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Remediation and Micro-Ecological Regulation of Cadmium and Arsenic Co-Contaminated Soils by Rotation of High-Biomass Crops and *Sedum alfredii* Hance: A Field Study

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Abstract: Rotation of high-biomass crops and hyperaccumulators is considered to be an effective, safe and economical method for the remediation of medium-mild heavy metal contaminated soil, but the present studies pay more attention to the removal efficiency rather than changes in soil micro-ecology. In order to explore the remediation effect of hyperaccumulators rotated with high-biomass crops on Cd and As co-contaminated soil, Cd hyperaccumulator ecotype (HE) *Sedum alfredii* Hance and crops were selected to construct a field experiment, five rotation modes including *Sedum alfredii* Hance-*Oryza sativa* L. (SP), *Sedum alfredii* Hance-*Sorghum bicolor* (L.) Moench (SS), *Sedum alfredii* Hance-*Zea mays* L. (SM), *Sedum alfredii* Hance-*Hibiscus cannabinus* L. (SK), *Sedum alfredii* Hance-*Trichosanthes kirilowii* Maxim. (ST), and investigated the effects of these modes on the removal efficiency, soil physiochemical properties and micro-ecological effects (soil nutrients, enzyme activities and microbial diversity) through a field experiment. The results showed that total soil Cd from the five rotation modes (SP, SS, SM, SK and ST) decreased by 25.1%, 20.3%, 34.5%, 6.3% and 74.3%, respectively, and total soil As decreased by 42.9%, 19.8%, 39.7%, 39.7% and 45.7%, respectively. The rotation significantly increased soil organic matter by 47.39–82.28%, effectively regulated soil pH value and cation exchange capacity. The rotation modes also significantly increased soil alkali-hydrolysable nitrogen by 9.09–50.91%, but decreased soil available phosphorus and rapidly available potassium. Except for urease, the soil enzyme activities increased overall. The Alpha diversity increased, and soil microbial structure optimized after rotation. ST mode was the most effective remediation mode, which not only reduces the content of Cd and As in the soil, but also effectively regulates the soil micro-ecology. The results from this study have shown that it is feasible to apply *Sedum alfredii* Hance and the high-biomass rotation method for the remediation of Cd and As co-contaminated soil.

Keywords: Cd and As co-contaminated soil; *Sedum alfredii* Hance; high-biomass crop; rotation mode; remediation effect

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1. Introduction

Accumulation of heavy metals has become one of the serious issues in soil contamination. Except for those naturally originating in the parent material of soil, contamination sources in soil are related to human activities [1]. With the continuous development of the country's industry and agriculture, heavy metal pollution is inevitably becoming increasingly serious. Heavy metals not only affect soil health, inhibiting the growth and development of crops, thus reducing their yield and quality, but also resulting in adverse effects on people's health through accumulation in the body [2,3]. Therefore, restoration of cultivated land contaminated by heavy metals is a necessity, and a challenging task that requires technology and effort.

Due to problems such as high cost or easy to create secondary pollution, phytoremediation is one of the best choices for farmland restoration [4]. Unfortunately, this technology also has some disadvantages. Studies have shown that that continuously growing a single crop for a long term may lead to various problems such as nutritional imbalance or root disease. In addition, hyperaccumulators usually have the disadvantage of low biomass and therefore the remediation efficiency is not high. Considering the defect of single hyperaccumulator continuous remediation, hyperaccumulators co-cropping with high biomass crops is worth trying. Through the rational use of time, rotation effectively reduces the allelopathic effects and autotoxicity of plants and avoids causing continuous cropping obstacles [5–7]. Since high biomass crops have the advantages of vigorous growth and easy cultivation, the rotation of such crops with hyperaccumulators not only fully exploit the contribution of crops to the removal efficiency and improve the overall remediation effect, but also increase the economics of rotation remediation methods to facilitate the large-scale application. The harvested crops can be resourcefully disposed, such as resources utilized for making biochar, bioethanol, plates and other resources, or by planting crops such as hemp that do not enter the food chain but have other economic value, making it possible to generate economic benefits and achieve a win-win outcome.

Several earlier studies on the combination use of hyperaccumulator and crops for phytoremediation have been reported, but more attention had been paid to the remediation efficiency of hyperaccumulators and heavy metal accumulation in crops. Rotation was reported to have positive effects on soil microorganisms, especially the beneficial microbial communities such as the nitrogen-fixing bacteria, and promoting more antagonistic microbial abundance [8–10]. In the study of Gao, plant diversity mitigated the toxicity of heavy metals to the microbial community, thus allowing the specific enzymatic activities [11]. Since soil microorganisms are sensitive to environmental changes, soil microbial flora and enzyme activities, such as soil biological indicators, are not only determinants of soil health, but they also predict changes in soil quality earlier. Variations in soil physicochemical properties and nutrients indicate the effects of land use on soil and whether or not the nutrients are utilized well [12]. Therefore, the dynamics of the soil environment reflect to a certain extent the remediation effect of soil heavy metal contamination.

Tang reported that *S. alfredii* rotated with water spinach and autumn cucumber decreased soil pH by *S. alfredii* rhizosphere secretion, thereby inhibiting fungal growth, and effectively remediated soil heavy metal contamination, increasing the soil enzymes activity while alleviating *Fusarium* wilt of autumn cucumber [13]. Fumagalli reported that the rotation of white lupin with industrial hemp had increased both active microorganisms and heavy metal bio-effectiveness in the soil, which was associated with metabolites secreted into the soil by lupine roots. In addition, rotation can enhance the uptake of nutrients and the use of environmental resources by crops and improve soil fertility [14]. Saad studied a rotation mode of nickel-hyperaccumulator *Alyssum murale* (*A. murale*) and a legume called *Vicia sativa* through pot experiments, and *Vicia sativa* increased the fixation of nitrogen in the soil, thus promoting the nutrient utilization and uptake by *A. murale*, which exhibited faster growth. The soil organic matter content, arylsulfatase and urease activities, and relative abundance of Phylum *Chloroflexi* increased, which are all favorable elements for plant growth and promoted plant extraction indirectly [15]. Another study by Saad also showed an increased Ni yield of *A. murale* root in rotation with leguminous crops [16]. The results of all these studies suggest that rotation remediation has a positive effect on soil factor changes, and these factor changes may strongly relate to soil heavy metal remediation. However, the studies mainly focused on the remediation effect and observed changes in some soil factors, but had not observed the changes in the overall soil environment. Therefore, it is necessary to study the overall changes in the soil environment along with the heavy metal contamination remediation.

