EFFECT OF CULTIVATION AND NATURAL RESTORATION ON SOIL MICROBIAL FUNCTIONAL STRUCTURE IN COLD-REGION WETLANDS

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Abstract. In order to reveal the soil cultivation and natural restoration patterns, bacterial microbial communities and functions in cold-region wetlands, the changes of soil bacterial microbial communities and functions driven by soil cultivation and natural restoration were studied by 16SrRNA Gene Fragments and their high-throughput sequencing, relying on the wetland and farmland soils at Hongxing Wetland National of Heilongjiang Province in China. The results showed that Actinobacteriota, Acidobacteriota, Proteobacteria, Chloroflexi and Firmicutes were the dominant bacteria in the tested soil samples. PCA and NMDS analysis showed that there was a significant separation between the bacterial communities in soil natural restoration treatments (CRMW and CSFW). Actinobacteria, Proteobacteria, Acidobacteria and Chloroflexi were the dominant bacterial species in the cultivation farmland (RF and SF). According to FAPROTAX algorithm and BugBase phenotype analysis, soil cultivation and natural restoration patterns can change the abundance of soil functional microorganisms. The dominant functional genes were mainly chemoheterotrophy and aerobic chemoheterotrophy bacteria. Soil cultivation and natural restoration patterns lead to significant differences between aerobic, facultatively anaerobic and stress tolerant bacteria abundances in wetland.

Keywords: *land uses, soil properties, bacterial community, functional structure, high throughput sequencing*

Introduction

Wetland soil, as one of the important environmental factors of wetland ecosystems, is highly significant for maintaining the balance and succession of wetland ecosystems (Pei et al., 2009). A change in the original function of wetland soil will lead to changes in the types of wetland vegetation, soil biology and wetland biodiversity, and ultimately to a change in the function of wetland ecosystems (Angeloni et al., 2006). Changes in soil cultivation and restoration patterns have led to changes in vegetation types. In recent years, researchers in China and throughout the world have focused on changes in different soil cultivation and restoration patterns in cold-region wetlands, such as the transformation of the natural soil of forestland and grassland into farmland soil (Zhao and Zhang, 2010; Davidson and Ackerman, 1993; Mclauchlan, 2006), or the conversion of farmland into natural vegetation (Knops and Tilman, 2000; Laganiere and Angers, 2020). The physical and chemical properties of the soil and its microbial environment have also changed (He et al., 2020; Wang et al., 2019). As one of the most important components in the wetland ecosystem, microorganisms play a critical role in the nutrient cycle of wetland soil media and soil composition and ecological restoration (Deng et al., 2019).

Soil microorganisms are the most active part of the soil, which are closely related to soil structure, ventilation, water, and nutrient status (Zhang et al., 2012) and play an important role in the process of litter decomposition, soil element conservation (Dan et al., 2014), the cycling of carbon and nitrogen (Horwath, 2017; Gu et al., 2018) and

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energy flow in wetland ecosystem, and maintaining the stability of the ecosystem (Yin et al., 2014). Jangid et al. (2011) found that transforming a wetland into a farmland caused significant changes in the bacterial abundance and species and that the change of land use pattern was the main factor determining the microbial community composition. Krashevska et al. (2015) compared the impact of a short-term land use pattern change on the microbial community and reported that when a natural rainforest was transformed into an artificial rubber plantation, the bacterial abundance significantly decreased. Mendes et al. (2015) revealed that the relative abundances of Acidophilus and Chlamydia were higher in the soil of a forestland, and the relative abundance of Actinomycetes was higher in the logging area of a forestland, while the relative abundance of nitrifying, thermophilic bacteria was higher in a farmland. Wood et al. (2017) showed that when oil palm was planted in a tropical woodland, the bacterial diversity increased and there were only slight differences in bacterial diversity and community composition between regenerated forest and the original woodland. Therefore, a comprehensive understanding of land use patterns, soil characteristics, and the interaction between the soil bacterial community structure and the diversity will improve the prediction and management of terrestrial ecosystems. Therefore, this study utilized three different soil cultivation and natural restoration patterns of wetland and farmland in the Hongxing National Nature Reserve of Heilongjiang Province, China. Changes in the richness and diversity of soil bacterial communities and the composition characteristics of related bacterial microbial functional groups were analyzed to clarify the mechanism of soil microbial communities and functions under the conditions of wetland land-use changes in cold regions and further explore the geochemical cycle process of wetland ecosystems. These results can provide a scientific theoretical basis to monitor and restore wetland soil degradation in cold regions.

