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Effects pf Cr Stress on Bacterial Community Structure and Composition in Rhizosphere Soils of *Iris Tectorum*

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Abstract: With the rapid development of industry, Cr has become one of the major heavy metal pollutants in soil, which has caused serious effects on the soil's ecological environment. However, the effects of Cr stress on bacterial communities in plant rhizosphere soils remain unclear. In this study, *I. tectorum* was selected as the research object, and 16S rRNA high-throughput sequencing technology was used to analyze the effects of Cr stress on the structure and diversity of the bacterial community in the rhizosphere soil of *I. tectorum*. The results showed that *I. tectorum* had strong tolerance and enrichment to Cr. However, under Cr stress, the diversity and abundance index of rhizosphere bacteria decreased by 8.5% and 6.8% on average, and the Sobs index decreased by 7.6%. Moreover, the bacterial community changed by 20.1% due to the addition of Cr, further leading to a 15.9% decrease in the common species of the bacterial community, among which *Proteobacteria*, *Actinobacteria*, *Chloroflexi* and *Acidobacteriota* accounted for more than 74.8% of the total sequence. According to the symbiosis network diagram, it was found that under a two-cultivated pattern, the synergizing effect between dominant bacteria was significantly enhanced, and the soil microenvironment was improved. Redundancy analysis showed that C, N, and P nutrient elements and Cr contents in uncontaminated and contaminated soils were the primary driving factors for the succession of *I. tectorum* rhizosphere bacterial community, and the response was stronger after Cr(VI) was added. In conclusion, the results of this study will provide insights into the response of rhizosphere bacterial communities to heavy metal Cr and the interactions between wetland plants and rhizosphere bacteria in wetland phytoremediation.

Keywords: Cr stress; rhizosphere bacterial community; *Iris tectorum*; 16S rRNA sequencing technology; Phytoremediation

1. Introduction

With the influence of human activities, chromium (Cr) has been widely used in tanning, battery manufacturing, fertilizer, textile, carpet, and electroplating industries[1]. This makes it the most common metal contaminant in groundwater, soil, and sediment. Hexavalent Cr^{6+} and trivalent Cr^{3+} are the two most stable and common forms of Cr in nature. Compared with Cr^{3+}, Cr^{6+} is more toxic to plants and microorganisms because of its higher solubility, mobility, and oxidation capacity[2]. It can damage the genetic material of all living things and is currently classified as A class carcinogen by the United States Environmental Protection Agency (USEPA)[3]. Studies have shown that heavy metals can inhibit plant growth and thus photosynthesis[4], destroy protein structure and even change cell morphology[5],[6]. Therefore, how to reduce the toxicity of Cr to the environment has become a concern of people.

As for the traditional heavy metal removal methods, bioremediation has the advantages of less investment, low operating cost, and no secondary pollution. Therefore, bioremediation is being widely used in Cr^{6+}-contaminated soil and water, and they mainly participate in the removal of Cr^{6+} through adsorption, bioaccumulation, and biotransfor-

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mation[7]. Among them, the rhizosphere is the main site of Cr migration and transformation, where plants promote the development of bacterial populations by releasing root exudates[6]. Moreover, the rhizosphere provides a variety of carbon-rich microhabitats that can provide a favorable environment for the colonization of beneficial bacterial populations[8][9].

In recent years, with the rapid development of high throughput sequencing technology, plant rhizosphere microflora research has achieved great progress[10][11], mainly reflected in plant rhizosphere microbial screening, the identification of genes that heavy metal tolerance, tolerance and the succession of microbial community characteristics and driving factors analysis, and artificial wetland biological strengthening technology[12][13]. The study found that plant growth-promoting bacteria (PGPB) under Cr stress can promote plant growth through mineral phosphate solubilization, nitrogen fixation, indole-3-acetic acid, siderophores, hydrogen cyanide, and ammonia production[14]. At the same time, the strains with Cr tolerance were screened out in previous studies, they are respectively Serratia sp. and *Arthrobacter* sp Serratia sp. And *Arthrobacter* sp.[15], (*Pseudomonas Alcaliphila* Strain NewG-2)[16], (*Pseudomonas* Strain CPSB21) (*Pratishtha* et al., *Bacillus* cereus (*Bacillus* cereus). They can alleviate Cr stress by their stress mechanism and promote plant growth. Furthermore, the removal rate of Cr^{6+} by *Bacillus cereus* was close to 100% when pH=7 and the temperature is 35℃[17]. Therefore, under the background of extensive research on rhizosphere microorganisms, it is of great significance to explore the response of the rhizosphere microbial community to Cr stress, as well as the protection of microorganisms on plants.

Constructed wetlands have been widely used in the treatment of Cr-containing wastewater because of their ecological and environmental advantages as well as economic and social benefits. More than 90% of Cr removal by constructed wetlands is fixed by substrates and microorganisms, and less than 10% of Cr is directly removed by plants, indicating that substrates and microorganisms are the keys to Cr removal by constructed wetlands[17],[18]. Wetland plants can fix Cr in the cell wall, cell membrane, and vacuole through their stress matrix, and exist in the form of chelates and complexes, thus reducing Cr toxicity[6],[19]. *I. tectorum* is a perennial herbaceous species with developed roots, strong stems, and rapid growth. At the same time, it has strong adaptability to the extreme living environment and has strong tolerance and enrichment of heavy metals. Therefore, it has been widely used in the heavy metal removal of constructed wetlands in recent years[20]. Under heavy metal stress, a large number of differentially expressed proteins appear in the roots of *I. tectorum*. These differentially expressed proteins mainly have functions such as signal transduction, ion transport, and REDOX, and are involved in amino acid synthesis, lignin synthesis, and glutathione metabolism, which play a crucial role in heavy metal stress[21]. Therefore, *I. tectorum* can serve as a good material base carrier under Cr stress, and at the same time combine with beneficial microorganisms to repair Cr-contaminated soil and water.

Taking *I. tectorum* as the research object, this paper uses 16S rRNA high-throughput sequencing technology to analyze the structure, composition, diversity, and intercommunity symbiosis network changes of microbial communities in soil under Cr pollution and discusses the law of the impact of Cr pollution on microbial community characteristics and reveals that under different cultivation modes, Symbiotic network patterns of microbial communities in Cr-contaminated soils. This study provided a theoretical basis for further understanding the characteristics of soil microbial communities under Cr stress and the biological remediation of chrome-contaminated soil.