Sedum alfredii Hance (*S. alfredii*), a typical Cd and zinc (Zn) hyperaccumulator which can absorb amounts of Cd, Zn and lead in the aboveground part of the plant and effectively reduce the pollution of heavy metals in the soil, was originally found in an ancient lead-Zn deposit in eastern China [17]. It is reported that *S. alfredii* can accumulate more than 2000 mg/kg Zn in shoots as well as uptake up to 1400 mg/kg Cd under field condition [18,19]. Over the past years, *S. alfredii* has been one of the most widely used plants for phytoremediation. Different from recent studies that only focus on the remediation efficiency, in this study, *S. alfredii* was selected to be rotated with high biomass crops, not only to observe the effect of these rotation modes on soil heavy metal remediation, but also to determine the changes in soil microecology after rotation, therefore investigating the effects of rotation remediation on soil environment overall.

2. Materials and Methods

2.1. Experimental Site

An experiment was conducted in a mild-moderate mixed cadmium (Cd) and mild arsenic (As) co-contaminated filed located in Huanggu Village, Liling, Hunan, China (27°58′ N, 113°22′ E). Before the experiment, unplanted soil was collected as the control group (CK). A five-point sampling method was used to collect fresh soil from a 0–20 cm depth of surface layer in the field, with about 1.5 kg weight of each sample.

2.2. Experimental Design

Cd hyperaccumulator ecotype (HE) *Sedum alfredii* Hance and different kinds of crops were selected to design five treatments, as shown in Figure 1. As for the selection of plants, there are the main principles as follows: *Oryza sativa* L. (paddy), *Sorghum bicolor* (L.) Moench (sorghum) and *Zea mays* L. (maize) are typical food crops with high biomass; *Hibiscus cannabinus* L. (kenaf) is not only an excellent fiber crop, but also has the potential to remove heavy metals [20]; *Trichosanthes kirilowii* Maxim. (*T. kirilowii*) is a kind of vine plant with high biomass. Its main production areas are often the locations where the concentrations of the heavy metals are seriously high [21]. Therefore, *T. kirilowii* is considered as a tolerant plant, but not much research has been conducted to understand why it is so tolerant to the heavy metals. In addition, these plants have high economic value and are easy for field management. Every treatment covered a cultivated area of 0.5 acres and was subject to conventional water management until harvest. The soil was plowed at the gap of the preceding plant harvest and succeeding plant cultivation. The fertilization rates for each treatment were 120 kg/acre of urea, 200 kg/acre of superphosphate, and 60 kg/acre of potassium chloride. Two-thirds of the urea was used as base fertilizer, one-third was used as top dressing, potassium chloride was divided equally for base fertilizer and top dressing, and superphosphate was used as a base fertilizer one time. The field experiment started in October 2017 and lasted for three years with three cycles of rotation, and soil samples were collected in mid-October 2020 after the rotation. Two groups were classified according to different plant types (Dicotyledonous and monocotyledonous), one of which was treated with Poaceae plants including SP, SS and SM, another group including SK and ST, and the unplanted soil was collected as CK treatment soil before the trial.

The collected soil samples were divided into two parts: one part was chilled on the ice immediately following collection in the field and transported in a cooler to the laboratory, then stored at −80 °C prior to DNA exaction; another was naturally air-dried, smashed with a hammer, and sifted through 10 or 100 mesh sieves according to the subsequent analysis requirements.

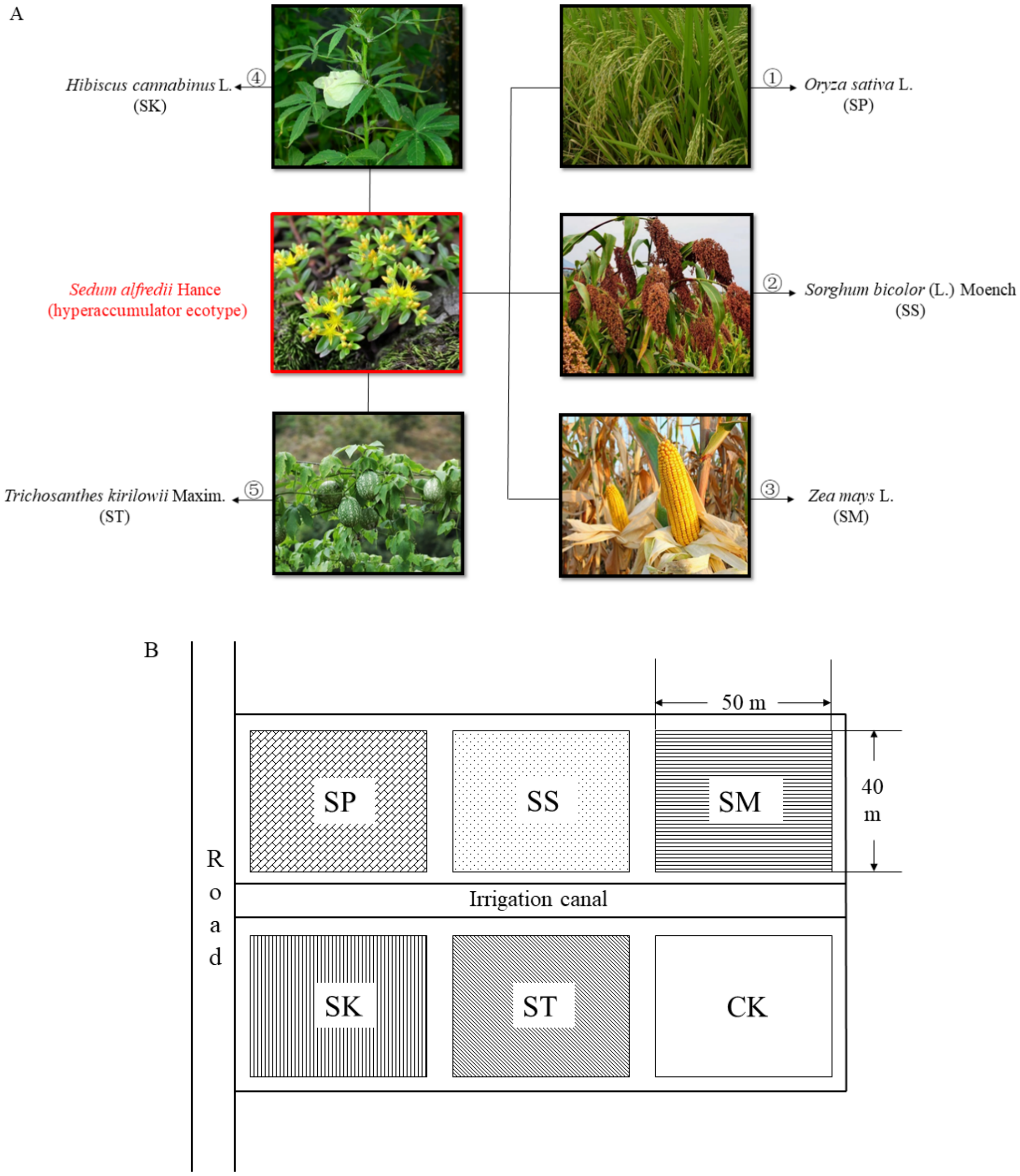

Figure 1. Experimental design for (**A**) crop selection and (**B**) site setup.

