Effects of Different Oilseed Sunflower Varieties on Rhizosphere Soil Enzyme Activity and Microbial Community Functional Diversity in Saline-Alkali Soil Postprint

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Abstract

Through pot experiments, the effects of four sunflower varieties (Xinkui 4, Xinkui 6, Xinkui 10, and American Dwarf) on soil physicochemical properties, enzyme activities, and microbial community functional diversity in saline-alkali soils were studied, aiming to screen for sunflower varieties more suitable for improving saline-alkali soil quality. The results showed that planting Xinkui 6 had the most significant effect on reducing rhizosphere soil pH and increasing total nitrogen content and sucrase activity in saline-alkali soils, while Xinkui 4 had the most significant effect on increasing the contents of alkali-hydrolyzable nitrogen, available phosphorus, and available potassium, as well as urease and phosphatase activities in rhizosphere soil; planting these four sunflower varieties all significantly (72 h, $P < 0.05$) increased the average well color development (AWCD) of rhizosphere soil microorganisms in saline-alkali soils for 31 carbon sources, following the pattern: Xinkui 4 > Xinkui 6 > American Dwarf > Xinkui 10 > CK. Planting these four sunflower varieties all increased the Shannon diversity index (H), Shannon dominance index (D), and carbon source utilization richness index (S) of rhizosphere soil microbial communities in saline-alkali soils to varying degrees, showing a similar pattern: the microbial diversity index was highest in Xinkui 4 rhizosphere soil and lowest in CK, and significantly higher than CK. Principal component analysis indicated that planting sunflowers improved the community composition of rhizosphere soil microorganisms in saline-alkali soils; carbohydrates, amino acids, carboxylic acid compounds, and polymers were the main carbon sources utilized by saline-alkali soil microorganisms. Therefore, planting sunflowers in saline-alkali soils can improve relevant soil physicochemical properties and enzyme activities, enhance microbial functional diversity, and optimize the community structure of saline-alkali soil microorganisms, with planting Xinkui 4 showing the most significant amelioration effect on saline-alkali soils.

Full Text

Preamble

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Effects of Different Sunflower (Helianthus annuus) Varieties on Rhizosphere Soil Enzyme Activities and Microbial Community Functional Diversity in Saline-Alkali Land of Xinjiang

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Abstract

A pot experiment was conducted to investigate the effects of four sunflower varieties (Xinkui 4, Xinkui 6, Xinkui 10, and American Aidadou) on the physical and chemical properties, enzyme activities, and microbial community functional diversity of saline-alkali soil rhizosphere, aiming to identify the most suitable variety for improving saline-alkali soil quality in Xinjiang. The results showed that Xinkui 6 most significantly reduced rhizosphere soil pH and increased total nitrogen content and invertase activity ($P < 0.05$). Xinkui 4 most significantly enhanced available nitrogen, available phosphorus, and available potassium contents, as well as urease and phosphatase activities. All four varieties significantly increased the average well color development (AWCD) of 31 carbon sources for rhizosphere microbes at 72 h ($P < 0.05$), following the order: Xinkui 4 > Xinkui 6 > Xinkui 10 > Aidadou > control (CK). The varieties also significantly improved Shannon diversity index (H), Simpson dominance index (D), and substrate richness (S) of the microbial community, with similar patterns observed across treatments. Xinkui 4 exhibited the highest microbial diversity index, significantly greater than the lowest CK treatment. Principal component analysis revealed that planting sunflowers improved the community composition of soil microbes, with carbohydrates, carboxylic acids, and polymers being the primary carbon sources utilized by saline-alkali soil microorganisms. Therefore, planting sunflower, particularly Xinkui 4, can significantly enhance soil physicochemical properties and enzyme activities, improve microbial functional diversity, and optimize microbial community structure in saline-alkali land.

Keywords: *Helianthus annuus*; soil enzyme activity; Biolog; saline-alkali soil; Shannon diversity index

1. Materials

The experimental soil was collected from saline-alkali land around Shihezi City, representing a mild saline-alkali sandy loam with a thickness of approximately 27 cm. Sunflower seeds of varieties Xinkui 4, Xinkui 6, Xinkui 10, and American Aidadou were provided by the Crop Research Institute of Xinjiang Academy of Agricultural Reclamation Sciences. Seeds were soaked in warm water overnight before planting. The pot experiment was conducted in a growth chamber (GXZ-430D) at 25°C with a light intensity of 450 mol•m ^{2}•s ^{1} and a 12 h photoperiod. After germination, seedlings were thinned to maintain consistent plant density. Soil samples were collected from the rhizosphere at the flowering stage, approximately 60 days after planting. Rhizosphere soil was obtained by shaking off loosely attached soil and collecting the soil tightly adhering to roots. Residual roots and debris were removed, and soil samples from replicate pots were mixed uniformly and passed through a 2 mm sieve before storage in a 4°C refrigerator for analysis. Unplanted soil served as the blank control (CK).