Materials and methods

Study area

The study area was located in the Heilongjiang Hongxing National Nature Reserve, which is located in the Yichun city at Heilongjiang Province, China (28°21’40”~128°53’30” N, 48°41’20”~49°11’00” E). The total area is 11,199.5 hm^2, and the wetland area is 7,669.2 hm^2. The reserve belongs to the northern temperate continental monsoon climate. The annual average temperature in the reserve is -1.3 °C; the annual average precipitation is 571 mm, and the average relative humidity is 68.3%. The Heilongjiang Hongxing National Nature Reserve is rich in biology and species, with typical water, swamp, meadow wetland, shrub and forest vegetation. The zonal soil is black soil, and the non-zonal soil includes grass, swamp soil, and peat soil. The soils in the sampled municipalities of Sucre are classified as Alfisols, Mollisols, Vertisols (Ding et al., 2022). The wetland park is divided into seven types of wetlands that include rivers, flood plains, herbs, mosses, swamp meadows, shrub swamps and forest swamps. Among them, the herb and swamp meadow wetlands constitute the main wetland landscape of the wetland park, which is surrounded by farms. The vegetation of the soil sample collection site is as follows: (1) converting rice farmlands to meadow wetland (CRMW) with the primary predominant plants *Deyeuxia angustifolia*, *Carex lehmanii*, *Carex meyeriana*, *Carex appendiculata*, *Caltha palustris*, *Cyamus flavicomus*, and *Pedialaris langiflora*, and this soil types has been naturally restored for ten years old (non-disturbance). (2) Converting soybean farmlands to forest wetland (CSFW): the primary forest with mixed coniferous and broad-leaved forests and broad-leaved forests as the main plant types. (3) Upland fields, large-scale cultivation (soybean farmland, SF): the main planting crop is soybean (*Glycine max* L.), which is continuously planted once a year. (4) Paddy fields, large-scale cultivation (rice farmland, RF): the main planting crop is rice (*Oryza sativa* L.), which is continuously planted once a year. At the end of each April, agricultural machinery tillage is conducted once, and the soybean is sown. Farm fertilization and field management are performed according to the local practices. The crop is harvested at the end of September each year, and the land is idle from the end of October to the middle of next April.

Sample collection

This experiment was conducted from August 20 to September 3, 2021, in the Key Laboratory of Wetland Ecology and Environmental Research in the cold regions of Harbin University (Harbin city, China) and the Heilongjiang Hongxing National Nature Reserve (Yichun city, China). According to the characteristics of land use in the nature reserve, four 100 blocks of meadow wetlands, forests and the surrounding upland and paddy fields were selected in a 100×100 m quadrat. Five sampling points were established according to the "S" type. A 5 cm soil shovel was used to collect soil samples that were 0 ~ 20 cm deep at each sampling point. The mixed soil samples were combined into one repetition, and each sample was collected three times. In each quadrat, after the fresh soil sample was passed through a 2 mm sieve, the root residues were removed and placed in a sampling box. Part of the soil samples were brought back to the laboratory for storage as soon as possible at -20 °C for analysis of the bacterial microbial community structure. Another part was stored at 4 °C to determine the physical and chemical properties of the soil.

Determination of soil samples

DNA extraction and high-throughput assay

Genomic DNA of the soil microorganisms was extracted with an Omega E.Z.N.A DNA Kit (Omega Bio-tek, Norcross, GA, USA). The extracted genomic DNA was detected by 1% agarose gel electrophoresis. PCR was performed on a Geneamp 9700 PCR system (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). The universal primers 515f (5'-gtgccagcmgcgg-3') and 907r (5'-ccgtcaattcmttragtt-3') were used to amplify the V3-V4 region of the bacterial 16S rRNA gene. The PCR products were quantified using a QuantiFluor$^{®}$ − ST fluorometer (Promega, Madison, WI, USA), and the samples were adjusted as needed for sequencing. Finally, they were sent to Shanghai Meiji Biotechnology Co., Ltd. (Shanghai, China) for high-throughput sequencing using an Illumina HiSeq 2500 PE250 platform (San Diego, CA, USA).

Statistical analysis

The R software package was used to visually analyze and describe the calculation β, which was modeled with vegan package in R Core Team (2022). The diversity was analyzed by a one-way analysis of variance (ANOVA) and the least significant difference. The data were statistically analyzed using Microsoft Excel 2007 (Redmond, WA, USA) and SPSS 22.0 (IBM, Inc., Armonk, NY, USA). The microbial functions of the soil bacteria were predicted by FAPROTAX and FUN Guide (Liang et al., 2020; Nguyen et al., 2016).