2.3. Soil Properties Analysis

Soil pH, organic matter content (OM), cation exchange capacity (CEC), and nutrient content including alkali-hydrolysable nitrogen (AN), available phosphorus (AP), and rapidly available potassium (AK) were measured. Soil pH was determined with a pH meter (PHS-3C, Leici, Shanghai, China) in a 1:2.5 soil:water ratio after stirring vigorously for 0.5 h. OM was determined by colorimetry after the soil samples were oxidized by potassium dichromate hydration heat [22]. CEC was assessed by the NH_4OAc extraction method and washed off with 95% ethanol [23]. The alkali-diffusion method was used for sample treatment and determined the released NH_3 to calculate the content of AN [24]. AP was determined by the molybdenum-antimony anti-spectrophotometric method after

HCl-NH_4F extraction; AK was extracted with NH_4OAc solution and determined on a flame atomic absorption spectrometer [25].

Mixed solution (concentrated hydrochloric acid (HCl) and concentrated nitric acid (HNO_3) in a volume ratio of 3:1) and concentrated perchloric acid ($HClO_4$) were used for soil digestion to analyze total Cadmium (TCd) and total Arsenic (TAs) content. Four metal fractions including acid-extractable Cadmium/Arsenic (EX-Cd/As), reducible Cadmium/Arsenic (RD-Cd/As), oxidizable Cadmium/Arsenic (OX-Cd/As) and residual Cadmium/Arsenic (RS-Cd/As) fractions were selected and estimated by BCR procedure [26] to clarify the changes of Cd and As mobility and the conversion between the forms.

The activities of four enzymes were measured as follows: potassium permanganate titration for catalase, phenol-sodium hypochlorite colorimetry for urease, phosphophenyl disodium colorimetry for acid phosphatase, and 3,5-nitrosalicylic acid colorimetry for sucrase [27].

2.4. Soil Microbe Analysis

The FastDNA TM SPIN Kit was used for DNA extraction (MPBio, Santa Ana, CA, USA). Total DNA of the samples was extracted, purified, quantified and homogenized for construction of a sequencing library and used for polymerase chain reaction (PCR). Specific procedures for PCR were the same as used by Milani [28]. Based on the Illumina HiSeq sequencing platform, 16s rRNA (V3 + V4) was used as the sequenced region and paired-end sequencing (Paired-End) was used to construct a small fragment library for sequencing. A rarefaction curve to compare OTU richness among all soil samples was constructed. Mothur (Version 1.30, https://www.mothur.org/, 5 December 2021) and QIIME (version 1.8.0, https://www.qiime.org/, 5 December 2021) were used to analyze the α and β diversity indices, respectively. Principal coordinate analysis (PCoA) was performed by dimensionality reduction of the original data matrix using R (Version 4.0.2) to visualize the differences in the bacterial community among the different groups. A redundancy analysis (Canoco, version 5.0, http://www.canoco5.com/, 5 December 2021) was conducted to examine which environmental factors significantly affected microbial community structure. The whole analysis was delivered by Biomarker Biotechnology Co., Ltd., Beijing, China (http://www.biomarker.com.cn/, 30 October 2021).

2.5. Statistical Analysis

An atomic absorption spectrophotometer (Thermo Fisher ICE3500, MA, USA) was used to determine the concentration of Cd and As, and an ultraviolet spectrophotometer (Shimadzu UV-1700, Tokyo, Japan) was used for absorbance determination. Microsoft Excel 2013 was used for data statistics, SPSS 24.0 was used for Duncan's multiple range tests and variance analysis, Origin 2018 for drawing and a Pearson correlation coefficient was performed to evaluate the correlation between each factor. The data were presented as the mean ± standard deviation.

3. Results

3.1. Total Cd/As and Fraction Contents

The TCd content of each treatment is shown in Figure 2A. Compared with CK, all treatments except SK had significant changes ($p < 0.05$), and the TCd between treatments was significantly different. The order of the content is: CK > SK > SS > SP > SM > ST, and the decreases in TCd content were 6.3%, 20.3%, 25.1%, 34.5% and 74.3%, respectively. ST treatment has the greatest remediation effect, and the SK treatment has the smallest remediation effect. The influence of each treatment on the Cd fraction and content is shown in Figure 2B and Table 1. The content of EX-Cd, RD-Cd and OX-Cd in the soil were decreased in all the treatment soils, which indicates the significant effect of plant extraction. It can be seen from the figure that the fraction of EX-Cd and RD-Cd in each treatment except SS was significantly higher than that of the other two fractions, indicating that Cd in the soil still exists in a bioavailable fraction. Compared with CK, the soil RS-Cd fraction increased significantly with SS treatment, reaching 39.4%. It is worth noting that although the TCd content of the SM treatment is lower than that of other treatments, its EX-Cd fraction was as high as 62.6%, indicating that this treatment has a high activation efficiency for soil Cd.

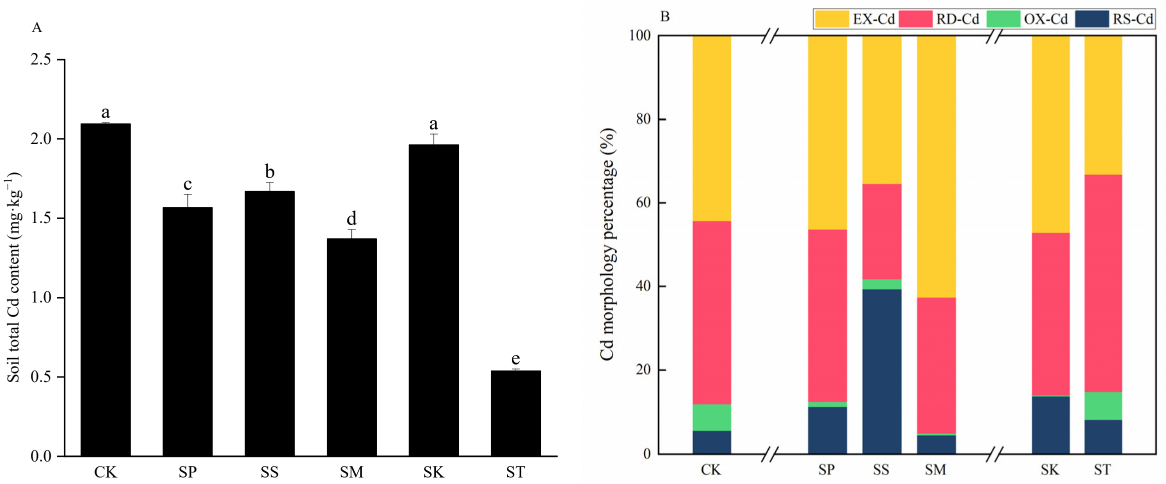

Figure 2. Total (**A**) Cd content and (**B**) Cd morphology fraction in soils under different rotation modes (means ± standard errors). Different letters over the error bars indicate significant differences ($p < 0.05$).