2. Materials and Methods

2.1. Experimental design

The background value of heavy metal Cr in Guizhou Province is 95.9 mg kg^{-1}[22]. The Cr content of farmland soil should be controlled below 350 PPM[23]. This study refers to the study of Wang et al.[24], and takes the intermediate concentration (200 mg kg^{-1}) as

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the Cr pollution concentration in our experiment. The *I. tectorum* seedlings used in the experiment were cultivated by the Plant Seedling company through strict hydroponics (The plants were suspended from the plate, part of the root system was inserted into the nutrient solution, and part of the root system was grown in the space between the nutrient solution and the plate). Firstly, after screening *I. tectorum* seeds of the same size, surface disinfection was carried out with 75% alcohol, the seeds were then washed five times with deionized water and placed in a damp petri dish to germinate. The germinated seeds were moved to the colonization plate and cultured with a nutrient solution. When they grew to 20-30 cm, we purchased these seedlings from the company and selected these seedlings with the same growth for the experiment. The seedlings were surface disinfected with 75% ethanol and 1% sodium hypochlorite for 10 s and 15 min[25], then carefully washed with deionized water 5 times, and then transplanted into greenhouse potted plants with 3 plants in each pot. A 1/4 concentration of 100 ml of Hoagland solution was added to each pot once a week during cultivation to ensure the nutrients needed for *I. tectorum* growth. Water depth was controlled with deionized water.

Experiments were performed in 32×28 cm (diameter×height) pots. *I. tectorum* was cultivated by a greenhouse pot experiment in a flooded condition. Each pot contained 20 kg of soil collected from a karst mountain in, southwestern China (106°37′36″E, 26°22′26″N), and multi-point mixed sampling was conducted to take soil samples from depths of 0-20 cm. weed stones were removed and passed through a 2 mm sieve. The pot of sole-cultivated pattern (ITI), two-cultivated pattern (ITII), and three-cultivated pattern (ITIII) was designed as the control group (original soil samples without Cr contaminated) and contaminated group (the exogenous addition of 0.1 mmol L^{-1} $K_2Cr_2O_7$ solutions made Cr(VI) content to be 200 mg kg^{-1} in the soil), and the un-planted blank samples (CK), Each group had three replicates. Details of the experimental grouping arrangement were shown in Fig. S1. The greenhouse ensured a constant temperature of 25 ℃ and moisture content of 50%, which reduced the interference of micro-meteorological factors on plant growth.

2.2. Sampling and chemical analysis

The pot culture started from the seedling (20-30 cm), and Cr(VI) was added to the pollution group after 30 days of domestication. After 3 months of pot experiment, destructive samples were taken. Randomly selected plants in potted plants were removed from the soil and collected the soil attached to the primary root (served as rhizosphere soil), and refrigerated to preserve the selected plants[26]; CK groups were potted to take soil samples of 0-5 cm from the upper layer. All of the samples were taken three times each time, a total of 21 samples. Finally, samples were frozen at -20 ℃ for DNA extraction, high throughput sequencing, and determination of Cr content[6].

The Cr content of uncontaminated soil was measured by atomic absorption spectrophotometer (Perkin Elmer Analyst 800, USA). soil pH was measured by the potentiometric method. Soil organic matter (SOM) and organic carbon (SOC) were measured by $K_2Cr_2O_7$-H_2SO_4 oxidation-external heating method[27]; Soil total nitrogen (TN) was measured by the Kjeldahl method of nitrogen determination[28]; Soil ammonium nitrogen (NH_4^+-N) and Soil nitrate nitrogen (NO_3^--N) were analyzed using 1 M potassium chloride (KCl), added sodium salicylate and hydrazine sulfate to produce a color reaction, and absorbance was measured spectrophotometrically at 660 nm and 550 nm[6]; Soil total phosphorus (TP) was measured by H_2SO_4 digest-Mo-Sb Anti spectrophotometer method[27]; Soil available phosphorous (AP) was measured by phosphomolybdenum blue spectrophotometric method[29]; The Cr concentration in plants was determined by atomic absorption spectrophotometry[30]. The physicochemical properties of the soils are available in Table S2.

2.3. DNA extraction, 16S rRNA gene sequencing, and shotgun Metagenome sequencing

Total genomic DNA from the IT rhizosphere and bulk soil was extracted using a FastDNA SPIN Kit for Soil (Qbiogene Inc., USA) following the manufacturer's instructions

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and sent Majorbio Bio-pharm Technology, Shanghai for sequencing. Sequencing interval: 338F 806R, The name of the primer was 338F/806R, Primer sequences: ACTCCTACGG-GAGGCAGCAG/ GGACTACHVG GGTWTCTAAT; Samples were randomly selected for pre-experiments to ensure that the majority of samples in the lowest number of cycles could be amplified to the appropriate concentration of products. After the preliminary experiment was completed, TransGen ap 221-02 was used in the formal PCR test: TransStart Fastpfu DNA Polymerase, 20 μL reaction system: As shown in Table S1. Using PCR instrument: ABI GeneAmp® 9700, PCR reaction parameters: a. 1×(3 min at 95 ℃), b. cycle number×(30 seconds at 95 ℃; 30 seconds at annealing temperature ℃; 45 seconds at 72 ℃), c. 10 minutes at 72 ℃, 10 ℃ until halted by the user. PCR products are detected by 2% agarose gel electrophoresis. The Majorbio Cloud platform was used to conduct OTU cluster analysis of sequencing results (based on Research software), optimize sequences to extract non-repeating sequences, select sequences with more than 97% similarity with representative sequences, calculate OTU number (that was, the observed abundance sobs value), and conduct Alpha diversity analysis to analyze species richness and diversity[6].

2.4. Statistical analysis

Normality and homogeneity of data were tested by KolmogorovSmirnov and Levene's test. The data that did not obey a normal distribution were transformed by the natural logarithm. Univariate analysis of variance (ANOVA) was used to test the significance of soil physicochemical properties and diversity index. All microbial visualizations (PCA, Veen, symbiotic network, species composition, etc.) were made using the online platform (www.majorbio.com). SPSS 26.0 statistical software was used for all statistical analysis, Origin 2021 was used for all histograms and boxplots, and Adobe Illustrator CC 2019 was used for schematics.

3. Results

3.1. Effects of Cr-stress on soil and plant properties

The Physicochemical properties of plant rhizosphere treatment groups and blank soil are analyzed (Table S2). pH, TN, and TP of rhizosphere soil are averagely lower than soil, whereas AP is the higher one. Furthermore, in terms of soil nitrogen form, the NH_4^+-N and NO_3^--N in each contamination group are more, and soil nitrogen mineralization is promoted. However, Especially there is more nitrogen nutrition assimilated by group CrITIII, with the Cr-stress and plant competition relationship, leading to the lowest TN values. TN($P < 0.05$), and TP ($P < 0.01$) perform a significant difference with the different cultivated pattern. Under Cr stress, SOM showed a significant upward trend ($p<0.05$) compared with the corresponding parameters in the control group, and the content of Cr in roots and leaves increased by 97.8% and 99.2%, respectively (Figure 1; Table S2). It is noteworthy that in the control group, Cr content in roots was significantly higher than that in leaves ($p<0.01$). However, the enrichment content in leaves was slightly higher than that in roots after Cr stress. These results indicate that *I. tectorum* has good tolerance and enrichment ability to Cr, and the root metabolic process is affected by Cr stress and plant competition while absorbing soil nutrients.