BugBase was used to annotate the functions of bacteria, and the operational taxonomic unit (OTU) table that clustered by sequences with 97.0% consistency was used as the input file. The OTU table was standardized by the predicted number of 16S copies, and the microbial phenotype was then predicted using the preprocessed database. The threshold was automatically selected by the BugBase tools. It can be divided into the following seven categories: Gram positive, Gram negative, Biofilm forming, Contains mobile elements, Aerobic, Anaerobic and Facultative anaerobic (Ward et al., 2017; Du et al., 2020).

Results and analysis

Variation of microbial community abundance

As shown in *Figure 1*, the composition of dominant bacterial species in different soil samples was similar, but the levels of abundance of bacterial microbial communities in the soil samples of cultivation and natural restoration patterns differed significantly from those of the phylum. At the taxonomic level of bacterial community in the soil samples tested, Actinobacteriota, Acidobacteriota, Proteobacteria, Chloroflexi and Firmicutes was dominant bacteria. RF compared with CRMW soil samples the proportions of Actinobacteriota, Acidobacteriota, Chloroflexi and Firmicutes was decreased by 6.68%, 2.30%, 3.51% and 2.86%, respectively.

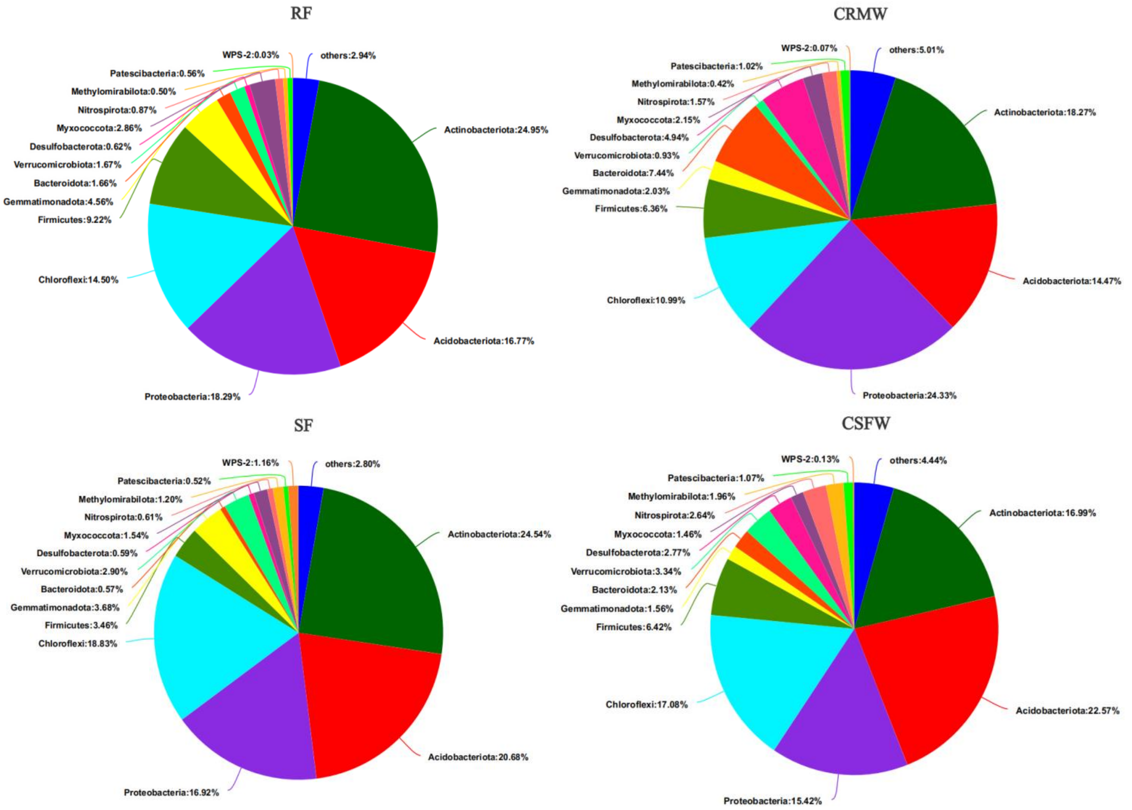

***Figure 1.** Relative abundance of soil bacterial communities at the levels of phylum. Note: The phylum abundance is less than 1% and merged into others. RF, rice farmland; CRMW, converting rice farmlands to meadow wetland; SF, soybean farmland; CSFW, converting soybean farmlands to forest wetland*

Proteobacteria was was increased by 6.04%. SF compared with CSFW soil samples the proportions of Actinobacteriota, Proteobacteria and Chloroflexi was decreased by 7.55%, 1.50% and 1.75%, respectively. Acidobacteriota and Firmicutes were increased by 1.89% and 2.96%.