Table 1. Soil EX-Cd/As, RD-Cd/As, OX-Cd/As, RS-Cd/As content of different rotation modes.

Treatments	CK	SP	SS	SM	SK	ST
EX-Cd	0.929	0.727	0.592	0.797	0.925	0.179
RD-Cd	0.919	0.647	0.381	0.413	0.763	0.280
OX-Cd	0.130	0.020	0.039	0.005	0.006	0.036
RS-Cd	0.117	0.175	0.657	0.057	0.269	0.044
EX-As	0.130	0.373	0.101	0.098	0.075	0.038
RD-As	1.098	0.727	0.808	0.764	0.773	0.164
OX-As	0.667	0.458	2.850	0.529	0.579	0.257
RS-As	27.916	15.461	20.163	16.605	16.555	15.740

As shown in Figure 3A, TAs content in the soil decreased significantly after remediation, and the decrease percentage was 45.7%, 42.9%, 39.7%, 39.7% and 19.8%, respectively, with the order of ST > SP > SK > SM > SS > CK. Except for the SS treatment, the difference in the soil TAs level between the treatments was not significant. The ST treatment had the most obvious removal efficiency with a 45.7% reduction of TAs, and the SS treatment had the smallest remediation effect with 19.8% reduction of TAs, which was clearly lower than the other treatments. The influence of each treatment on the As fraction and content are shown in Figure 3B and Table 1. The RD-As and RS-As content in the soil were significantly decreased after rotation, particularly the RD-As content in SP treatment soil, which dropped by nearly half. Except for the SS treatment, the changes of As fraction in each treatment was not significant, and the fraction of RS-As was high on the whole, reaching a level of more than 80%, which is probably because most of the activated As has been extracted by the plant. Compared with other treatments, the fraction of RS-As in the SS treatment was as low as 84.2%, and the TAs of this treatment was relatively high, which might be due to a part of the As in the soil being activated but not extracted yet; the RS-As content in the ST treatment accounts for the increased fraction, which might be because most of the available As had been extracted by the plants.

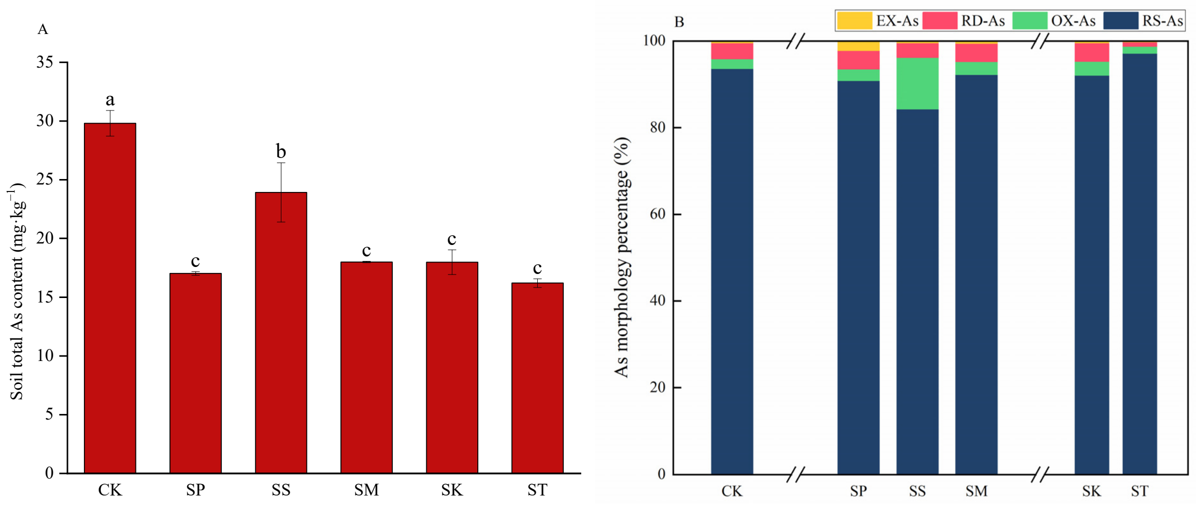

Figure 3. Total (**A**) As content and (**B**) As morphology fraction in soils under different rotation modes (means ± standard errors). Different letters over the error bars indicate significant differences ($p < 0.05$).

3.2. Soil Physicochemical Characteristics

The effects of different rotation modes on soil physicochemical properties are shown in Table 2. Except the pH of the SP treatment, which increased to 6.14, the pH of all other treatments decreased compared to CK. The pH of the SK treatment significantly decreased to 4.98, the lowest pH among the treatments. The soil OM content showed an upward trend, the SP, SS, and SM treatments increased by 47.4%, 52.9%, and 62.1%, respectively, and the SK and ST treatments increased by 72.9% and 82.3%, respectively. The increase of OM content in the Poaceae treatment group (SP, SS and SM) was lower than that of another treatment group (SK and ST). The soil CEC showed different degrees of decline, among which the SS and SM treatments decreased significantly, reaching a drop rate of 17.1% and 17.2%, respectively. Compared with other treatments, the CEC and OM content of the ST treatment were the highest, showing a great soil fertility status.

Table 2. Physicochemical properties of the soil samples.

Treatment	**Soil Properties**		
	pH	**OM (mg/kg)**	**CEC (cmol$^+$/kg)**
CK	5.97 ± 0.01 b	18.17 ± 0.06 e	12.05 ± 0.35 a
SP	6.14 ± 0.05 a	26.78 ± 0.61 d	11.46 ± 0.27 b
SS	5.44 ± 0.12 c	27.79 ± 1.41 cd	9.99 ± 0.31 c
SM	5.84 ± 0.13 b	29.46 ± 0.16 bc	9.98 ± 0.23 c
SK	4.98 ± 0.12 d	31.42 ± 2.19 ab	10.35 ± 0.23 c
ST	5.85 ± 0.04 b	33.12 ± 1.62 a	11.55 ± 0.05 b

Data representation means ± SE ($n = 3$). Different letters indicate significant differences among treatments (Duncan's multiple range test, $p < 0.05$).

3.3. Soil Nutrient Content

The effect of different rotation modes on soil nutrient content is shown in Figure 4. The soil nutrients after rotation had changed significantly ($p < 0.05$). The soil AN content in each treatment was significantly increased, among which SP, SS and SM treatment increased by 27.3%, 16.4%, 49.1%, respectively, and SK and ST treatment increased by 50.9%, and 9.1%, respectively. Except for a slight increase in the SK treatment, the AP and AK content in other treatments declined, especially in the SP treatment, where they decreased the most significantly (with a reduction by 32.14% and 16.51%, respectively); this indicates that rotation had overall positive effects on the conversion and utilization of soil nutrients.

