THE COMPREHENSIVE EFFECT OF MICROBIAL METABOLIC DIVERSITY ON CARBON COMPONENT CHANGES IN SALINE ALKALI FARMLAND

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Abstract. In the face of the reduction of biodiversity in saline alkali farmland and its adverse effects on soil carbon components, experiments were conducted using different sludge treatment gradients (0, 25, 50, 100, 200 t/ha). The changes in functional groups in soil aggregates were quantitatively analyzed using infrared spectroscopy technology, and microbial activity was measured using chemical analysis methods. The results showed that the application of sludge significantly increased the soil macroaggregates' mass fraction, which was 48.9% in untreated soil and increased to 51.1% after the highest treatment gradient. The absorption peak intensity of 3617 cm^{-1} caused by hydroxyl functional groups significantly increased after application, especially at 150 t/ha. In addition, there was a slight increase in microbial activity in soil aggregates ranging from 0.25 to 2 mm, while microbial activity was significantly enhanced in aggregates larger than 2 mm. The soil salt content indirectly affected the microbial metabolic rate by affecting soil sucrase activity and soil organic carbon, with a path coefficient of -0.42. Salt is a key factor affecting microbial metabolic diversity. Reasonable application of sludge can effectively improve the metabolic diversity of microorganisms in saline alkali soil, promote the formation of soil aggregates, and enhance the fixation soil organic matter. This has practical value in improving the soil structure of saline alkali farmland and promoting sustainable soil management.
Keywords: *saline alkali land, microorganisms, metabolism, carbon components, ecosystem*

Introduction

Soil salinization is a global environmental problem that seriously restricts agricultural productivity and ecosystem service functions (Akimbekov et al., 2020; Titilawo et al., 2020.). The unique environmental conditions of saline alkali soil might affect structure and function of microbial communities. This makes the microbial mediated carbon cycling process and its contribution to carbon component changes a research focus (Li et al., 2021a; Liu and Wang, 2021). Therefore, it is necessary to explore in depth how microbial metabolic diversity in saline alkali soils affects the transformation and stability of carbon components. This is crucial for understanding global carbon balance and promoting ecological restoration of saline alkali land (Feng et al., 2023; Li et al., 2021b). In the face of salt alkali stress, interdisciplinary strategies have been proposed to comprehensively evaluate the interaction between microbial metabolic diversity and soil carbon components. This strategy combines high-throughput sequencing and chemical analysis, innovatively applying microbial ecology techniques to reveal carbon cycling mechanisms. This method highlights the connection between microorganisms and the macro environment, providing scientific support for saline alkali soil management. It can promote soil health and agricultural sustainability, assist in soil improvement, enhance productivity and carbon sequestration capacity, and improve global carbon balance.

The impact of microbial metabolic diversity on carbon cycling in saline alkali soil environments has become a hot topic in ecological and soil science research. Li et al.

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(2023) proposed that supplementing with Rhamnolipids (RLs) could improve saline alkali soil quality, promote microbial growth, nutrient cycling, and plant seed germination. These results confirmed that RLs supplementation improved soil aggregate stability and promoted the formation of macroaggregates. The improvement reduced the pH value of alkaline soil and the salt content of saline soil. This changed the microbial community structure and promoted the proliferation of beneficial bacteria. Wu et al. (2019) proposed that nitrifying spirochetes and Pseudomonas were dominant microorganisms in the saline alkali soil along the coast of Wudi, Shandong, closely related to soil physicochemical properties. These results confirmed that soil enzyme activity gradually increased with soil depth, catalase activity was directly correlated with soil total potassium content, and Urease (URE) activity was directly correlated with soil Total Nitrogen (TN) content. Total Phosphorus (TP) can affect bacterial communities seriously, while URE can affect fungal communities seriously. Liu et al. (2022) proposed that soil physicochemical properties and archaeal-fungal community structure had obvious changes during vegetation succession in the Songnen saline alkali grassland. These results confirmed that soil physicochemical properties improved with vegetation succession stages. The structure of archaeal-fungal communities varied with vegetation succession stages, and different succession stages exhibited significantly enriched Anodic Stripping Voltmetry (ASV) and functional pathways. Except for cellulase activity, all soil physicochemical properties might affect community structure. Li and Liu (2020) proposed that soil respiration in saline alkali land was influenced by vegetation type, soil properties, and climate factors. These results confirmed that the halophytic plant communities of Tamarix and Goji berries significantly reduced soil salinity, slightly decreased soil bulk density, increased soil Soil Organic Carbon (SOC), and promoted microbial communities. This provided a new understanding of the spatiotemporal changes and driving factors of soil respiration in saline alkali land, which was helpful for vegetation restoration and carbon cycling research in saline alkali land. Wang et al. (2021b) proposed planting five halophytes, such as wolfberry, to improve saline alkali soil and increase soil organic matter, TN, TP, available phosphorus, and alkaline nitrogen content. These results confirmed that Proteobacteria was crucial for the degradation of soil organic matter. In addition, the root exudates of halophytes might promote the growth of Proteobacteria, especially in Suaeda salsa and Sophora japonica.

