Effects of Hyperaccumulator *Sedum Plumbizincicola* Intercropped With Maize and Castor on Soil Microbes and Enzyme Activities under Field Conditions

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Abstract. The effects of *Sedum plumbizincicola* intercropped with maize and castor on soil microbial populations and enzyme activities were investigated under field conditions. The quantity of bacteria found under maize/*Sedum plumbizincicola* intercrops were significantly reduced by 68.8% when the soil under *Sedum plumbizincicola* was assessed. The amount of fungi and actinomycetes significantly increased by 70.5% and 620.6%, respectively, for *Sedum plumbizincicola*, while they decreased by 23.2% and 10.7% for maize as compared to counts found in monoculture models. The urease activities of intercropped maize and *Sedum plumbizincicola* were significantly increased by 63.0% for *Sedum plumbizincicola*, but no significant change for other intercrop combinations was observed as compared to the enzyme activity of the monoculture. The activities of catalase under maize/*Sedum plumbizincicola* intercropping were significantly promoted by 16.7% and 28.8%, respectively, but no significant changes were seen under castor/*Sedum plumbizincicola* intercropping. The activities of invertase on sucrose were enhanced significantly with all intercropping patterns as compared to that observed in the monoculture models. Together, this demonstrates that intercropping patterns are important for improving microbial count and enzyme activity in soil.

1. Introduction
Cultivated land polluted by heavy metals in China spans nearly $2 \times 10^7$ hm$^2$, which accounts for about 20% of the total cultivated land available in the country [1]. At present, phytoremediation has been globally recognized as a green technology for remediation of heavy metal pollution in soil [2]; however, phytoremediation takes a long time to clean contaminated soil, during which agricultural production must be disrupted. These conditions are in conflict with China's land resource shortage, and often face challenges to being accepted and applied by farmers. Therefore, it is important to study alternative technologies involving safe agricultural production in heavy metal-contaminated soils [3].

Intercropping is one of the main practices of traditional agriculture in China [4]. Recently, it has been discovered as a new method to restore heavy metal-contaminated soils by selecting appropriate heavy metal enriched plants which then form a crop-hyperaccumulator intercropping system, allowing farmers to continue production while reclaiming contaminated soil. Studies have shown that hyperaccumulator
plants which are intercropped with non-enrichment plants are able to provide certain protection mechanisms for the non-enrichment plants [5, 6].

Several studies have proposed that intercropping can improve soil microbial communities, as well as boost enzyme activities in soil, when compared to monocropping [7-9]. For example, peanut/maize intercropping could promote the population of microorganisms associated with nitrogen fixation in the rhizospheric soil [10]. A two-year experiment was conducted to investigate the effect of a pepper-garlic intercropping system on the soil biology and nutrient profile. This study, performed by Khan [11], revealed that the concentration of soil bacteria and actinomycetes was respectively higher after one month of garlic intercropping as compared to when only pepper was grown. In contrast, the fungal population exhibited a diminishing trend, while soil enzyme activity (invertase, urease, and alkaline phosphatase) also showed dynamic changes after the intercropping of garlic. Intercropping of peanut with Atractylodes lancea effectively increased soil urease and invertase activities, and increased the gram-negative bacterial population by 31.2–79.9 % [12].

Based on the results of previous study, we hypothesized that intercropping would alter the soil microecology. Therefore, in this study, corn and castor were intercropped with the Cd hyperaccumulator Sedum plumbizincicola in order to study the changes in microbial count and enzyme activity in soil compared with monoculture and to provide the research basis for phytoremediation of heavy metal pollution by intercropping hyperaccumulator plants with agriculturally valuable crops.

2. Materials and methods

2.1. Study area
The experimental site was farmland polluted by heavy metals in Xinjing village, Jinding Town, Lanping County, Nujiang Prefecture, Yunnan Province. The soil pH was 6.42, and organic matter was 36.2 g · kg⁻¹. The total N, P and K was 1.31 g · kg⁻¹, 0.60 g · kg⁻¹ and 24.7 g · kg⁻¹ respectively. The alkali hydrolyzed N was 75.7 mg · kg⁻¹, the effective P was 71.9 mg · kg⁻¹, and the available K is 383 mg · kg⁻¹. The total Cd was 4.18 mg · kg⁻¹, exceeding the standard for soil pollution risk management and control of agricultural land (Trial) (GB 15618-2018, 2.00 mg · kg⁻¹).