2. Analysis of Soil Physicochemical Properties and Enzyme Activities

Soil pH was measured using a pH meter (FE20K) with a soil-to-water ratio of 1:2.5. Total nitrogen was determined by the perchloric acid-sulfuric acid digestion method. Available nitrogen was extracted using the alkaline hydrolysis distillation method. Available phosphorus was measured by the 0.5 mol/L NaHCO extraction method. Available potassium was determined by flame photometry after NH OAc extraction. Urease and phosphatase activities were measured according to the methods described by Guan Songyin [21]. Invertase activity was determined following standard procedures for soil enzyme assays.

3. Analysis of Soil Microbial Functional Diversity

Microbial community functional diversity was assessed using Biolog EcoPlates (Biolog-ECO Plate) containing 31 different carbon sources. Fresh soil equivalent to 10 g dry weight was added to a 250 mL flask containing 90 mL of 0.05 mol/L phosphate buffer solution (PBS) and shaken for 30 minutes. The suspension was diluted to 10 ^{3}, and 150 L of the diluted solution was inoculated into each well of the EcoPlate. The plates were incubated in the dark at 28°C, and absorbance was read at 590 nm using a microplate reader at 12 h intervals for up to 144 h. The average well color development (AWCD) was calculated as an indicator of overall microbial metabolic activity. Shannon diversity index (H), Shannon evenness index (E), Simpson dominance index (D), and substrate richness (S) were calculated according to established formulas [22-24]. AWCD was calculated as the sum of (Ci - Ri) divided by 31, where Ci is the absorbance value of each well and Ri is the absorbance of the control well. Pi was calculated as (Ci - Ri) divided by the sum of all (Ci - Ri) values. H was calculated as - (Pi × ln Pi), E as H divided by ln S, D as 1 - (Pi^2), and S as the total number of carbon sources utilized.

4. Data Processing

Experimental data were analyzed using Excel and SPSS 16.0 software. One-way ANOVA was performed to compare means among treatments, with significant differences determined at $P < 0.05$. Principal component analysis (PCA) and cluster analysis were conducted to evaluate patterns of carbon source utilization. Figures were generated using appropriate statistical software.

1. Effects of Different Sunflower Varieties on Soil Chemical Properties

All four sunflower varieties exhibited significant rhizosphere effects on soil physicochemical properties. Xinkui 6 showed the most significant reduction in rhizosphere soil pH. Compared with the control, Xinkui 4 significantly increased rhizosphere soil available nitrogen, available phosphorus, and available potassium contents, with the best performance observed for this variety. Xinkui 6 produced the highest total nitrogen content, significantly higher than the control. All varieties reduced rhizosphere soil pH, with Xinkui 6 showing the most pronounced effect. Xinkui 10 resulted in the lowest available nitrogen content, which was significantly different from other treatments. The detailed results are presented in .

2. Effects of Different Sunflower Varieties on Rhizosphere Soil Enzyme Activities

Xinkui 6 produced the highest invertase activity in rhizosphere soil, significantly higher than Xinkui 10, Aidadou, and the control. Xinkui 4 had the greatest effect on urease activity, with its rhizosphere soil urease activity significantly higher than other treatments. Xinkui 4 and Aidadou significantly increased phosphatase activity compared to the control, with Xinkui 4 showing the highest phosphatase activity among all varieties. These results demonstrate that different sunflower varieties have distinct effects on soil enzyme activities, with Xinkui 4 generally showing the most significant enhancement of enzyme activities. Detailed enzyme activity data are shown in .

3. Effects of Sunflower Varieties on Microbial Carbon Source Utilization

The AWCD value represents the overall ability of soil microorganisms to utilize carbon sources and reflects their biological activity [25]. During the 144 h incubation period, rhizosphere microorganisms from all sunflower treatments began metabolizing single carbon sources after 0-12 h of lag phase. The AWCD values showed significant differences among treatments ($P < 0.05$). Rhizosphere microorganisms from Xinkui 4 entered the logarithmic growth phase earlier (after

24 h) and reached stable metabolic activity by 144 h. The control treatment showed the lowest AWCD throughout the incubation period. Planting sunflower varieties significantly enhanced microbial metabolic activity, with Xinkui 4 producing the most pronounced effect, increasing AWCD by 113.83% compared to the control at 72 h. The temporal changes in AWCD are illustrated in

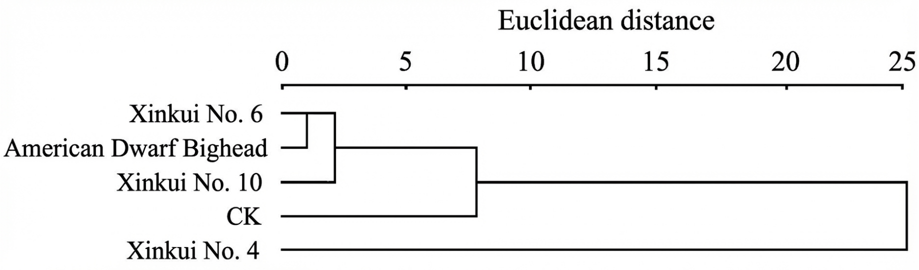

Figure 1: Figure 1

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4. Effects of Sunflower Varieties on Soil Microbial Diversity Indices