Analysis on the difference of soil microbial composition

As shown in *Figure 2,* Comparing the bacterial community structures of different soil types, Actinobacteria, Proteobacteria, Acidobacteria and Chloroflexi were the dominant bacterial species in the RF and SF. After farmland converting measures, the proportions of Actinobacteria and Chloroflexi was average decreased by 28.74% and 16.78% ($P < 0.001$), respectively. The proportions of Acidobacteria in RF was increased than CRMW, but the proportions changes are opposite in SF and CSFW. The change proportions of Proteobacteria contrary to Acidobacteria.

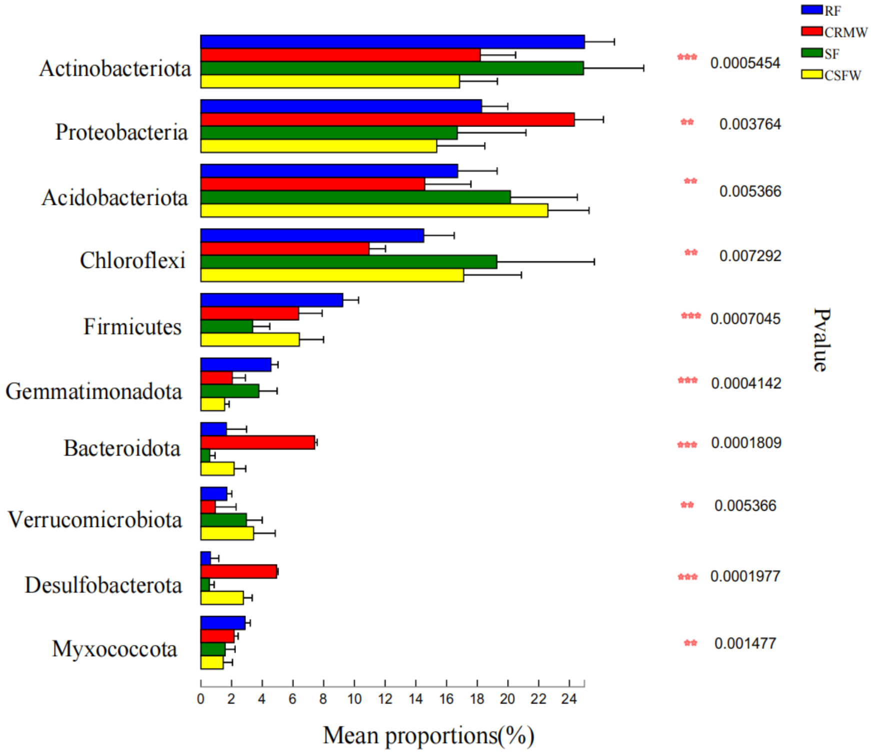

Figure 2. *Relative proportions of soil bacterial communities at the levels of phylum. Note: The Y-axis represents the name of a species at a certain taxonomic level, the X-axis represents the average relative abundance of different groups of species, and the columns of different colors represent different groups; the far right is the p-value, **P < 0.01. ***P < 0.001. Analysis of variance (Duncan's multiple comparison test) was used to test the significance of differences. RF, rice farmland; CRMW, converting rice farmlands to meadow wetland; SF, soybean farmland; CSFW, converting soybean farmlands to forest wetland*

Beta diversity analysis of soil bacterial microbial

The beta diversity for all treatments based on analyses of dimensionality reduction demonstrated the variation of bacterial communities between different treatments (*Fig. 3*). The PCA plot showed that the bacterial communities of different treatments were separated. Gaps between the bacterial communities for the CRMW and CSFW treatments were larger than those for RF and CF. The NMDS analysis showed that there was a significant separation between the bacterial communities in natural restoration treatments (CRMW and CSFW) and the farmland (RF and SF), and the bacterial communities in different treatment groups were also significantly separated, indicating that both cultivation and natural restoration treatments had a significant impact on the composition of soil bacterial communities (R = 0.956, P = 0.001, stress = 0.049). The results indicated obvious variation of the bacterial communities among all treatments.