2.2. Plant materials
Sedum plumbizincicola was provided by the Nanjing Soil Research Institute of Chinese Academy of Sciences. The castor and maize (Huidan 4) were purchased from the market.

2.3. Experimental design
Five treatments were set up in the experiment: corn monoculture, Sedum plumbizincicola monoculture, castor monoculture, corn intercropping with Sedum plumbizincicola, and castor intercropping with Sedum plumbizincicola. Each treatment was repeated in triplicate. 15 plots (4m × 5m) were used in total.

2.4. Soil samples
Soil samples were collected after the plants matured on October 13, 2018. Three soil samples were randomly collected from each plot, at a depth of 0-20 cm. After the soil samples were mixed, about 1 kg of fresh sample was taken to the laboratory in plastic bags. A subsample of this was taken while the soil was fresh to determine the microbial count. The rest of the soil samples were ground after air drying, and passed through 0.25 mm and 1 mm sieve holes before being stored in a cool, dry place until testing.

2.5. Biological properties of soil
The number of microorganisms was determined using the dilution plate method. Beef extract peptone agar medium was used for bacteria, Martins Bengal erythromycin medium was used for fungi, and modified Gaoshi No.1 medium was used for actinomycetes. The bacterial, fungal and actinomycete colonies on the medium were counted after 2, 3 and 5 days of incubation, respectively.
The activity of urease was determined by phenol-sodium hypochlorite colorimetry, and the activity of catalase in soil was determined by potassium permanganate titration, while the activity of invertase was measured by 3,5-dinitrosalicylic acid colorimetry.

2.6. Statistical analysis
The mean value, standard deviation, and t-tests were calculated using SPSS 21.0 data processing software. The significance was analyzed at the level of \( p < 0.05 \) and 0.01, respectively. The graphics in this paper are drawn by origin 9.0 software.

3. Results and discussions

3.1. Changes of bacterial quantity in soils from intercropping of different crops and Sedum plumbizincicola
Soil microbiology plays an important role in many ecological processes, such as nutrient cycling, organic matter decomposition, and soil structure formation; however, agricultural management techniques such as intercropping systems can change the soil microbiome [13]. Previous studies have shown that intercropping changes microbial community structures in soil in comparison with monocultured crops [14, 15]. In this study, the bacterial count in the soil of monoculture maize under field conditions was found to be \( 14.3 \times 10^9 \) CFU·g\(^{-1}\) dry soil. Maize/Sedum plumbizincicola intercropping did not change the number of bacteria in corn soil as compared to monoculture (\( p > 0.05 \)), but it significantly reduced bacteria quantity for Sedum plumbizincicola to 68.8% less than that seen with monoculture. In the castor/Sedum plumbizincicola intercropping treatment, the bacterial count in the intercropping soil was significantly less than that found in soil from the monoculture of Sedum plumbizincicola and castor (70.8% and 81.2% lower, respectively) (Figure 1).

![Figure 1. Bacterial quantity in soils from intercropping of different crops and Sedum plumbizincicola](image)

3.2. Changes of fungi quantity in soils from intercropping of different crops and Sedum plumbizincicola
Between the single cropping and intercropping conditions, the number of fungi in the corn-planted soil was not significantly different (\( 8.2 \times 10^5 \) CFU·g\(^{-1}\) soil and \( 6.3 \times 10^5 \) CFU·g\(^{-1}\) soil, respectively). Sedum plumbizincicola intercropping with corn significantly increased the amount of fungi in the soil (70.5% for Sedum plumbizincicola). Sedum plumbizincicola intercropping with castor significantly improved the amount of fungi in soil by 60.2% and 60.0%, respectively. The fungal count in the soil after intercropping with other plants, with the exception of corn, shows a significant increase (Figure 2). The number of fungi in the soil increased significantly after intercropping in all cases except for soil from maize (Figure 2).
3.3. Changes of actinomycetes quantity in soils from intercropping of different crops and *Sedum plumbizincicola*