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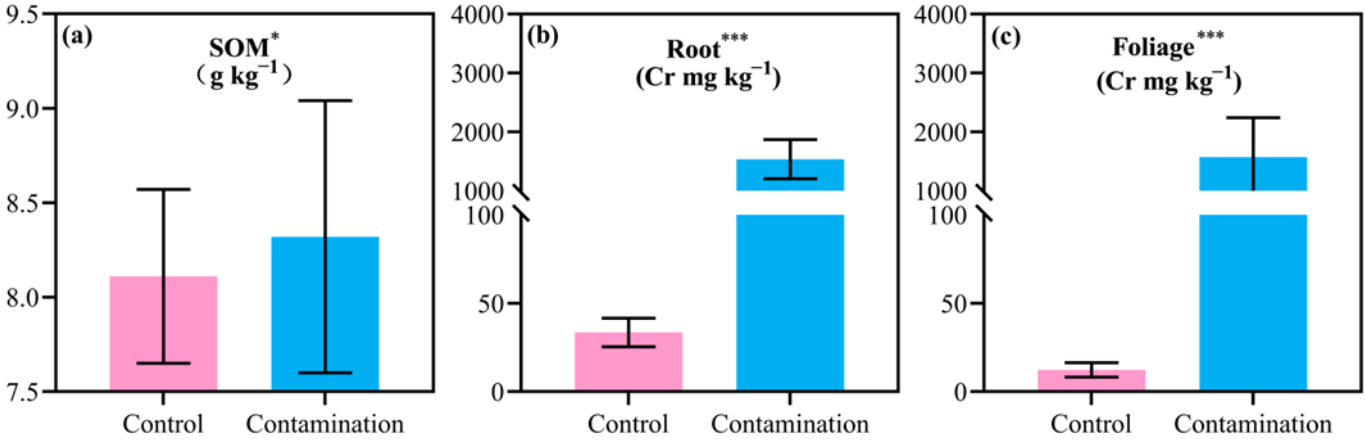

Fig. 1 Comparison of physicochemical properties between control and contaminated groups. (a) SOM; (b) Foliage Cr; (c) Root Cr. *p < 0.05, **p < 0.01, and ***p< 0.005, The results showed that there were significant differences in physicochemical properties between the control group and the polluted group. Error bars refer to standard errors.

3.2. rhizosphere bacterial diversity

A total of 1239,259 valid sequences were obtained and 3008 bacterial taxa (OTUs) were identified by high-throughput sequencing technology after removing unrecognized gene base sequences and chimera. The coverage of OTU sequences was above 98%. The slopes of the dilution curves of all samples were close to saturation (Figure S2). Under Cr stress, compared with the control group, the Sobs index, Shannon index, and Ace index of the rhizosphere bacterial community of *I. tectorum* decreased by 7.6%, 8.5%, and 6.8% respectively (Figure 2a-c; Table S3). Therefore, exogenous Cr supplementation reduced the diversity and abundance index of the rhizosphere soil bacterial community of *I. tectorum*.

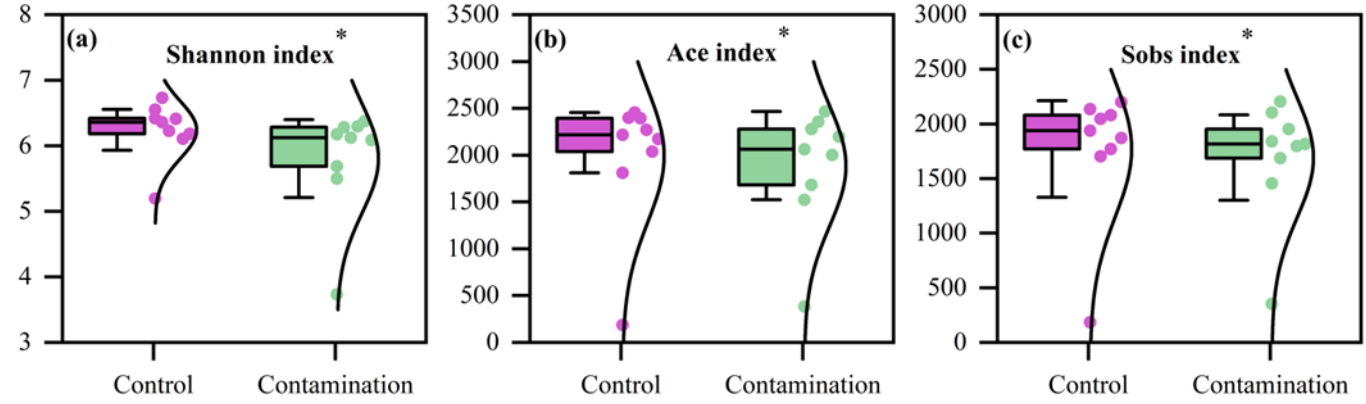

Fig. 2 Comparison of bacterial community diversity and abundance index between control and contaminated groups. (a) Shannon index; (b) Ace index; (c) Sobs index. *p < 0.05, **p < 0.01, and ***p< 0.005, The results showed that there were significant differences between the control group and the contaminated group. The error bar is the 95% confidence interval.

3.3. Soil bacterial community structure

Veen diagram showed species in the polluted group were significantly affected under Cr stress, and the total number of species in the control group and the polluted group were 49.8% and 41.9%, respectively (Figure S3a and b). To further understand the differences in bacterial community structure under Cr pollution, (PCoA) and (NMDS) were conducted on the OTU level for each group of samples to explore the spatial and temporal distribution pattern of the bacterial community in soil (Figure 3a and b). The bacterial