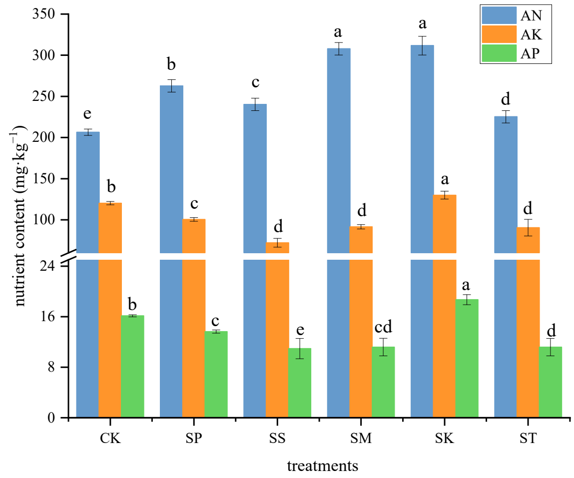

Figure 4. Soil available nutrient content under different rotation modes. Different letters over the error bars indicate significant differences ($p < 0.05$).

3.4. Soil Enzyme Activities

The effect of different rotation modes on soil enzyme activities is shown in Figure 5. Soil catalase activity decreased slightly in the SS and SK treatments, but increased slightly in other treatments; the urease activity showed a slight downward trend as a whole, while the acid phosphatase was the opposite; the sucrase activity decreased significantly in the SS treatment but increased significantly in the SM and ST treatment. The SS treatment had much lower enzyme activities than other treatments. Its activities of catalase, urease, acid phosphatase and sucrase decreased by 9.2%, 25%, and 31.4%, respectively.

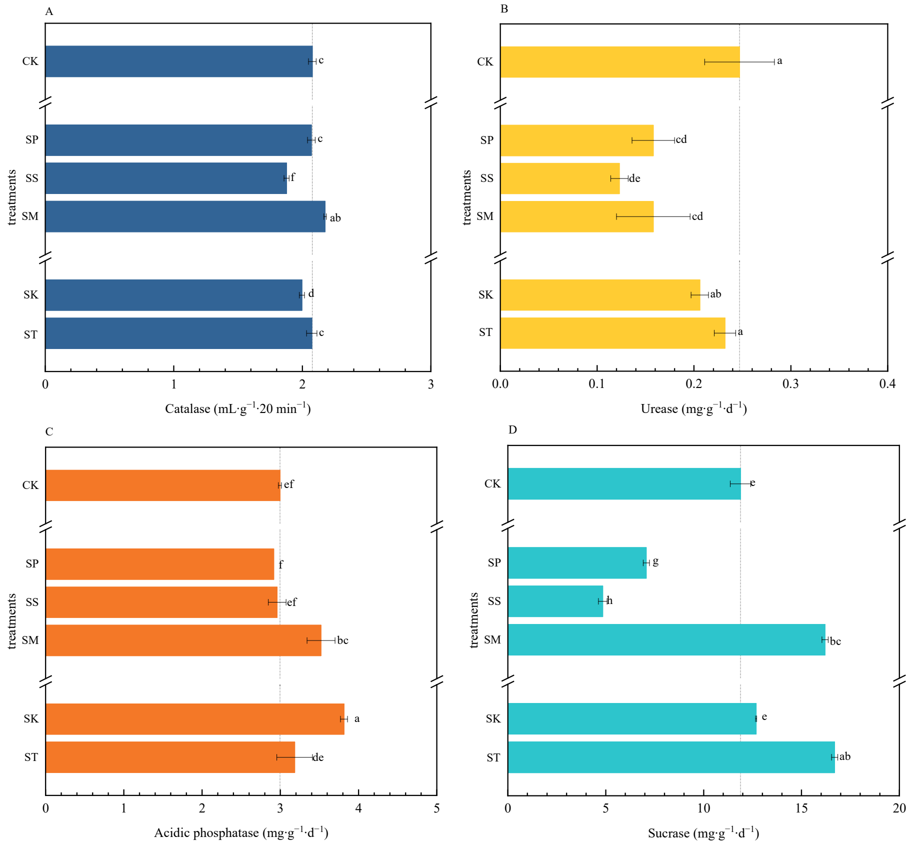

Figure 5. Effects of rotation modes on activities of soil catalase (**A**), urease (**B**), acidic phosphatase (**C**), sucrase (**D**). Different letters over the error bars indicate significant differences ($p < 0.05$).

3.5. Soil Bacterial Properties

3.5.1. Microbial Community Composition

The 16S rRNA amplicons were taxonomically classified to different levels, resulting in 30 phyla, 84 classes and 165 orders, respectively. As shown in Figure 6A, the top 10 most abundant phyla in each soil sample were selected. Phylum *Proteobacteria* (24.91–34.22%) accounted for the highest abundance, followed by phylum *Acidobacteria* (17.92–22.59%) and *Chloroflexi* (10.48–22.17%). The remaining dominant phyla are listed as *Gemmatimonadetes* (5.67–14.21%), *Actinobacteria* (2.77–12.00%), and *Nitrospirae* (0.93–7.68%), respectively. There was a significant difference between different treatments (Figure 6C). From the perspective of overall changes after treatment, comparison revealed that the relative abundance of *Acidobacteria* and *Rokubacteria* decreased, while *Gemmatimonadetes* increased. In addition, the abundance of many bacteria in soil was also significantly different between Poaceae-treated ones and the others. For example, the abundance of *Chloroflexi*, *Nitrospirae* and *Firmicutes* in the soil of Poaceae treatments was significantly higher than that of the other treatments, while the abundance of *Proteobacteria*, *Gemmatimonadetes* and *Actinobacteria* was just the opposite.