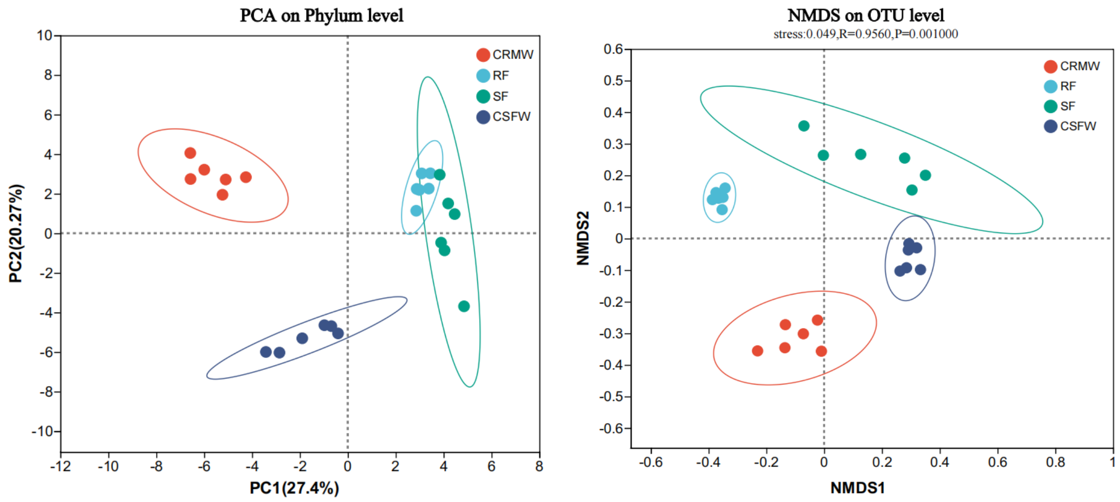

Figure 3. *Combined plot integrated with the PCA and NMDS plots of the bacterial communities corresponding to beta diversity. Note: the length of the branches in the community clustering tree represents the distance between the samples. Different groups can be presented in different colors. The X-axis and Y-axis in the PCoA diagram represent the two selected main coordinate axes, and the percentage represents the interpretation value of the main coordinate axis to the difference in sample composition; Color or shape points represent samples of different groups, the closer the two sample points are, the more similar the species composition of the two samples. The PCA and NMDS analysis based on Bray-Curtis distance at the operational taxonomic unit level. RF, rice farmland; CRMW, converting rice farmlands to meadow wetland; SF, soybean farmland; CSFW, converting soybean farmlands to forest wetland*

Changes in the microbial functions of soil bacteria

The FAPROTAX algorithm selected the top nine functional groups in the soil bacterial community according to their sequence ranking, and statistically analyzed the functional bacterial abundance. As shown in *Figure 4*, soil cultivation and natural restoration patterns can change the abundance of soil functional microorganisms. The dominant functional genes of bacterial community in the tested soil samples were chemoheterotrophy and aerobic chemoheterotrophy. The abundance of chemoheterotrophy in the CRMW and CSFW was 4.11% and 2.21% higher than that in the RF and SF, respectively, and there was significant difference among the groups ($P < 0.01$). The abundance of animal parasites or symbionts in the RF and CF soil samples differed significantly from those in CRMW and CSFW ($P < 0.01$). The abundance of aerobic chemoheterotrophy bacteria in the CRMW and CSFW soil

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samples was 7.16% and 9.13% higher than those in the RF and CF, respectively (*P* < 0.001).