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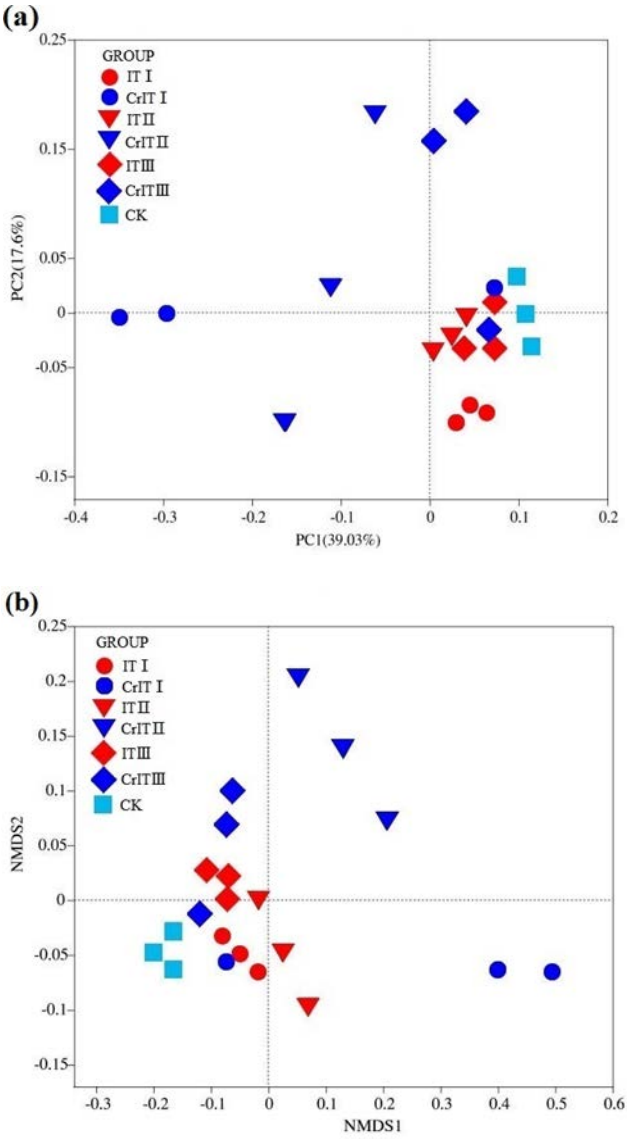
community structure was significantly different between the control group and the contaminated group. The similarity analysis of all samples showed that Cr pollution had a significant impact on soil bacterial community structure (R=0.1623, P<0.05, Figure. S4). PLS-DA analysis was carried out to prove the contribution of Cr stress on the change of the *I. tectorum* rhizosphere bacterial community (Figure 3c), in which the samples were divided into control and pollution groups. The results showed that the bacterial community changed by 20.1% due to Cr stress, which led to the control group and the contaminated group showing two obvious divisions. Therefore, exogenous Cr supplementation can significantly affect the spatial pattern and composition of bacterial communities.



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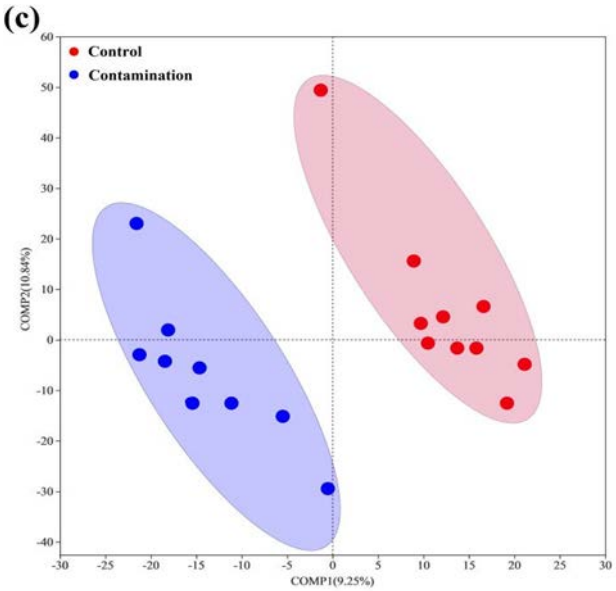

Fig. 3 NMDS analysis on Bray-Curtis distance (a), PCoA analysis on weighted UniFrac distance (b), PLS-DA analysis (c), which based on OTUs at a 97% similarity level. PLS-DA performance of differentiation judges the potential interference factors, followed by OTUs at a 97% similarity degree. Comp1 and Comp2 respectively represent the suspected affecting factors for the deviation of microbial composition. The unplanted and bulk soil (CK), sole-cultivated pattern (ITI), two-cultivated pattern (ITII), three-cultivated pattern (ITIII), Under Cr stress, sole-cultivated pattern (CrITI), two-cultivated pattern (CrITII), three-cultivated pattern (CrITIII). Three duplicate samples.

3.4. Soil bacterial community composition

Our study identified 3008 bacterial taxa belonging to 36 phyla, 90 classes, and 830 genera. The dominant phyla were *Proteobacteria* (average relative abundance 38.7%), *Actinobacteria* (17.5%), *Chloroflexi* (10.0%), *Acidobacteria* (8.6%), *Gemmatimonadetes* (7.7%), *Bacteroidetes* (4.3%) and *Firmicutes* (4.1%) (Figure 4). In the control group, *Proteobacteria* showed an obvious upward trend under the three-Cultivated pattern. *Actinobacteria* and *Chloroflexi* have the highest abundance in the sole-Cultivated pattern (even higher than that in the blank group). *Acidobacteria* and *Gemmatimonadetes* had the highest abundance in the three-Cultivated pattern, whereas they were lower than the blank group. However, compared with the control group, the abundance of *Proteobacteria* increased significantly under the two-cultivated pattern and three-cultivated pattern after Cr addition. The variation trend of *Actinobacteria* and *Chloroflexi* was consistent with that of the control group, but their abundance was slightly lower than that of the control group. *Acidobacteria* and *Gemmatimonadetes* also have the highest abundance in the three-Cultivated pattern, but the abundance is still lower than that in the control group. These results showed that the dominant bacteria were not significantly affected under Cr stress, while some less abundant bacteria (such as *Bacteroidetes*、*Firmicutes*, and *Patescibacteria* et al) fluctuated greatly, and the bacterial community abundance was significantly improved under a two-cultivated pattern and three-cultivated pattern.

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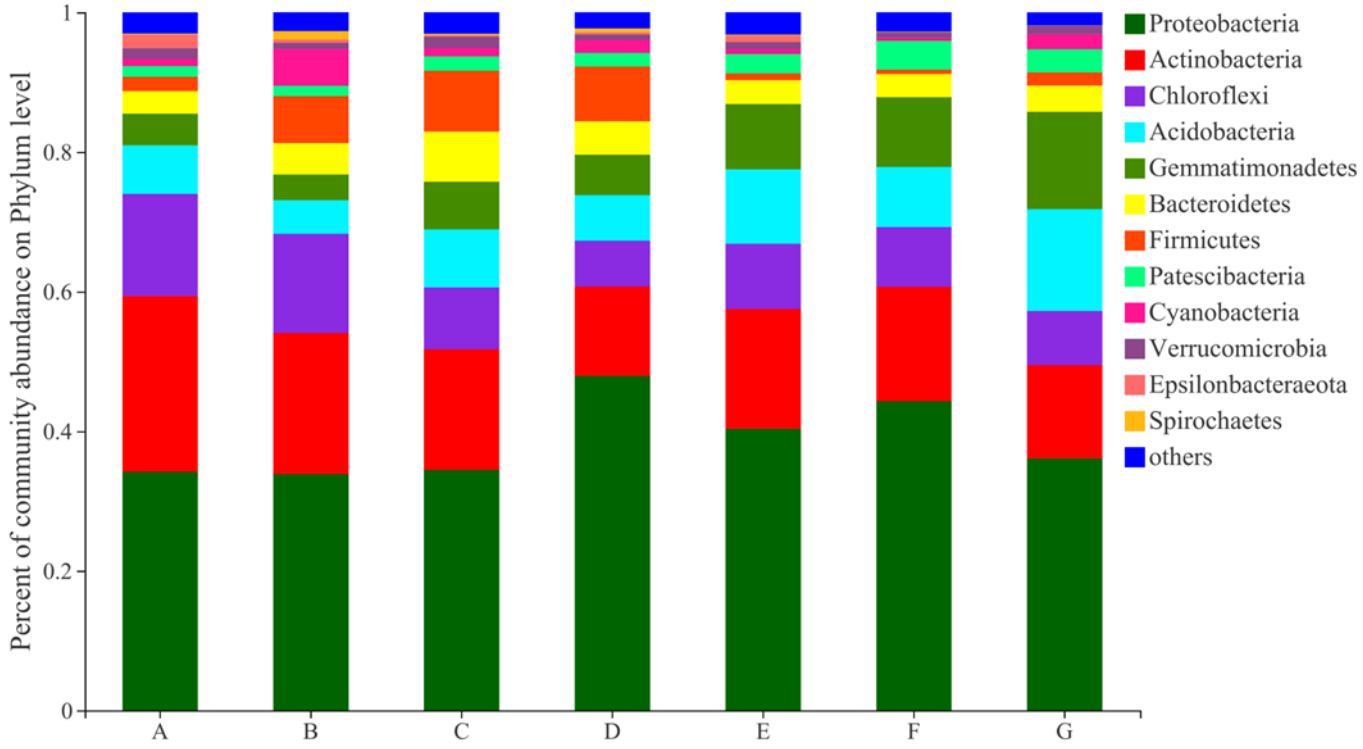