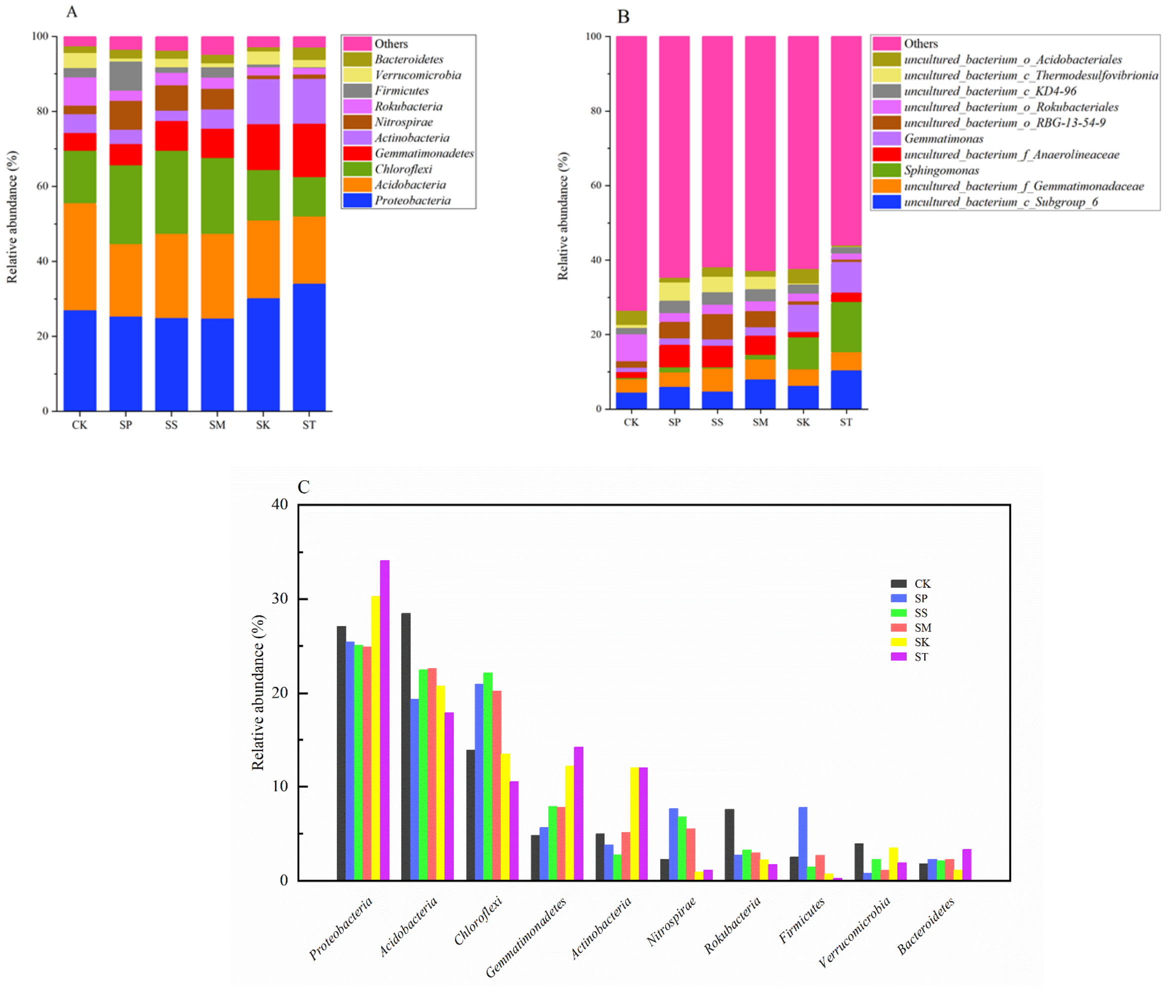

Figure 6. Relative abundance of predominant phyla (**A**) and genera (**B**), phyla with significant differences (**C**) among different treatments.

Gemmatimonas, *uncultured_bacterium_c_Subgroup_6*, *Sphingomonas*, and *uncultured_bacterium_f_Anaerolineaceae* were dominant at the genera level (Figure 6B). It was found that the abundance of *Sphingomonas* in SK and ST treatments was at the forefront reaching more than 10%, while the three Poaceae-treated soils was hardly observed.

3.5.2. Diversity of Bacterial Community

The effect of different rotation modes on soil microbial diversity is shown in Table 3. From the table, it can be seen that the Chao1 and ACE indexes increased after the rotation treatments, indicating that the treatments were beneficial to increase the abundance of soil microbial communities. The Simpson index of the Poaceae treatment group was significantly higher than another group, while the Shannon index was also significantly lower, indicating that the Poaceae treatment had a more even distribution and higher diversity of soil microbial communities than other treatments. It is worth noting that the diversity of the SK treatment was slightly lower than that of the CK. It might be due to its high content of soil heavy metals, which had an adverse impact on the microbial community.

Table 3. The Alpha diversity indices of microbes in the soil samples.

Treatment	Richness Estimators		Evenness Indices		Coverage
	ACE	Chao1	Simpson	Shannon	
CK	1416.14 ± 9.96 d	1447.95 ± 7.58 d	0.0081 ± 0.0014 b	5.8040 ± 0.11 b	1.0
SP	1691.82 ± 14.96 a	1693.15 ± 14.33 a	0.0036 ± 0.0004 e	6.4780 ± 0.04 a	1.0
SS	1531.99 ± 16.41 bc	1565.51 ± 16.43 bc	0.0043 ± 0.0003 d	6.2668 ± 0.05 a	1.0
SM	1663.51 ± 2.91 a	1674.78 ± 7.56 a	0.0037 ± 0.0002 e	6.4557 ± 0.01 a	1.0
SK	1550.34 ± 33.46 b	1574.52 ± 42.2 b	0.0079 ± 0.0020 c	5.9639 ± 0.12 b	1.0
ST	1466.50 ± 31.66 cd	1499.36 ± 29.5 cd	0.0141 ± 0.0047 a	5.7576 ± 0.16 b	1.0

Data representation means ± SE (n = 3). Different letters indicate significant differences among treatments (Duncan's multiple range test, $p < 0.05$).

3.5.3. Relationship between Microbial Properties and Environmental Variables

Soil bacterial community structural changes after plant cultivation were clearly observed on principal coordinate analysis (PCoA). As shown in Figure 7, the explanation variances of the first principal component (PC1) and the second principal component (PC2) were 44.62% and 23.45%, respectively. The treatments with Poaceae plants were grouped together at PC1, while the other two treatments were biased towards the positive axis. The position of all soil samples after planting was shifting towards the positive axis of PC2 in comparison to CK, indicating that the microbial community structure had been enhanced from phytoremediation.

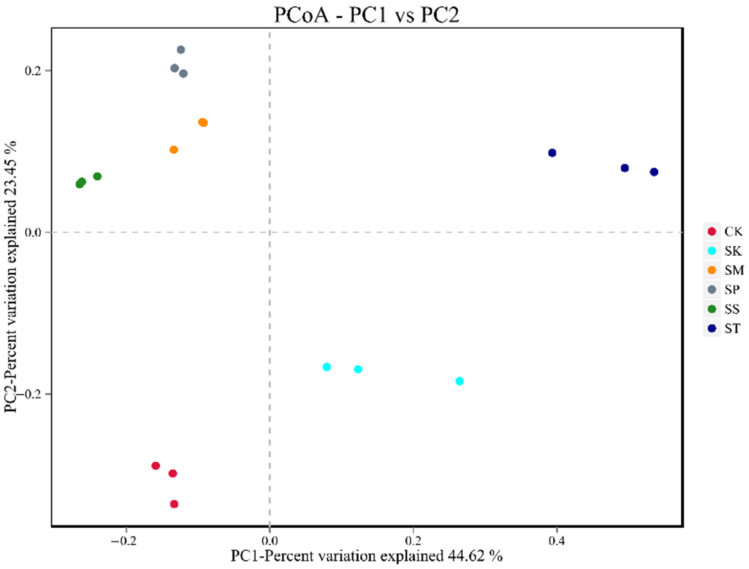

Figure 7. Principal coordinate analysis of soil bacterial communities based on Bray-Curtis distance. Soil samples are colored by different treatments. The percent variation explained by each private channel is indicated on the axis.