Soil microorganisms are crucial for in the fixation, transformation, and release of carbon, and their diversity and function directly determine the steady-state and changes of carbon components in saline alkali farmland. Xiang et al. (2021) proposed that long-term antibiotic pollution altered the composition and carbon fixation pathways of carbon fixed microbial communities in karst rivers, leading to selective changes in the coding genes of carbonic anhydrase. These results confirmed that the selection of carbon fixation pathways might be one of the adaptive strategies for microbial communities to cope with antibiotic pollution pressure and develop antibiotic resistance. Akimbekov et al. (2022) proposed that microorganisms in coal environments had rich functional potential and played an important role in the production and remediation processes of the coal industry. These results confirmed that microorganisms in the coal environment played an important role in the production and remediation processes of the coal industry, including coal mining, coal processing, and coal pollution remediation. Ren et al. (2021) compared locations along a 45-year time-series of Robinia pseudoacacia afforestation. Then metagenomics was used to study the trends of microbial carbohydrate active enzymes and their reactions to the decomposing biomass. The abundance of CAZyme was higher,

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correlated with microbial metabolic activity. This indicated that bacterial derived components were invested more in microbial carbon turnover after afforestation. Ye et al. (2020) used EPS as a carbon source to enrich microorganisms with different particle sizes. The changes in organic components and microbial communities during EPS degradation were studied using fluorescence Excitation Emission Matrix (EEM) and Illumina sequencing. The phyla Proteobacteria, Firmicutes, and others had correlation with changes in organic matter through humification. Li et al. (2022) investigated the soil carbon emission characteristics and underground microbial composition in six different succession stages of peatlands. These results confirmed that peat moss peatland had the highest carbon emissions, and its soil enzyme activity was closely related to the type of aboveground vegetation cover. As the soil moisture content decreased, the microbial community structure became simpler and looser. Bacteria exhibited a random distribution in nutrient rich soil environments and transition to a deterministic distribution as water and nutrient content decrease.

In summary, the current understanding of the complex relationship between microbial metabolic diversity and soil carbon components in saline alkali environments is not comprehensive. There is an urgent need for in-depth mechanism research and more refined methodological exploration. In response to this issue, the experiment enhances the integration and application of interdisciplinary research methods and designs more accurate experimental plans. It is hoped to contribute new scientific data and theoretical basis for the ecological role of microorganisms in saline alkali soil and soil carbon cycling processes. It is hoped to provide reference for soil carbon balance research and ecosystem management in the context of global change.

The research will be conducted in four parts. Firstly, the comprehensive effect of microbial metabolic diversity on carbon component changes in saline alkali farmland is summarized. Secondly, the comprehensive effect of microbial metabolic diversity in saline alkali farmland on carbon component changes is studied. Then, there is experimental verification for the second part. Finally, there is a summary of the research content and an indication of shortcomings.