Fig. 4 Bacterial community structure and differences. Different colored columns represent different species, and the length of the column represents the size of the proportion of that species. A, C,and E were the control group without Cr in *I. tectorum* single, double and three plants, respectively. B, D,and F were polluted groups with *I. tectorum* single, double and triple Cr treatments, respectively. G is for unplanted and bulk soil (CK).

According to the histogram analysis of species abundance difference (Figure S5), the abundance of *Actinobacteria* and *Deltaproteobacteria* was the highest in the sole-Cultivated pattern, and the abundance of the polluted group was significantly lower than that of the control group. *Gammaproteobacteria* and *Alphaproteobacteria* had the highest abundance under a two-cultivated pattern, and Cr stress induced an increase in their abundance. *Actinobacteria* in the three-cultivated pattern showed an opposite situation to that in the sole-cultivated pattern, and the beneficial bacteria population, *Gemmatimonadetes*, appeared again as the dominant species.

3.5. Interspecific symbiotic network of soil bacterial communities

The symbiotic network diagram of bacterial communities was used to evaluate the correlation between different bacterial communities, reflect the coexistence pattern of bacterial communities in specific habitats, and explore the interspecific relationship of soil bacterial communities under Cr stress (Figure 5a-c). The colinear network diagram indicated that bacterial community structure among all samples was similar (Figure S6). The dominant species are *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, *Gemmatimonadetes*, and *Nitrospinae*. Through further analysis, we found that the dominant species in the sole-Cultivated pattern are mainly symbiosis, but the competition between the dominant species is still prominent. The symbiosis is not concentrated. *Proteobacteria*, *Actinobacteria*, and *Chloroflexi* are mainly divided into three blocks (Figure 5a). Under the Three-Cultivated pattern, the symbiotic relationship between dominant species is significantly weakened, and the symbiotic system is increasingly dispersed (Figure 5c). Under the two-cultivated pattern, the symbiotic relationship between dominant species is significantly enhanced, while the competition relationship is significantly reduced. Moreover, the relationship between species is closer, and the symbiotic system is highly concentrated (Figure 5b; Table S5). Therefore, the results show that the symbiotic system formed among dominant species in the two-cultivated pattern is more conducive to resisting Cr stress.

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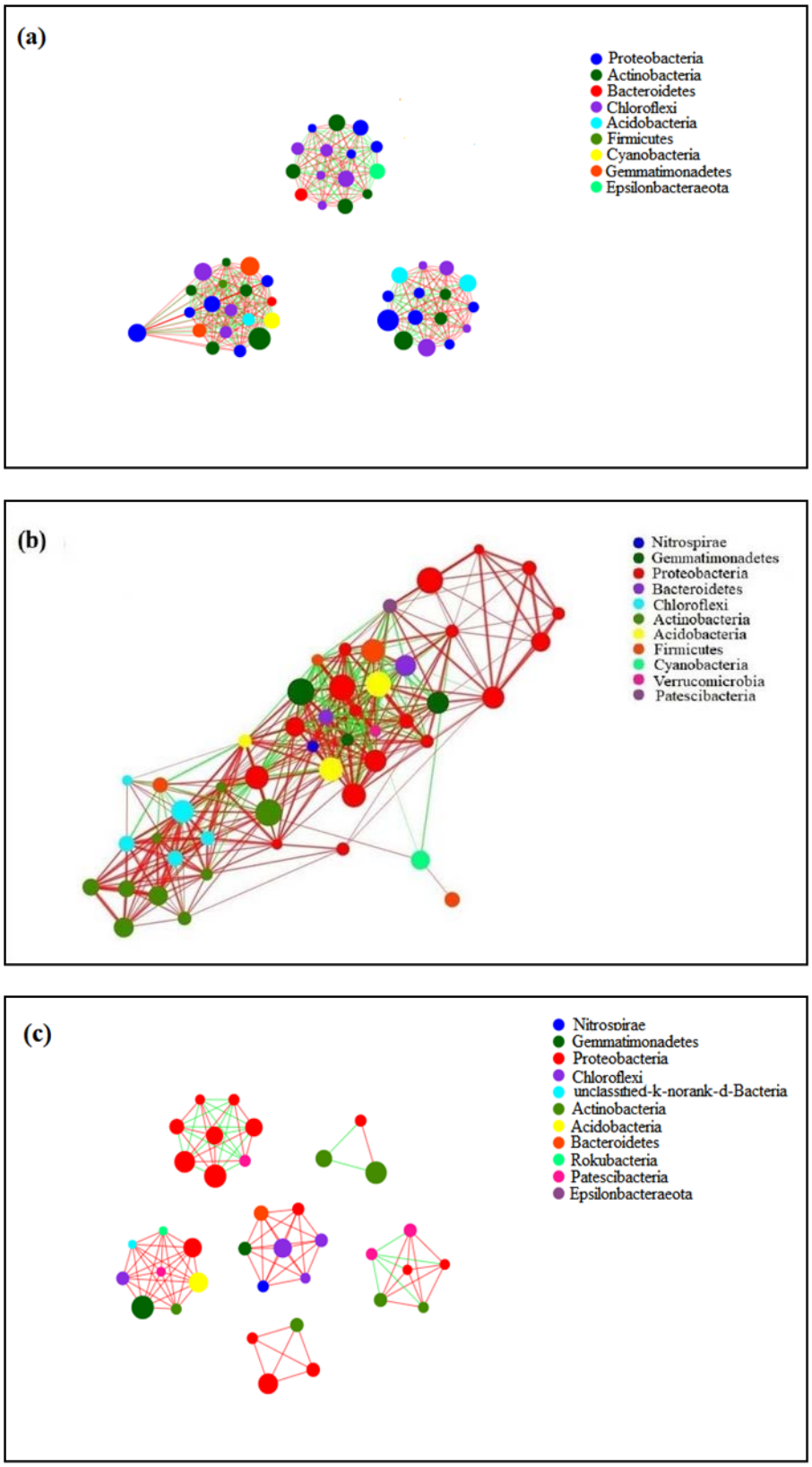