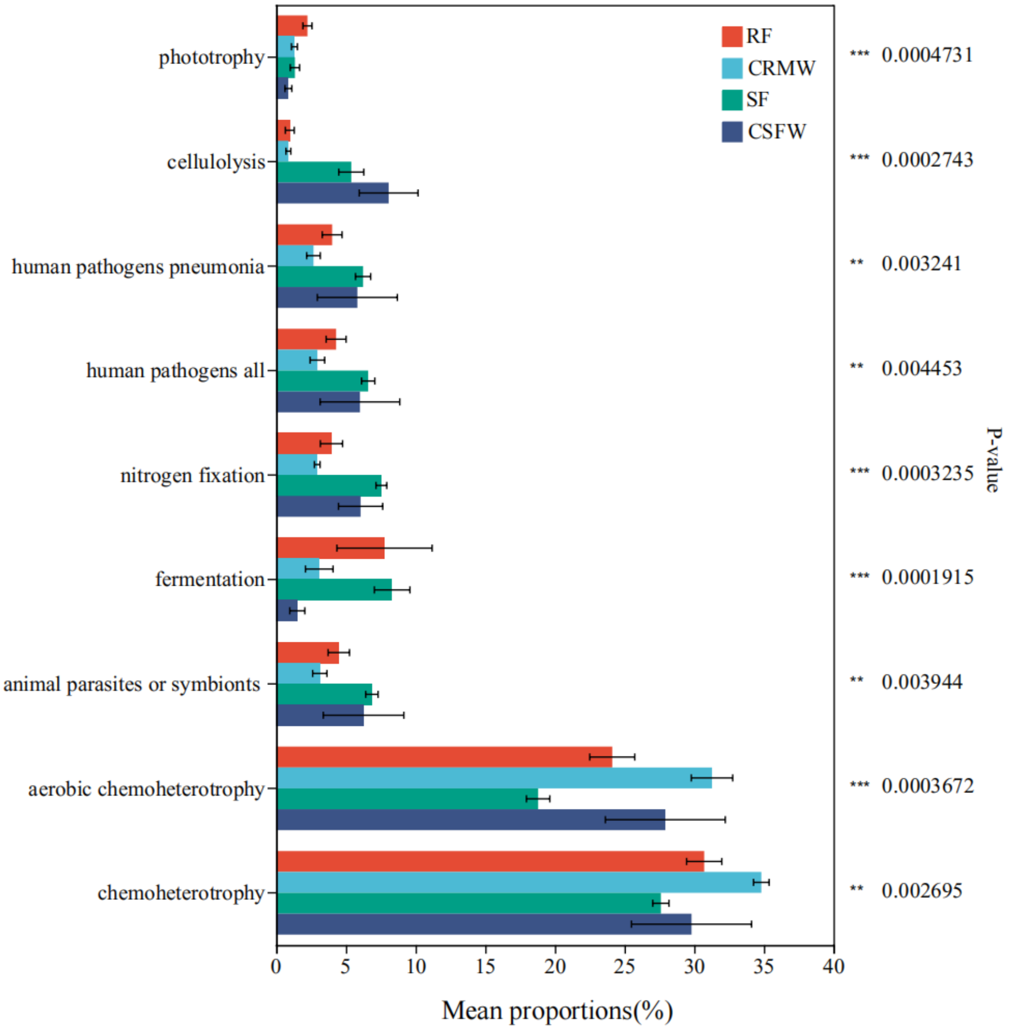

Figure 4. *The abundance of soil bacterial community predicted by functional genes composition. Note: Analysis of variance (Duncan's multiple comparison test) was used to test the significance of differences. **P < 0.01, ***P < 0.001. RF, rice farmland; CRMW, converting rice farmlands to meadow wetland; SF, soybean farmland; CSFW, converting soybean farmlands to forest wetland*

BugBase phenotype prediction

BugBase was used to predict the abundances of seven bacterial phenotypes based on their different land use modes (*Fig. 5*). At the level of microbial community, the abundance of aerobic bacteria in the CRMW and CSFW soil samples was significantly higher than that in the RF and SF (*P* < 0.001). The abundance of anaerobic bacteria in the CRMW soil samples was significantly higher than that in the RF (*P* < 0.001), but in the SF soil samples was significantly higher than that in the CSFW (*P* < 0.001). The abundance of facultatively anaerobic bacteria in the RF was higher than in the CRMW (*P* < 0.01). The bacterial abundance of phylum that contain mobile elements in the

CSFW soil samples was significantly higher than in the SF ($P < 0.05$). The bacterial abundance of those that form biofilms in the CRMW was significantly higher than that in the RF ($P < 0.001$), but there were no significant differences with SF and CSFW. The abundance of gram-negative bacteria in the RF and CF soil samples was higher than those in the CRMW and CSFW, which significantly correlated with them ($P < 0.001$). The abundance of gram-positive bacteria in the RF soil samples was higher than in the CRMW, but in the CSFW soil samples was significantly higher than that in the SF ($P < 0.05$). The changes in bacterial abundance potentially pathogenic abundance variation is opposite to gram positive. The abundance of stress tolerant bacteria in the CSFW soil samples was higher than in the SF ($P < 0.001$), but there were no significant differences with RF and CRMW.