Materials and methods

Overview of the experimental area

The field experiment is conducted in Dawa County, Panjin City, Liaoning Province. Dawa County belongs to a typical coastal saline alkali land, with a relatively flat terrain. The research soil is alkaline. The experimental area belongs to a temperate monsoon climate with distinct four seasons. The average annual pH value of precipitation is 8.20, indicating alkaline precipitation. There are 90 cloudy days, 120 sunny days, and 75 rainy days throughout the year. The precipitation in Dawa County is mainly concentrated in June and July, with a total rainfall of 550 mm. The annual average high and low temperatures are 24°C and -5°C.

Experimental design and soil collection

A detailed experimental design is studied and planned based on the saline alkali land conditions in Dawa County. This design aims to evaluate the impact of different soil improvement materials on soil quality and their potential agricultural value. *Table 1* presents an overview of the experimental design.

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Table 1. *Overview of experimental design*

Parameter	Description
Experimental site	Dawa County, Panjin City, Liaoning Province
Experiment area size	16 m² (4 m × 4 m)
Design type	Completely randomized block design
Treatment groups	Living Sewage Sludge (S), Vermicompost Improved Sludge (V), Biomass Vinegar Residue ®
Application rates	Control (0), low (50 t ha^{-1}), medium (100 t ha^{-1}), high (150 t ha^{-1})
Replications per treatment	3 replicates
Total number of subplots	12 (4 treatments × 3 replicates)
Application timing	Prior to the crop growth period as per local agricultural practice
Soil collection timing	After crop harvest
Soil collection depth	0-20 cm
Soil sample analysis	Air-dried, ground, and sieved for analysis
Soil sample storage temperatures	Physicochemical properties (room temperature), enzyme activity (4°C), microbial analysis (-80°C)

In *Table 1*, experiments were conducted using sludge of different concentrations (0, 50, 100, 150 t/ha), and microbial regulator Vermicompost Improved Sludge, V was used as a method for improving saline alkali soil. The reason for choosing manure is that it can provide soil microbial diversity and activity, thereby promoting the formation of soil aggregates and the fixation of soil organic matter. Earthworm manure, as an organic amendment, contains beneficial microorganisms and nutrients that help enhance soil biological activity and fertility, thereby improving soil structure and promoting plant growth. *Table 2* shows the expected soil parameter testing projects.

Table 2. *Expected soil parameter testing project*

Test item	Test purpose
Soil salinity content	Evaluate the amelioration effect on saline-alkali soil
Soil pH value	Assess the acid-base balance of the soil
Soil electrical conductivity	Estimate the concentration of soluble salts in the soil
Soil organic matter content	Evaluate the contribution of organic amendments to soil organic carbon
Soil total nitrogen, phosphorus, and potassium content	Evaluate nutrient status
Soil microbial community structure Analysis (16S rRNA)	Identify soil microbial diversity and its impact on organic matter metabolism
Soil enzyme activity test	Assess the activity of biochemical processes in soil
Soil respiration rate	Evaluate the ability of soil microorganisms to decompose organic matter

To ensure that the experimental design meets local conditions, close cooperation has been established with the local agricultural extension department. According to the local crop planting cycle and agricultural management practices, the application and sampling time have been carefully adjusted to promote the smooth progress of the experiment without interfering with local agricultural production. Soil sampling after harvest can

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evaluate the specific effects of different improvement materials and their application rates for saline alkali land improvement and crop growth.

Testing indicators and methods

Soil collection and analysis are conducted on saline alkali farmland in Dawa County during the experiment. Firstly, soil samples are obtained at designated locations. Some soil samples are sieved to determine the physicochemical properties of soil aggregates with different particle sizes, while the remaining soil samples are stored in an ultra-low temperature refrigerator at -80°C for subsequent microbial testing (Yang et al., 2021, 2023). The determination of physical and chemical properties is a key of soil quality assessment, including soil pH value, Electrical Conductivity (EC), salinity, SOC, TN, and TP indicators. The analysis of soil aggregates is carried out using the Cary 610/670 micro-infrared spectrometer, which covers a spectral range of 7800-375 cm^{-1}, with a wavenumber accuracy of over 0.1 cm^{-1} and a transmittance accuracy better than 0.1% T. When processing soil samples, they are placed in a refrigerator at 4°C, dried with silicone desiccant to a moisture content of about 10%, and then sieved using a 5 mm sieve. 100 g of soil sample with a moisture content of about 10% is taken and placed into the Octagon 200 soil aggregate dry sieve. The soil aggregates larger than 2 mm and 0.25-2 mm are separated by vibrating at maximum amplitude for 2 min, and the remaining soil components' particle size distribution on the sieve is determined.