Fig. 5 Symbiotic network diagram of soil rhizosphere microbial community (a: *I. tectorum* sole-cultivated pattern under Cr stress b: *I. tectorum* two-cultivated pattern under Cr stress; c: *I. tectorum* three-cultivated pattern under Cr stress). The size of nodes in the figure indicates the abundance of species, and different colors indicate different species. The color of the line indicates a positive and negative correlation, red indicates a positive correlation, and green indicates a negative correlation. The thickness of the line indicates the correlation coefficient. The thicker the line, the higher the correlation between species. The more lines, the more closely related the species is

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to other species. B, D, and F were polluted groups with *I. tectorum* single, double and triple Cr treatments, respectively. The network diagrams of B, D, and F are (a), (b) and (c) respectively. B represents the network diagram under single planting mode Cr treatment, D represents the network diagram under double planting mode Cr treatment, and F represents the network diagram under three planting mode Cr treatment.

3.6. Correlation analysis between rhizosphere soil bacterial community and physicochemical properties

RDA analysis showed that for the rhizosphere bacterial communities in uncontaminated soil and contaminated soil, the interpretation rates of the first two main axes for the total coefficient of variation of I. tectorum bacterial communities were 80.34% and 85.78%, respectively (Figure 6). It can be seen from the figure that SOM, TN, NH_4^+-N, and pH are the main environmental factors driving the succession of rhizosphere bacterial communities in uncontaminated soil, followed by TP, AP, and NO_3^--N. In addition, *Proteobacteria*, *Acidobacteria*, and *Gemmatimonadetes* were positively correlated with TP, TN, NH_4^+-N, and SOM, and negatively correlated with pH. SOM was the main influencing factor of *Actinobacteria, Firmicutes* and *Patescibacteria* were positively correlated with NO_3^--N and negatively correlated with AP. After adding Cr(Ⅵ), NO_3^--N, SOM and Cr contents were the main environmental factors driving rhizosphere bacterial community succession, followed by NH_4^+-N and pH. Meanwhile, *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Acidobacteria* are the most abundant bacterial groups in polluted soil. *Proteobacteria* is positively correlated with Cr content. It was negatively correlated with SOM, TN, TP, NH_4^+-N, and pH. AP was the main influencing factor of *Firmicutes*. In addition, the community structure of the uncontaminated and contaminated groups was similar, but the abundance was significantly different.

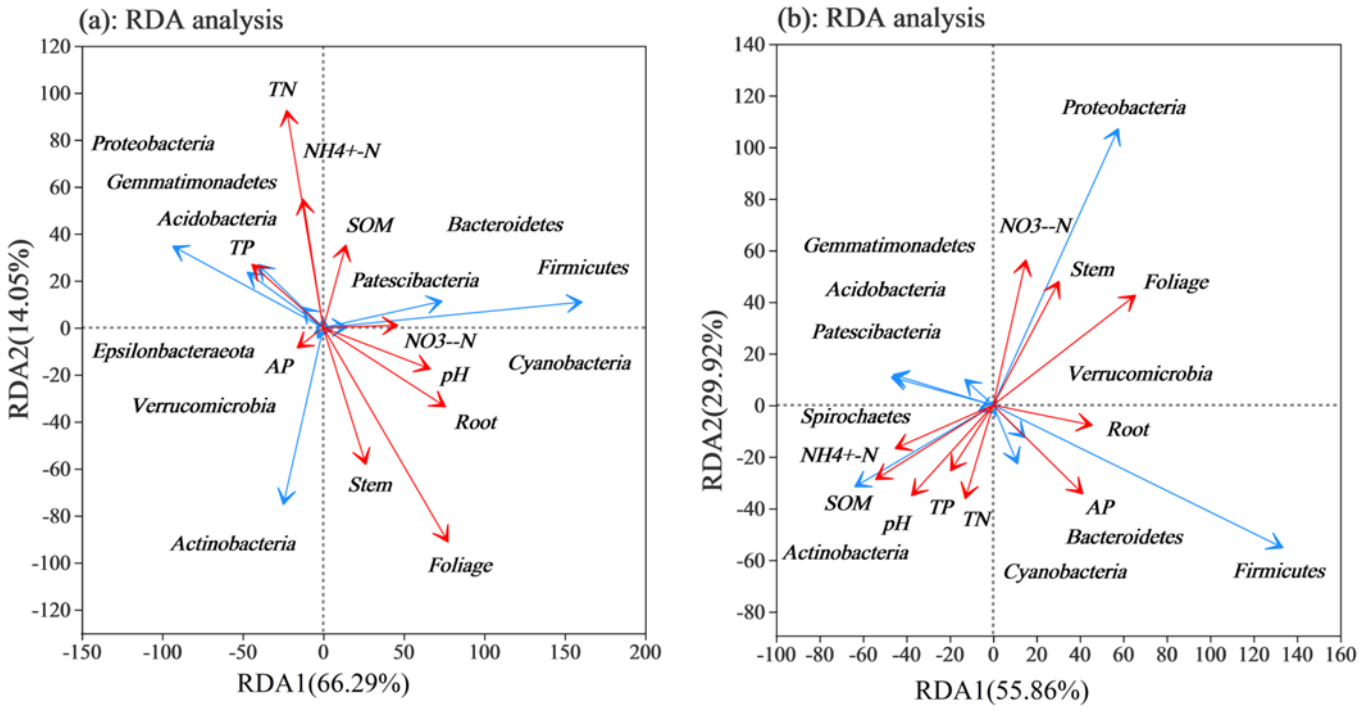

Fig. 6 RDA analysis of bacterial communities in the rhizosphere of *I. tectorum* (a: uncontaminated soil b: Cr contaminated soil). The red arrow represents quantitative environmental factors, and the length of the environmental factor arrow can represent the impact degree (interpretation quantity) of environmental factors on species data. The Angle between the arrows of environmental factors represents positive and negative correlations (acute Angle: positive correlation; Obtuse Angle: negative correlation; Right Angle: no correlation).