Both in the single cropping and intercropping conditions, the *Actinomycetes* counts in the corn-planted soil were $5.6 \times 10^6$ CFU·g$^{-1}$ soil and $5.0 \times 10^6$ CFU·g$^{-1}$ soil, respectively, which were not significantly different ($p>0.05$). In *Sedum plumbizincicola* intercropping with maize, the number of actinomycetes in soil increased significantly for *Sedum plumbizincicola* to 6.2 times that of the monoculture. In *Sedum plumbizincicola* intercropping with castor, the number of actinomycetes in soil increased significantly as well, to 8.2 times higher than that of the monoculture, however the number of actinomycetes decreased significantly in comparison with that of monocultures for castor (from $6.5 \times 10^6$ CFU·g$^{-1}$ soil with monoculture to $4.0 \times 10^6$ CFU·g$^{-1}$ soil with intercropping). From this, it can be seen that the number of actinomycetes increased significantly ($p<0.01$) in the intercropping system for *Sedum plumbizincicola* (Figure 3).
3.4. Effects of crops intercropping with Sedum plumbizincicola on soil enzyme activities

The urease activity was significantly enhanced (63%) for Sedum plumbizincicola, but there was no significant change for maize under maize/Sedum plumbizincicola intercropping conditions when compared with the monoculture. In the intercropping of castor/Sedum plumbizincicola, there was no significant change of urease activity in the soil for either plant (Figure 4).

![Figure 4. Effects of crops intercropping with Sedum plumbizincicola on soil urease activities](image)

The activity of catalase had the similar trend for urease activity; it was significantly enhanced to 4.56 mL·g⁻¹ soil and 5.45 mL·g⁻¹ soil for maize and Sedum plumbizincicola, respectively, under intercropping conditions. In the intercropping of castor/Sedum plumbizincicola however, there was no significant change of catalase activity (Figure 5).

![Figure 5. Effects of crops intercropping with Sedum plumbizincicola on soil catalase activities](image)

The activity of invertase was significantly increased (by 18.13% and 28.23%, respectively) in the intercropping of maize/Sedum plumbizincicola, and augmented (by 37.1% and 26.1%, respectively) by the intercropping of castor/Sedum plumbizincicola (Figure 6).
3.5. The correlations between microbial quantities and enzyme activity in the soil of crops/ Sedum plumbizincicola intercropping

In maize monoculture, the correlation coefficient between fungi quantity and urease activity was significantly negatively correlated (-0.984), but the correlation coefficient between the number of bacteria and actinomycetes was significantly positive (0.932). In the intercropping of maize/Sedum plumbizincicola, there was a significant positive correlation between invertase, urease, and catalase activity with the fungal count in the maize soil. When Sedum plumbizincicola was planted in monoculture, there was a significant correlation between invertase and urease activity, and a significant positive correlation between fungal and actinomycete count. Castor monoculture had a significant correlation between invertase and urease activity, and fungal and actinomycete count. When the castor was intercropped with Sedum plumbizincicola, there was a significant linear correlation between the enzyme activity and microorganism count for the castor soil; at the same time, there was a significant positive correlation between the enzyme activities, though there was no significant linear correlation between microorganism counts.

The increase in the number of soil microorganisms indicates the formation of a more active microbial community, as well as the secretion of more enzymes into the soil [11]. This augmented enzyme activity could improve the soil nutrient status and provide nutrients for the growth of soil microorganisms, improving nutrient transformation during plant growth, and promoting the plant root system to secrete more enzymes. Therefore, there was a significant positive correlation between soil enzyme activity and microbial count in this study.

4. Conclusion

Here it was demonstrated that, compared with monoculture conditions, the intercropping of maize/Sedum plumbizincicola and castor/Sedum plumbizincicola significantly increased the number of actinomycetes and fungi in the soils but decreased the number of bacteria. There was also a significant increase in urease activity for Sedum plumbizincicola, along with an increase in the catalase activity in the soil for maize and Sedum plumbizincicola and an increase in the invertase activity in the soil for maize, castor, and Sedum. After the plants were intercropped, the changes of microbial counts and enzyme activities in soils observed provided a theoretical basis for explaining the mechanisms of plant remediation for heavy metal contaminated soils seen with intercropping.

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