When evaluating soil aggregates' stability in saline alkali farmland in Dawa County, Mean Weighted Diameter (MWD) and Geometric Mean Diameter (GMD) are usually calculated. MWD can reflect the relationship between the average diameter of soil aggregates on different sieves and their mass percentage. GMD is determined based on the average diameter of soil aggregates and their percentage of total mass (Wang et al., 2021a; Zhu et al., 2021). MWD is represented by *Equation 1*.

$$MWD = \sum_{i=1}^{n}(Wi,Wi)\Big/\sum_{i=1}^{n}Wi \quad \text{(Eq.1)}$$

In *Equation 1*, *Xi* is aggregates' average diameter (mm) on different sieves. *W* means the percentage of corresponding particle size aggregates. *i* is a different soil aggregate. GMD is represented by *Equation 2*.

$$GMD = \sum \exp\left(\sum_{i=1}^{n} WiloXi\right)\Big/\sum_{i=1}^{n}Wi \quad \text{(Eq.2)}$$

In *Equation 2*, *Xi* is *i* particle size aggregates' average diameter (mm). *W* means the percentage of particle size aggregates corresponding to *Xi* in total mass.

The total microbial activity of soil is determined by the Fluorescein Diacetate (FDA) hydrolysis method, and the results are expressed in units of fluorescein produced per gram of dry soil per hour (μg) (Zhong et al., 2020; Zhang et al., 2019). In addition, enzyme activities closely related to carbon, nitrogen, and phosphorus cycling in soil are determined using 3, 5-dihydroxysalicylic acid colorimetry, phenol-sodium hypochlorite colorimetry, and phosphobenzene colorimetry. These enzymes include Sucrase (SUC), URE, and Alkaline Phosphatase (ALP). The activity determination of these enzymes is crucial for understanding how soil microorganisms participate in and affect the transformation of nutrients such as carbon, nitrogen, and phosphorus in soil under saline

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alkali conditions. The collection and analysis of data will be based on the actual soil samples collected, ensuring that the measurement method accurately reflects the unique saline alkali soil conditions of Dawa County. Furthermore, the mechanism by which microbial metabolic diversity in saline alkali soil affects carbon component changes is explored in depth (Heo et al., 2022; López-Mondéjar et al., 2020).

Extraction of total DNA from soil

The FastDNA rotation kit was used to extract genomic DNA from 0.25 grams of dry soil samples from each treatment of saline alkali farmland. The extraction process was strictly carried out in accordance with the manufacturer's manual (Gajda et al., 2020; Zhu et al., 2020). The extracted DNA is dissolved in anhydrous ethanol and stored at -20°C for subsequent PCR amplification and high-throughput sequencing analysis. This step provides high-quality molecular data for studying the microbial metabolic diversity of saline alkali soil and its impact on carbon component changes.

High throughput sequencing of bacteria and fungi

To investigate the comprehensive impact of microbial metabolic diversity on carbon composition changes in saline alkali farmland, high-throughput sequencing analysis of bacterial and fungal community composition is conducted using Illumina MiSeq sequencing technology (Chang et al., 2021; Gupta et al., 2021). Firstly, total DNA is extracted from the collected saline alkali soil samples and operated according to standardized procedures using a professional soil DNA extraction kit to ensure that the quality and purity of DNA meet subsequent analysis requirements. For bacterial community analysis, specific primer pairs such as 515F and 806R are selected to perform PCR amplification on the V4 region of bacterial 16S rDNA. For fungal community analysis, PCR amplification is performed using specific primers targeting the ITS region. These PCR products are mixed at equimolar amounts and purified using Ampure XP beads to prepare for high-throughput sequencing. After sequencing is completed, the obtained raw sequence data are subjected to systematic quality control, sequence merging, and analysis to ensure the accuracy of these data. Through these data analyses, this study will explore the changes in microbial communities in saline alkali farmland under different organic amendments and their effects on soil carbon components and microbial metabolic functions.