4. Discussion

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4.1. Physiological response of I. tectorum to Cr stress

Cr hardly participates in the metabolic function of plants, but it is potentially toxic and can adversely affect its morphophysiological properties and antioxidant defense mechanisms[31]. In the control group, Cr content in *I. tectorum* roots was significantly lower than that in leaves (Figure 1b and c). This may be because when plants grow in Cr-contaminated soil, the roots are preferentially exposed to heavy metals in the soil, which leads to the accumulation and transport of a large amount of Cr in the soil in the roots of plants[32]. After the exogenous addition of Cr, the content of Cr in the roots and leaves of *I. tectorum* was significantly higher than that in the control group (Figure 1b and c). This may be because wetland plants use their removal mechanism to fix Cr in the cell wall and vacuole of plant cells, to purify the soil contaminated by Cr[6]. Moreover, previous studies have shown that hemicellulose (HC-1) in plant cell walls binds heavy metals to the greatest extent under heavy metal stress, thus reducing the toxicity of heavy metals to plants[33].

The significant increase in SOM under Cr contamination (Figure 1a) may be due to the following three reasons: 1) Cr would compete with plants for sulfate channels, thus affecting the absorption of water and nutrients by plants[34]. 2) Damage to plasma membrane integrity, resulting in damage to root tip cells and more organic matter transport to the soil[35]. 3) It may also be related to dead bacteria in the soil, which is due to the inanimate bacterial biomass accumulating in the soil over time. Meanwhile, Cr stress-sensitive bacterial communities provide the soil with a large amount of organic matter[36]. From the above results, we can see that Cr stress has a certain amount of influence on plants and rhizosphere bacterial communities, but *I. tectorum* can still enrich Cr in large quantities in extreme environments. Therefore, it can be seen that *I. tectorum* has its advantages in Cr removal.

4.2. Effects of Cr stress on soil bacterial community structure

Under Cr stress, it is very important to study the complex bacterial community structure and species diversity in plant rhizosphere soil for understanding the regulation mechanism of the ecosystem itself[37],[38]. In this study, exogenously added Cr reduced the α diversity index (Sobs, Shannon, and Ace index) of the bacterial community in the rhizosphere of *I. tectorum* (Figure 2), This may be because the high concentration of Cr(VI) inhibited the nitrification and denitrification of the bacterial community, thereby reducing the diversity and abundance index of the bacterial community[39]. Moreover, under the action of reductase or reductive substances, Cr(VI) in cells will produce a large number of reactive oxygen species (ROS) in the process of reduction to Cr(III), which will cause DNA damage after binding with DNA, resulting in cell deformation, genetic variation and even death[40]. This harmed the diversity and abundance index of the bacterial community. In addition, the analysis of PCoA and NMDS showed that Cr stress significantly changed the spatial structure of the bacterial community in the rhizosphere soil of *I. tectorum* (Figure 3a and b), which has also been confirmed in previous studies, such as *Windmill Grass*[6] and *Oryza sativa*[41]. In addition, PLS-DA (Figure 3 c) and Veen (Figure S3 a and b) analysis again proved that exogenous Cr supplementation significantly affected the structural composition and species number of bacterial communities. As can be seen from the above results, the addition of exogenous Cr significantly reduces the diversity of the rhizosphere bacterial community of *I. tectorum* and changes the structure of the rhizosphere bacterial community, causing damage to the macroecology of *I. tectorum*.

4.3. Effects of Cr stress on rhizosphere bacteria

Bioremediation relies on the universality and diversity of bacterial communities and responds to Cr stress through its REDOX system, extracellular adsorption, and efflux mechanisms[40],[42]. In this study, it was found that *Proteobacteria*, *Actinobacteria*, and *Chloroflexi* were significantly more abundant in the control group than in the blank group

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(CK) (Figure 4), which may be because root exudates (polysaccharides, phenolic compounds, flavonoids, and organic acids), It provides more nutrients for the survival of the bacterial community (Figure 7), thus promoting the growth of bacteria[43]. Under Cr stress, compared with the control group, the growth of Proteobacteria was not inhibited, but showed an upward trend (Figure 4). This may be because *Proteobacteria* are Gram-negative bacteria, and the gram-negative cell envelope is composed of an outer membrane (containing anion lipopolysaccharide, phospholipid, and outer membrane protein) and peptidoglycan, which plays a key role in heavy metal binding[44]. Moreover, bacteria possess polysaccharide slime layers, which readily offer amino, carboxyl, phosphate, and sulfate groups for metal binding[45]. Compared with the control group, the growth of *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, and *Gemmatimonadetes* was restricted under Cr stress (Figure 4). Although these four dominant bacteria can alleviate heavy metal stress through strong secondary metabolism, more metabolic functions, and self-regulation of energy supply[46]. *Actinobacteria* as a typical Gram-positive bacterium exhibits remarkable Cr resistance, which plays a direct role in the reduction and removal of Cr[47].

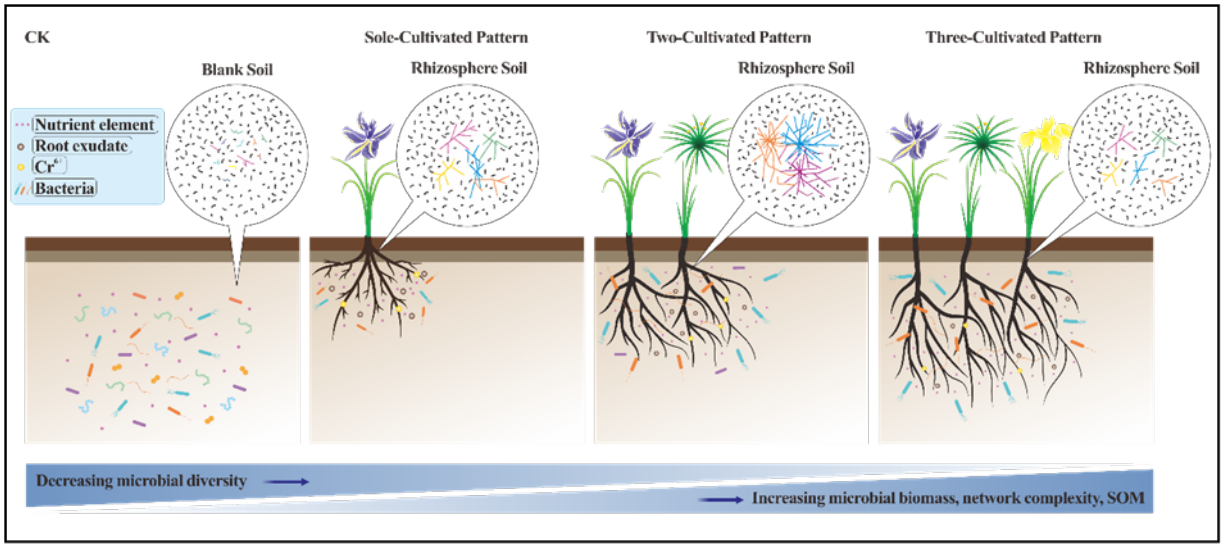

Figure 7 Response mechanism of rhizosphere bacterial community under different cultivation modes. Blank soil has a uniform bacterial community without external interference. Sole-cultivated pattern: During plant growth, organic substances are secreted by the root system to make bacterial communities gather around the root system. Moreover, due to the addition of exogenous Cr, a symbiotic network is established among rhizosphere bacterial communities to jointly resist Cr stress. Under a two-cultivated pattern, the increase of plants can significantly improve the soil microenvironment, increase the rhizosphere bacterial community biomass, and build a more complete symbiotic network. Under the three-Cultivated pattern, the soil microenvironment is improved, but the symbiotic relationship between bacteria is weakened. The effects of different cultivation patterns on the soil microenvironment showed that diversity decreased, biomass increased, network complexity increased, and soil organic matter increased, thus effectively improving the soil microenvironment.