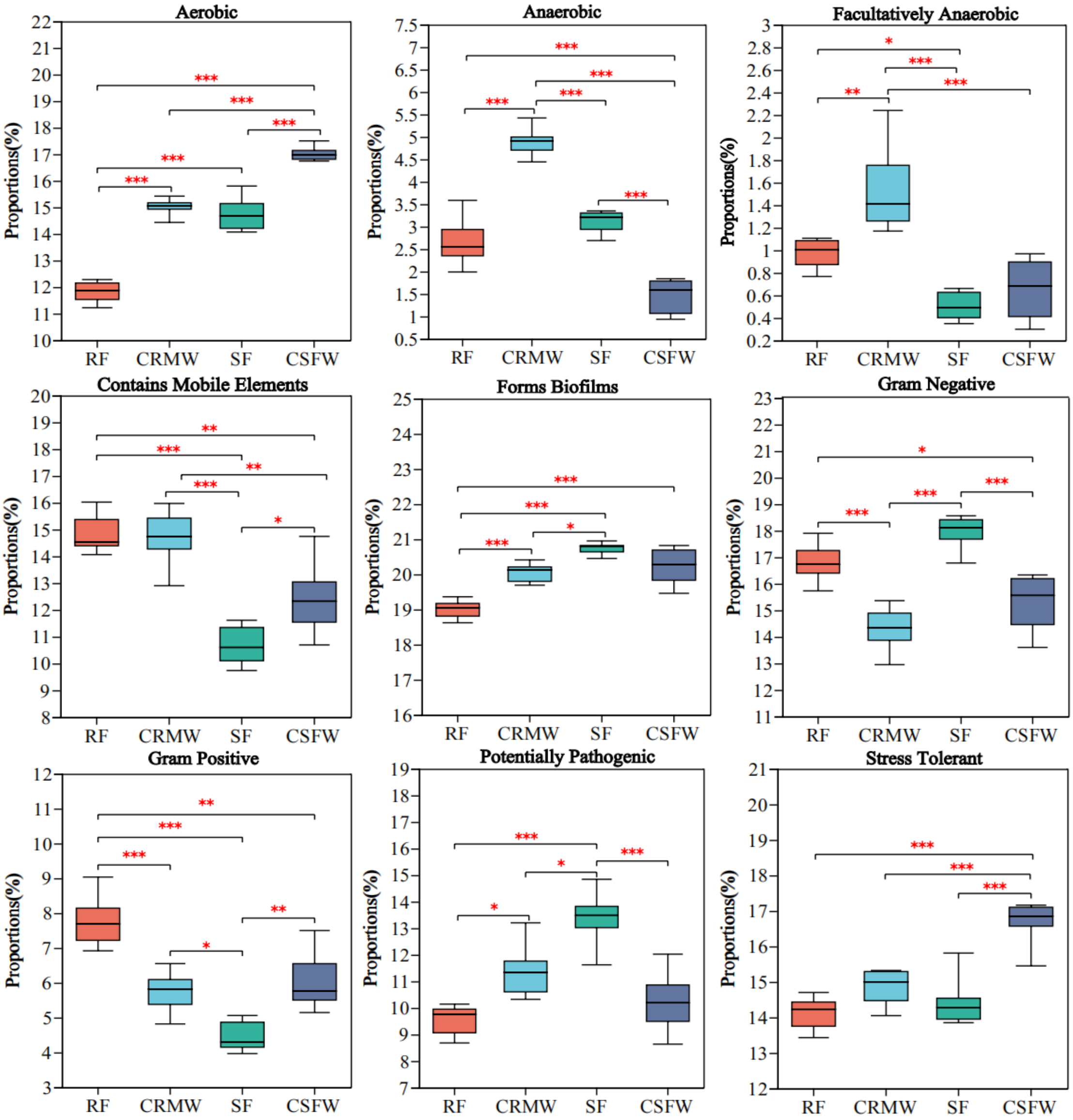

***Figure 5.** Prediction of bacterial BugBase phenotype. Note: Analysis of variance (Duncan's multiple comparison test) was used to test the significance of differences. *P < 0.05. **P < 0.01. ***P < 0.001 is marked. RF, rice farmland; CRMW, converting rice farmlands to meadow wetland; SF, soybean farmland; CSFW, converting soybean farmlands to forest wetland*

Discussion

The diversity of soil microorganisms represents the stability of microbial communities and reflects the effects of soil ecological mechanisms on communities (Yang et al., 2008). We compared the degree of restoration of diversity and richness of the bacterial community following different natural restoration period and found that samples in CRMW and CSFW were extremely close to those of normal farmland. These results supported the findings of a previous investigation of the effects of different cultivation methods and periods on soil microorganisms (Elad et al., 2011). Microbial community succession was found in restored soils, and the ratios of dominant bacterial communities in the soil ecological system from restored land successively changed. The role of the dominant bacterial community is crucial in recovering soil quality. As the most active part of wetland ecosystems, soil microorganisms can respond more sensitively to changes in land use and can be used to evaluate the quality and health level of wetland soil. The soil microbial community structure was significantly affected by land use patterns (Fu et al., 2019). The analysis of the composition of soil microbial community from the cultivation and natural restoration mode shows that the dominant species of soil microbial community in wetland nature reserves were Actinobacteriota, Acidobacteriota, Proteobacteria, Chloroflexi and Firmicutes, which can account for more than 80% of the total bacterial community in the soil samples tested. Proteobacteria and Acidobacteria are they have important ecological functions, such as degrading plant residue polymers and participating in iron cycles, the light absorption of Fenna-Matthews-Olson (FMO) proteins under plant photosynthesis, and in the metabolism of single carbon compounds (Pankratov et al., 2011; Lu et al., 2010; Blöthe et al., 2008; Müh et al., 2007; Radajewski et al., 2002; Kalyuzhnaya et al., 2008). Acidobacteria, as the second largest group of soil bacteria in northern China, is much less abundant in forestland soil (Liu et al., 2015). At the phylum level, different relationships between bacterial communities and the soil quality of wetlands were observed after different restoration periods. Changes in the soil Acidobacteria were studied in different types of vegetation in Germany, and the abundance of soil Acidobacteria in meadows was found to be significantly higher than that in forestland soils, indicating that there were significant differences in the community structure of Acidobacteria in different places in the same ecological environment (Naether et al., 2012). Acidobacteria, as the second largest group of soil bacteria in northern China, is much less abundant in forestland soil (Zhang et al., 2014). The abundance of Acidobacteria also decreased significantly after the Amazon virgin forest in South America was transformed into farmland (Navarrete et al., 2013). High-throughput sequencing in samples from the study area showed that the change of abundance of farmland soil was significantly higher than that of the wetland at the level of Acidobacteria, which could be caused by environmental factors (Wu et al., 2007). With the changes in the diversity of the bacterial community, its functionality in soil ecological systems varied accordingly (Knelman et al., 2012; Zhang et al., 2016). The bacterial phylum whose relative abundance changed significantly were the key factors that leaded to the variation in the diversity of the bacterial community. Thorough analyses of the differential bacterial phylum might help to clarify the direction and details of variation of the bacterial community, which reveals its subsequent potential changes of functions. Furthermore, the analyses can lead to the identification and screening of efficient functional strains based on the correlation analysis of the functional orientation of the bacterial community and key differential bacterial phylum.