Statistical analysis

SPSS 19.0 version is used for one-way ANOVA of soil physical and chemical properties, enzyme activity, Shannon diversity index, and microbial community structure. Duncan multiple range test is applied to determine the treatments' differences at the 5% significance level (Ari et al., 2023; Ghernaout et al., 2020). QIIME software is used to remove low-quality sequences, and sequences with a similarity of over 97% are clustered into Operational Taxonomic Units (OTUs). Subsequently, OTUs are annotated based on the Greengenes database. After data dilution analysis, Principal Coordinates Analysis (PCoA) is conducted using R language to explore the differences in microbial community composition and structure among different treatment groups. R package Phyloseq and ggplot2 are used to draw heatmaps, showing the dominant microbial groups with higher average relative abundance in different treatments (Barnett et al., 2021). A Structural Equation Modeling (SEM) is constructed to evaluate

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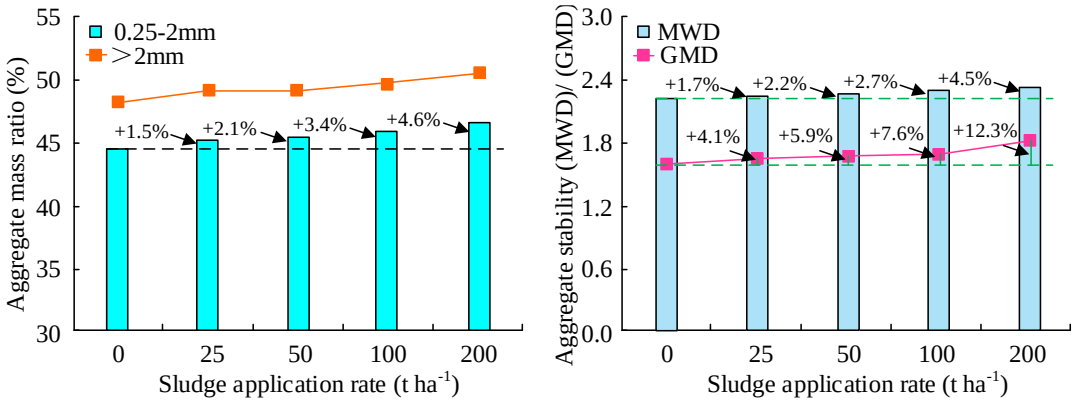
how soil physicochemical properties, enzyme activity, and microbial community diversity affect soil carbon composition.

Results and discussion

Particle size distribution and stability of soil macroaggregates

In the study of the effect of domestic sludge on soil aggregates in saline alkali land, the application of domestic sludge significantly promoted the composition and stability of soil macroaggregates, as shown in *Figure 1*. In *Figure 1a*, the mass fraction of 0.25-2 mm soil aggregates increased with the increase of sludge application amount. Compared to the soil without sludge application (mass fraction of 44.7%), after sludge treatment, the mass fraction of aggregates in this range increased to 45.4%, 45.7%, 46.2%, and 46.8%, with growth rates of 1.5%, 2.1%, 3.4%, and 4.6%, respectively. The growth effect was particularly significant, especially when the sludge application reached 100 and 200 t/ha. For aggregates larger than 2 mm, their mass fraction also showed a positive correlation increase. In different treatment groups of sludge application, the mass fraction of these aggregates increased from 48.9% of the untreated group to 49.6%, 49.9%, 50.1%, and 51.1%. This increase was particularly prominent at sludge application quantities of 100 and 200 t/ha. In *Figure 1b*, the application of domestic sludge also affected soil aggregates' MWD and GMD positively. After using domestic sludge, both MWD and GMD increased as the application amount increased. The MWD of 0.25-2 mm aggregates increased from untreated 2.23 mm to 2.27, 2.28, 2.29, and 2.33 mm in different treatment groups, with growth rates of 1.7%, 2.2%, 2.7%, and 4.5%, respectively. The increase in GMD was more significant, with untreated 1.70 mm increasing to 1.77, 1.80, 1.83, and 1.91 mm, with growth rates of 4.1%, 5.9%, 7.6%, and 12.3%, respectively.