4.4. Effect of different cultivation patterns on rhizosphere bacteria

Through species analysis between groups, it can be seen that Actinobacteria is the dominant species in the sole-Cultivated pattern, but its abundance is lower than that of the control group. For two-cultivated and three-cultivated patterns, the abundance of *Gammaproteobacteria*, *Gemmatimonadetes*, *Alphaproteobacteria*, and *Actinobacteria* the abundance was not only the highest but also significantly higher than that of the control group. (Figure S5). This may be due to the redistribution of bacterial communities in the soil caused by human disturbances (i.e., different cultivation patterns) or root exudates of wetland plants (Figure 7; [48]). According to the symbiotic network analysis that although the bacterial community structure among all samples is similar, the correlation between dominant bacterial communities is weak in the sole-cultivated pattern and three-cultivated pattern, and the symbiosis pattern is relatively scattered (Figure 5a and c; Table S5). This

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may be caused by the sole-cultivated pattern, the plant itself and its rhizosphere bacterial community have a weak ability to cope with Cr stress. In the three-cultivated pattern, a large number of plant roots and their exudates compete for soil microbial resources. This results in the dispersion of the rhizosphere bacterial community, which makes the symbiotic relationship between bacteria weaker. In the two-cultivated pattern, the correlation between dominant species is significantly enhanced, the relationship between species is more close, and the symbiotic system is highly concentrated (Figure 5b). It was mentioned in the study that environmental changes played a dominant role in the formation of plant rhizosphere bacterial communities[49][50].

4.5. Effects of soil physicochemical properties on rhizosphere bacterial communities

Previous studies have shown that the growth and development of rhizosphere microbial communities can be affected by soil's physical and chemical properties[6]. In this study, we found that C, N, and P elements were the primary driving factors of the rhizosphere bacterial community in uncontaminated soil, which may be because the rhizosphere bacterial community would transform and utilize soil nutrient elements in normal life activities, to maintain soil microecological balance and its life activities[11]. In addition, during the utilization of the N element by nitrifying and denitrifying bacteria, the intermediate products produced will significantly change the contents of NO_3^--N and NH_4^+-N, and H^+ will also be released in this process, thus leading to the change of pH in the rhizosphere soil of *I. tectorum*[51]. After the addition of Cr(Ⅵ), NO_3^--N, SOM and Cr contents were the main environmental factors driving rhizosphere bacterial community succession, at the same time, it is also related to the stress mechanism of rhizosphere bacterial community[6]. Previous studies have shown that the detoxification of Cr by the bacterial community itself is mainly through intracellular or extracellular binding and reduction of heavy metals to reduce their heavy metal toxicity. At the same time, it can also activate its stress mechanism and adjust its nutrient recycling mode to better survive[52]. In this study, it was found that Proteobacteria became the most abundant bacterial group in contaminated soil after the addition of Cr(Ⅵ), and showed a significant positive correlation with Cr content. This may be because Proteobacteria has high biotransformation efficiency for Cr(Ⅵ), which can reduce Cr(Ⅵ) to Cr(Ⅲ) with low toxicity. Greatly reduces Cr mobility and bioavailability in the environment[53]. The content of SOM is an important indicator of soil fertility and a necessary substance for the survival of rhizosphere microorganisms. It has been found in this study that *Actinobacteria*, *Firmicutes*, and *Acidobacteria* can survive well under Cr stress and become the dominant bacteria in polluted soil. This may be due to their ability to adjust their nutrient use in extreme environments, where Gram-positive bacteria such as Actinobacteria can sustain their survival through robust secondary metabolism[54], *Firmicutes* and *Acidobacteria* can reduce their growth restriction by actively regulating their energy supply forms and using C, N, and P elements in extreme environments[55][56]. Not only that, but pH also plays an important role in the growth of rhizosphere bacterial communities, For example, the removal rate of Cr^{6+} by *Bacillus* cereus was close to 100% when pH=7, the temperature is 35℃[17]. The results showed that soil physicochemical properties could drive the succession of rhizosphere bacterial communities, and the dominant rhizosphere bacteria could also improve the microenvironment by changing their nutrient recycling patterns.

5. Conclusions

This study focused on the interference of Cr stress on the rhizosphere microenvironment of *I. tectorum*. In conclusion, the addition of exogenous Cr significantly reduced the abundance and diversity index of the bacterial community in the rhizosphere soil of *I. tectorum*, significantly changed the spatial pattern of the bacterial community, and affected the composition of the rhizosphere bacterial community. With the increase in plant

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diversity, the abundance of dominant bacteria in the soil microenvironment increased significantly, and beneficial bacteria appeared. Among them, the two-cultivated pattern effectively changes the symbiosis relationship between dominant species, obviously strengthens the synergy between dominant flora, and forms a more concentrated symbiosis network. Through RDA analysis, we also found that C, N, and P nutrient elements and Cr content were the primary driving factors for shaping the structure and diversity of the bacterial community in the rhizosphere of *I. tectorum*, and the response was stronger after Cr(Ⅵ) was added. The results of this study are helpful to further understand the effects of Cr stress on rhizosphere bacterial communities and provide a theoretical basis for bio-remediation of Cr-contaminated soils.

6. Patents

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1 20 μL reaction system. Fig. S1 *I. tectorum* pot experiment design. Fig. S2 Bacterial community dilution curve of soil sample. Fig. S3 Venn diagram (a、b). Fig. S4 ANOSIM analysis (R=0.1623, P<0. 05). Fig. S5 Wilcoxon rank-sum test bar plot on the Class level. Fig. S6 Collinear network graph.

Author Contributions: For research articles with six authors, a short paragraph specifying their contributions must be provided. The following statements should be used "Conceptualization, Zhu Gu and Zhao; methodology, Zhu and Wang.; software, Zhao and Xia.; validation, Zhao and Xia; formal analysis, Zhao Xia and Yang; investigation, Zhao Xia and Yang.; resources, Zhu and Zhao.; data curation, Zhao and Xia; writing—original draft preparation, Zhao; writing—review and editing, Zhu and Gu.; visualization, Zhao and Zhu; supervision, Zhu and Gu.; project administration, Zhu.; funding acquisition, Zhu. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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