A redundancy analysis (RDA) showed that there was a strong correlation between the microbial community structure and the environmental variables. As shown in Figure 8, soil pH, CEC and soil nutrient content were distributed on the two sides of two axis, showing a significant negative correlation between them. The arrow lengths of urease, sucrase, and OM are relatively long, indicating that these environmental variables had a significant impact on the soil microbial community. The arrow angles of *Acidobacteria* and EX-Cd, *Nitrospirae* and CEC, *Firmicutes* and pH, *Proteobacteria* and sucrase were very small, indicating a significant positive correlation between them. Poaceae treatments tended to be aggregated positively along axis 1, and other treatments gathered towards a negative direction along axis 1. In addition, only CK was significantly biased towards the positive orientation of axis 2.

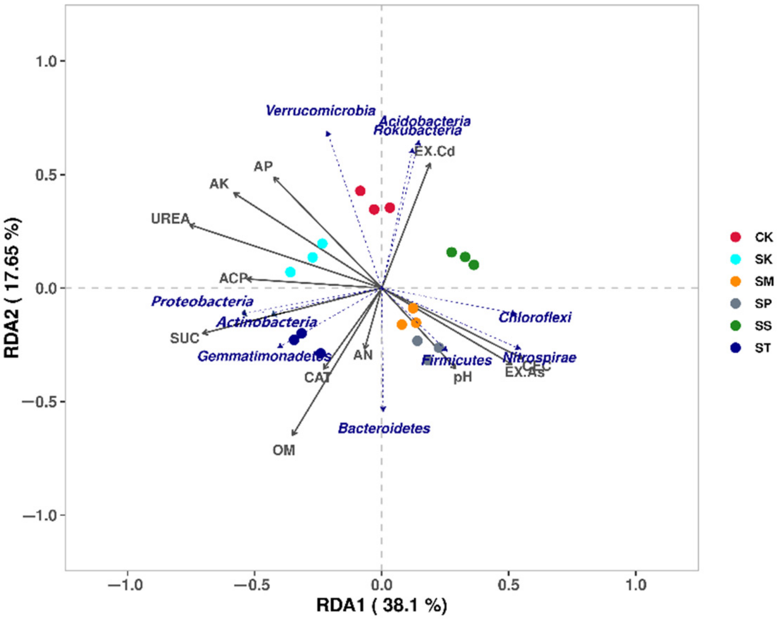

Figure 8. Redundancy analysis of the relationships between dominant phyla, different treatments and environment variables (soil properties, soil nutrient content and EX-Cd/As).

4. Discussion

4.1. *Effect of Rotation Modes on Soil Cd/As Content*

Changes in soil physiochemical properties directly affect physical and chemical reactions of soil heavy metals [29]. For example, pH directly affects the adsorption and analysis of soil heavy metal ions or precipitation and dissolution. With the exception of SP treatment, soil pH of all treatments decreased in the present study, which was contrary to the previous finding that observed an increase in soil pH after rotation [30]. The decrease of soil pH may be due to some special exudates secreted by the root and rhizosphere microorganisms of plants, including organic acids, amino acids, sugars, and some allelopathy exudates [31]. The decrease in pH increased the bioavailability of Cd and enhanced phytoextraction, thus the EX-Cd content in all treatments was substantially reduced in comparison to CK.

As the soil pH decreased in treatments, the soil CEC showed a similar variation trend. Root exudates regulate the heavy metal stress through a reduction the soil medium pH and the disrupted equilibrium of desorption-adsorption between heavy metals and soil colloid [32], and the soil cation exchange capacity is related to the change of the adsorption site of the soil colloid. Therefore, the change in the pH of the rhizosphere soil reduces the cation exchange capacity, which is consistent with the decrease in soil CEC in the present study. The decrease of CEC led to some heavy metal ions adsorbed by the soil colloid being released into the soil solution, increasing their mobility and making it easier to be extracted by plants, therefore promoting the decrease of soil EX-Cd content.

The soil OM content of each treatment increased significantly compared to CK, which might be related to the artificial fertilization management and plant nutrient utilization that changed the soil fertility. In general, organic matter affects the presence of heavy metals in soil, because the humic acid and organic ligands of organic matter can undergo complex reactions with heavy metals to form more stable substances, and the organically bound substances cause a reduced bioavailability of heavy metals in the soil. In this study, except for RS-Cd, the soil Cd fraction content and the soil organic matter content showed a significant negative correlation. The pH level and OM content of the ST treatment were high, and the significant changes in the fraction of soil EX-Cd and RD-Cd in this treatment also showed that in addition to being extracted by plants, soil Cd also occurred to stabilize transformation under the influence of high OM content. However, different from TCd, which has different decreasing amplitudes, there is no significant difference between the TAs content of each treatment except for the SS treatment. This might be attributed to the fact that most of the activated soil As has already been extracted.

4.2. Effect of Rotation Modes on Soil Microecology

The carbon and nitrogen source of soil microorganisms mainly derive from the soil nutrients. Microorganisms absorb the nutrients in the fertilizer and turn them into organic substances, which can reduce the nutrient loss caused by leaching and volatilization. Generally speaking, the relationship between the change of AN and OM is of the most significant, because the content of AN in soils with rich OM and high ripening degree is higher [24], as can be seen in this study, where AN and OM showed a similar trend of change. The contents of AP and AK decreased in some of the treatments, which might be due to the absorption and utilization capacity of different plants. However, although the soil AN content increased in the present study, the soil urease activity decreased slightly. Saad observed the same results in his study, speculating that mineral fertilization had a negative effect on urease because the high input of organic and mineral nitrogen in the soil could reduce microbial community activity [15]. In addition, Ajwa observed a decrease in soil urease activity with the importation of nitrogen fertilizer [33]. Therefore, a similar phenomenon occurred in the present study.

Different from nutrients that are utilized in the soil biological activities, soil enzymes are a group of catalysts secreted by living animals, plants and microorganisms, released into the soil by decomposition of animal and plant residues or remains. In general, microorganisms are responsible for the mineralization of organic matter and the fixation of nutrients such as nitrogen, while enzymes are responsible for the mineralization of organic fertilizers into available nutrients, and the two constitute the main framework for soil nutrient cycling [34,35]. Therefore, soil enzyme activity is closely related to nutrient utilization and soil microbial bioactivity. As the soil AP and AK content decreased in the present study, the enzyme activities related to nutrient utilization decreased as well. The enzyme activity of the SS treatment was the lowest. It can be seen that the soil OM, available nutrients and Alpha diversity of this treatment were relatively low, while the contents of TCd and TAs in the soil remain high, indicating that the remediation effect of this treatment was not significant, and the toxic effects of heavy metals on microecology remain high, resulting in poorer microecological health relative to other treatments.