(a) The effect of sludge on the mass fraction of soil aggregates

(b) The effect of domestic sludge application rate on soil aggregate MWD and GMD

Figure 1. *Application of sludge and earthworm manure on soil macroaggregates' formation and the effects of MWD and GMD in saline alkali soil*

Characteristics of organic carbon components in soil macroaggregates

Figure 2 shows the changes in SOC components in soil aggregates of 0.25-2 mm and > 2 mm after the application of domestic sludge. In *Figure 2a*, the application of domestic sludge had a significant impact on the SOC functional groups of soil

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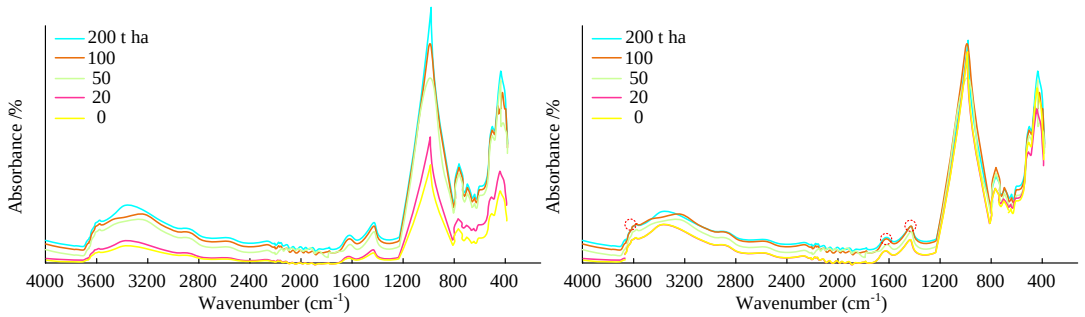
aggregates in saline alkali soil, ranging from 0.25-2 mm and > 2 mm. Infrared spectroscopy analysis confirmed that the absorption peak of aromatic C-H functional groups was enhanced in the range of 600-900 cm^{-1}. As the sludge application rate increased from 100 t/ha to 150 t/ha, this characteristic absorption peak also increased. In *Figure 2b*, the absorption peak at 1010 cm^{-1} corresponded to alcohol and ether functional groups, and its intensity also increased with the increase of sludge dosage. The absorption peak of 1435 cm^{-1} indicated the presence of methyl functional groups, and its intensity increased after sludge application. The absorption peak of 1640 cm^{-1} was attributed to the stretching vibration of olefin C = C, indicating an increase in olefin content after sludge application. The absorption peak of 3617 cm^{-1} was caused by hydroxyl functional groups, and its intensity significantly increased after application, especially at a dosage of 150 t/ha.



(a) Changes in organic carbon components in 0.25-2mm soil aggregates after application of household sludge

(b) Changes in organic carbon components in soil aggregates>2mm after application of household sludge

Figure 2. *Changes in organic carbon components in soil aggregates of 0.25-2 mm and > 2 mm after application of domestic sludge*

Figure 3 shows the changes in SOC components in soil aggregates of 0.25-2 mm and > 2 mm after the application of microbial regulators. In *Figure 3a*, after the application of earthworm manure sludge, the characteristic absorption peaks of SOC functional groups in soil macroaggregates of 0.25-2 mm and > 2 mm in saline alkali soil showed significant changes in the infrared spectrum. In the range of 600-900 cm^{-1}, the out of plane bending vibration absorption peak of aromatic C-H functional groups was enhanced, indicating that the content of aromatic compounds increased with the increase of application amount. In *Figure 3b*, the absorption peak near 1010 cm^{-1} was related to alcohol and ether functional groups, and its intensity increased with increasing application rate, especially reaching its maximum value when the application rate reached 200 t/ha. The absorption peak at 1435 cm^{-1} was caused by the vibration of methyl functional groups, and its intensity also increased as the application amount increased. The absorption peak of 1640 cm^{-1} was related to the stretching vibration of olefin C = C, indicating that the earthworm manure sludge made the unsaturated bonds in soil aggregates increased. The absorption peak at 3617 cm^{-1} was caused by the stretching vibration of hydroxyl functional groups. After the application of earthworm manure sludge, this absorption peak was significantly higher than the control group (CK), especially at 100 and 200 t/ha.

