community Structure and Seasonal Dynamics in Alpine Grassland Under Climate Change. Master's Thesis, Lanzhou University, Lanzhou, China, 2022. [\[CrossRef\]](#)
47. Du, F.; Rong, X.Y.; Xu, P.; Yin, B.F.; Zhang, Y.M. Effects of precipitation on bacterial community diversity and construction process in Gurbantunggut Desert. *Biodiversity* **2023**, *31*, 141–154. [\[CrossRef\]](#)
48. Luan, L.; Jiang, Y.; Cheng, M.; Dini-Andreote, F.; Sui, Y.; Xu, Q.; Geisen, F.; Sun, B. Organism body size structures the soil microbial and nematode community assembly at a continental and global scale. *Nat. Commun.* **2020**, *11*, 6406. [\[CrossRef\]](#)
49. Liu, X.; Yang, T.; Shi, Y.; Zhu, Y.; He, M.; Adams, J.M.; Chu, H. Strong partitioning of soil bacterial community composition and co-occurrence networks along a small-scale elevational gradient on Zijin Mountain. *Soil Ecol. Lett.* **2021**, *3*, 290–302. [\[CrossRef\]](#)
50. Li, X.; Yan, Y.; Lu, X.; Fu, L.; Liu, Y. Responses of soil bacterial communities to precipitation change in the semi-arid alpine grassland of Northern Tibet. *Front. Plant Sci.* **2022**, *13*, 1036369. [\[CrossRef\]](#)
51. Hernandez, D.J.; David, A.S.; Menges, E.S.; Searcy, C.A.; Afkhami, M.E. Environmental stress destabilizes microbial networks. *ISME J.* **2021**, *15*, 1722–1734. [\[CrossRef\]](#)
52. Wang, S.; Wang, X.; Han, X.; Deng, Y.; Fortin, M.J. Higher precipitation strengthens the microbial interactions in semi-arid grassland soils. *Glob. Ecol. Biogeogr.* **2018**, *27*, 570–580. [\[CrossRef\]](#)
53. Li, J.; Benti, G.; Wang, D.; Yang, Z.; Xiao, R. Effect of alteration in precipitation amount on soil microbial community in a semi-arid Grassland. *Front. Microbiol.* **2022**, *13*, 842446. [\[CrossRef\]](#) [\[PubMed\]](#)

54. Hu, Z.; Xiao, M.; Ding, J.; Ji, J.; Chen, J.; Ge, T. Response characteristics of soil microbial community under long-term film mulching. *Environ. Sci.* **2022**, *43*, 4745–4754. [[CrossRef](#)]
55. He, D.; Shen, W.; Eberwein, J.; Zhao, Q.; Ren, L.; Wu, Q.L. Diversity and co-occurrence network of soil fungi are more responsive than those of bacteria to shifts in precipitation seasonality in a subtropical forest. *Soil Biol. Biochem.* **2017**, *115*, 499–510. [[CrossRef](#)]

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