It is common that the root exudates of plants after planting contain some nutrients that are often needed for soil microbial activities and can promote the excretion or decomposition of soil microorganisms to increase, thus promoting the increase of soil available nutrients. Therefore, the Alpha diversity of each treatment increased significantly. In this study, the Shannon index of the SK treatment with the highest EX-Cd content was relatively low, indicating that high Cd content had an inhibitory effect on microbial activity, which was similar to the previous study [36]. However, even the TCd content of ST treatment had a significant decrease, while the Alpha diversity of this treatment was relatively low and similar to the previous research results of Hussian J., who speculated that this could be caused by the antibacterial effect of garlic in the rhizosphere [37]. Because of its medicinal value, it can be inferred that *T. kirilowii* has similar antibacterial properties [38].

Bacteria is the main component of microorganisms, and its community changes and structural characteristics reflect the dynamics of the soil microbial environment to a certain extent. As the main participants in the utilization of soil nutrients, various bacterial populations bear different responsibilities and functions. Since the soil microbial community is be affected by plant species, the microbial populations in the rhizosphere of different plants are different, and thus plant combinations can make the soil nutrient to be more efficiently utilized by the microbes [39]. Since *Acidobacteria* is a phylum that prefers an acidic environment, it can be seen that the abundance of *Acidobacteria* was relatively high in the SS, SM and SK treatment, which had a decrease in pH value. However, the abundance of *Acidobacteria* was decreased significantly in all the treatment soils, and this result was consistent with the previous study that showed that the abundance of *Acidobacteria* was much higher in continuous cropping soil in comparison to rotation [40]. Moreover, *Acidobacteria* was found to be soil oligotrophic and sensitive to heavy metals [41,42]. Luo observed that the abundance of *Acidobacteria* increased with the increase of Cd content in the range of moderate pollution (0.3–3.0 mg/kg) [43]. Therefore, the decrease in Cd content might have had a greater effect than the decrease in pH on *Acidobacteria*, making the abundance of *Acidobacteria* decrease overall. *Rokubacteria* and *Acidobacteria* were found to have the ability to produce natural products that are antibiotic and fungicidal, so they are considered as the beneficial bacteria [44]. In the RDA diagram, soil EX-Cd and abundance of *Acidobacteria* and *Rokubacteria* had a significant and negative correlation with soil OM and AN, and the significant increase of soil OM and AN indicated that treatments decreased EX-Cd through an increase of the soil OM and AN, thus decreasing the high Cd bioavailability stress and the abundance of Cd sensitive phylum *Acidobacteria* and *Rokubacteria*. This phenomenon was the most significant in the ST treatment.

The genus *Sphingomonas* is a type of gram-negative bacterium, a special microorganism that is commonly used as a biological control agent [45,46]. It has been proven to lessen H_2O_2 content via enhanced GSH concentration and relevant gene expression, thus improving Cd tolerance [47,48]. Therefore, it is considered as a genus that can greatly improve the efficiency of phytoremediation. In addition, Luo reported that endophytes of *S. alfredii* could transmit from shoot to root and soil, and their abundance was closely related to the increase of branch biomass and heavy metal accumulation, and most of these families from phylum *Actinobacteria* that was found to be very abundant in heavy metal contaminated soils [49,50]. The abundance of the genus *Sphingomonas* and phylum *Actinobacteria* were both highly observed in the SK and ST treatment, although their soil TCd content was quite different. However, the abundance of genus *Sphingomonas* and phylum *Actinobacteria* was low in the SP, SS and SM treatments. In the study of Baderna, the toxicity of mixed heavy metal pollution to monocotyledonous and dicotyledonous plants was different [51]. Therefore, it can be speculated that mixture contamination contributed various toxicity levels to different plants in this study, thus making the soil microbial community colonization of two root types highly different.

4.3. Cost-Benefit Analysis

Conventional crop cultivation costs include seeds and field management (water, fertilizer, pesticide and labor costs), and the field management process was consistent for the five treatments in the present study, with total expenditures roughly around 800 CNY per acre (*S. alfredii* + the crop). Since the yield of *S. alfredii* was basically the same among treatments, the difference in economic benefits between treatments was mainly due to the different species of high biomass crops. The estimated economic benefits of each treatment are shown in Table 4. The resourceful disposal of harvested crop residues mainly include three methods: making biomass fuel, biochar and bioethanol. Crop residues for biomass fuel application do not require in-depth treatment and therefore have lower and similar market prices but high market demand, while the other two applications have more complex pretreatment processes and relatively low market demand but higher market prices. It can be seen from the table that *T. kirilowii* is high-biomass and has high dry weight yield per acre, and the economic benefits of ST treatment are considerable. The yield of sorghum is relatively high and the economic benefits of SS treatment are second to that of the ST. In contrast, the economic benefits of SK treatment are lower due to the lower yield of kenaf relative to the other crops. Therefore, as far as the overall comparison is concerned, with the higher yield of crops, the higher the economic benefits.

Table 4. The benefit analysis of five treatments.

Treatment	Dry Weight (kg/arce)	Economic Benefit (CNY/arce)		
		Biomass Fuel	Biochar	Biothanol
SP	925.3	1388.0	2705.9	3198.4
SS	1323.5	1984.5	3937.9	3246.9
SM	830.1	1245.2	2193.6	1819.4
SK	717.7	1076.6	2111.3	1746.4
ST	1561.8	2342.7	4326.3	4348.0

Economic benefit = Dry weight × Utilization rate × Unit price. Dry weight including the weights of *S. alfredii* and the crop.

5. Conclusions

The results of a field experiment and soil index analysis showed that the decreasing rate of soil total Cd of each mode was 74.3%, 34.5%, 25.1%, 20.3% and 6.3%, in the order of ST > SM > SP > SS > SK, respectively. The decreasing rate of soil total As was 45.7%, 42.9%, 39.7%, 39.7% and 19.8%, in the order of ST > SP > SK > SK > SS, respectively. Soil pH value and cation exchange capacity decreased slightly, while soil organic matter content increased significantly by 47.39–82.28%. The content of soil alkali-hydrolysable nitrogen increased by 9.1–51.0%, and the content of available phosphorus and available potassium decreased by 16.5–4.01% and 15.5–32.2%, respectively. Except for a slight decrease in SS treatment, soil enzyme activities increased overall. The soil microbial community structure and the microbial diversity had significantly improved by the rotation treatments, and the abundance of the microbial population might have been affected by the plant species. The mode that *Sedum alfredii* Hance rotated with *Trichosanthes kirilowii* Maxim. (ST) has decreased the soil heavy metal content most significantly, and the soil indices were at high levels overall. All the rotation modes had reduced soil heavy metal content, regulated soil basic properties and improved the diversity of soil microorganisms overall, indicating that *Sedum alfredii* Hance rotated with high-biomass crops could effectively remediate the soil Cd and As co-contaminated soil, and significantly improve the soil micro-ecological environment. It is feasible to promote rotation remediation methods in practical applications.

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