The Shannon diversity index (H) is widely used to assess microbial community functional diversity [26], while substrate richness (S) indicates the number of carbon sources utilized [27]. All four sunflower varieties significantly increased H, D, and S indices compared to the control ($P < 0.05$), following similar patterns. Xinkui 4 produced the highest diversity indices, significantly greater than the control. The Shannon evenness index (E) was lowest for Xinkui 4, indicating distinct community structure. These results demonstrate that planting sunflowers significantly enhances soil microbial community functional diversity, with Xinkui 4 showing the most substantial improvement. The diversity indices for different treatments are shown in [FIGURE:2].

5. Principal Component and Cluster Analysis of Microbial Carbon Source Utilization

Principal component analysis (PCA) was performed to evaluate differences in carbon source utilization patterns among treatments. The first three principal components explained 52.8%, 24.2%, and 13.9% of the variance, respectively, with a cumulative contribution rate of 90.9%. The first principal component (PC1) was strongly correlated with phenolic compounds, carboxylic acids, and polymers. PC2 was associated with amine compounds and carbohydrates, while PC3 correlated with amino acids and carbohydrates. Xinkui 4 scored highest on PC1, indicating its significant impact on microbial carbon metabolism. The PCA results demonstrate that planting different sunflower varieties significantly alters the metabolic characteristics of soil microbial communities in saline-alkali land.

Cluster analysis grouped the treatments based on their carbon utilization profiles. Xinkui 4 formed a separate cluster with large Euclidean distances from other treatments, indicating its distinct effect on microbial community function. Xinkui 6 and Xinkui 10 clustered together, while Aidadou showed intermediate characteristics. This classification reflects the differential impacts of sunflower varieties on rhizosphere microbial communities, consistent with the results from soil physicochemical and enzyme activity analyses. The cluster analysis dendrogram is presented in [FIGURE:3].

1. Effects of Sunflower Varieties on Soil Biochemical Properties

Planting salt-tolerant plants effectively ameliorates saline-alkali soils and improves soil fertility [28]. This study found that all four sunflower varieties improved rhizosphere soil physicochemical properties to varying degrees. Xinkui 4 most significantly increased available nitrogen, phosphorus, and potassium contents, while Xinkui 6 most effectively reduced pH and increased total nitrogen. The differential effects likely stem from variations in root exudate composition and quantity among varieties [36-37], which significantly influence rhizosphere enzyme activities and microbial metabolism. Xinkui 4 and Xinkui 6 showed the strongest effects on soil properties, suggesting they produce distinct root exudate profiles that substantially modify the rhizosphere microenvironment.

2. Effects on Microbial Functional Diversity

The AWCD value reflects microbial metabolic capacity and activity [38]. This study demonstrated that planting sunflower varieties shortened the microbial adjustment phase and accelerated entry into logarithmic growth. Xinkui 4 produced the highest AWCD values, indicating enhanced microbial metabolism. The Shannon diversity indices (H, D, S) were all significantly increased by sunflower planting, with Xinkui 4 showing the greatest enhancement. These improvements in functional diversity suggest that sunflower root exudates provide novel carbon sources and create niche differentiation for microbial communities in the nutrient-poor saline-alkali soil, thereby restructuring the microbial ecosystem.

3. Principal Component and Cluster Analysis

PCA effectively reduces dimensionality to identify key variables [39-40]. The analysis revealed significant differences in carbon source utilization among treatments, with carbohydrates, carboxylic acids, and polymers being the primary carbon sources metabolized by saline-alkali soil microbes. The distinct clustering of Xinkui 4 confirms its unique impact on microbial community function, consistent with findings from soil property and enzyme activity analyses. These results align with previous studies showing crop-specific effects on soil microbial communities [41-42], demonstrating that different sunflower varieties selectively enrich for distinct microbial functional groups through differential rhizosphere modification.

4. Conclusion

Soil microorganisms are among the most active components in soil, influencing soil formation, organic matter decomposition, humus formation, and nutrient cycling [45-47]. Soil enzymes, derived from microbial activity [48], serve as critical indicators of soil health and fertility. This study demonstrates that planting sunflower varieties, particularly Xinkui 4, effectively improves saline-alkali soil by: (1) reducing rhizosphere pH and increasing total nitrogen, available nitrogen, phosphorus, and potassium; (2) significantly enhancing urease, phosphatase, and invertase activities; and (3) increasing microbial utilization of carbon sources and functional diversity indices. Principal component and cluster analyses confirm that Xinkui 4 has the most substantial impact on microbial community structure and function. Therefore, Xinkui 4 is recommended as a priority variety for saline-alkali land remediation, as it promotes nutrient cycling, enhances microbial metabolism, and optimizes microbial community functional diversity in these degraded soils.

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