Through PCA and NMDS analysis, the soil cultivation and natural restoration can lead to changes in soil microbial community types, which will also lead to changes in functional microbial community.

The results showed that the chemoheterotrophic and aerobic chemoheterotrophic bacteria were primarily involved in the soil carbon cycle under different soil cultivation and natural restoration patterns, which was consistent with the results of previous research (Siles and Margesin, 2016). The abundance of nitrification and aerobic ammonia oxidation functional genes involved in the soil N cycle in wetland was significantly higher than in the farmland (Wu et al., 2021). The dispersion of soil particles could affect the availability of oxygen. The farmland (RF and SF) soil types primarily contain Acidobacteria, which grows best in anaerobic and microaerobic environments, and the acidic soil is suitable for the growth of some subpopulations of Acidobacteria (Wang et al., 2016). Most species of Proteobacteria are facultative or obligate anaerobic and heterotrophic and can rely on the energy of photosynthesis for self-sufficiency (Zhao et al., 2021). The abundance of facultative anaerobic bacteria is substantially affected by the rich organic carbon and TN content of wetland soil (Velmourougane et al., 2017). This study identified a positive correlation between mobile elements and antibiotic resistance genes in soil. As the main source of antibiotics, Actinomycetes cause the abundance of mobile elements in wetland soil to be significantly higher than those in farmland. Bacteria that form biofilms can degrade refractory compounds, enhance the effects of bioremediation, contribute to plant growth and root and stem restoration and protect moisture in the soil (Ligi et al., 2014). The functional groups in the biofilm forming bacteria in the farmland were relatively abundant, indicating that their bacterial functional groups are promising for the restoration of ecological sites (Ghorbanzadeh et al., 2020). Simultaneously, facultative anaerobic and biofilm forming bacteria negatively correlated with the soil pH values. High soil pH values can lead to changes in the structure of soil particles, thus, forcing differences in microbial phenotypes (Sunil and Padmakumari, 2020). The relative abundance of gram-negative bacteria in the farmland was significantly higher than in the wetland ($P < 0.01$). Gram negative bacteria primarily obtain their carbon nutrients from plant residues (Wong et al., 2009). The abundant litter in farmland provided a rich source of carbon by application of artificial chemical fertilizer for gram negative bacteria. The results of this study show that the change in soil cultivation and natural restoration patterns leads to the heterogeneity of the living environment of microorganisms. The physical and chemical properties of the soil and the composition, functional groups and phenotypic differences of soil microorganisms changed. Soil microorganisms can balance the distribution of resources in terms of their growth, survival and reproduction, produce selective traits or functional responses, and then regulate the phenotypic changes of microorganisms to enhance the adaptability of environment (Zhao et al., 2021).

Conclusion

This experiment shows that different soil cultivation and natural restoration patterns in a cold-region wetlands lead to differences in soil the phylum level of dominant microbial communities and the relative abundance of functional genes. The analysis was primarily driven by soil cultivation and natural restoration patterns in the changes of soil biological micro-environment. In the classification of soil bacterial community in soil cultivation and natural restoration, Actinobacteriota, Acidobacteriota, Proteobacteria, Chloroflexi and Firmicutes were the predominant bacteria. Soil cultivation and natural restoration patterns can change the abundance of soil functional microorganisms. The dominant functional genes were primarily for chemoheterotrophy and aerobic chemoheterotrophy bacteria. The BugBase phenotype analysis predicted that there are differences in the relative abundance of seven phenotypic functional bacteria and their primary microbial communities.

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