Figure 4 shows how the vinegar residue affects soil macroaggregates' SOC characteristics. The application of vinegar residue significantly affected the SOC functional group characteristics of 0.25-2 mm and > 2 mm macroaggregates in saline alkali soil. In the infrared spectrum, the out of plane bending vibration absorption peak

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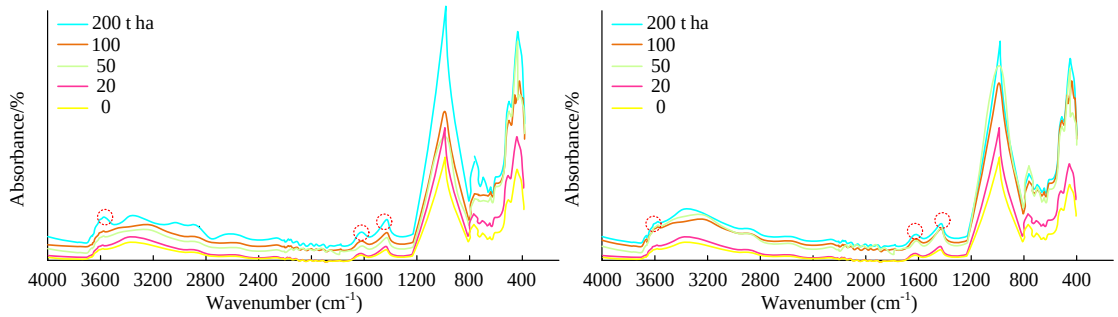
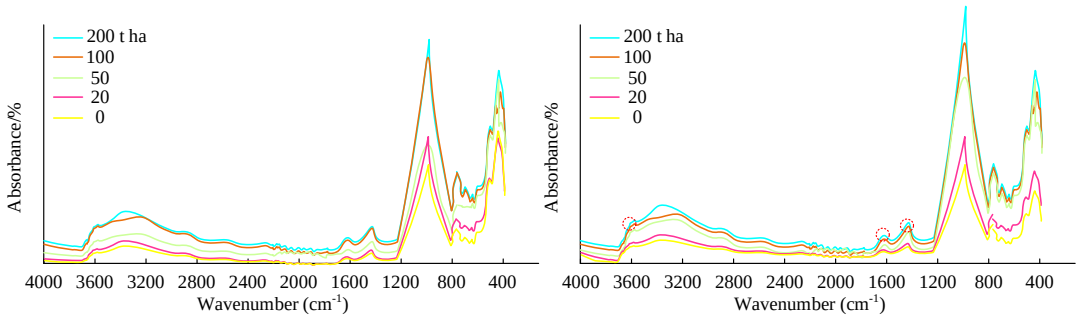
intensity of aromatic C-H functional groups in the 600-900 cm^{-1} region increased with the increase of vinegar residue application. It indicated an increase in the soil macroaggregates' aromatic compounds. At around 1010 $cm^{-1,}$ vinegar residue treatment increased the intensity of absorption peaks related to alcohol and ether functional groups, especially when the application rate reached 200 t/ha, this absorption peak reached its maximum value. The absorption peak at 1435 cm^{-1} reflected the vibration of methyl functional groups, and the increase in intensity also indicated an increase in methyl content in soil with the increase of vinegar residue application. For the absorption peak at 1640 $cm^{-1,}$ the stretching vibration of the C = C bond in the reaction alkene was observed. After treatment with vinegar residue, the absorption peak was enhanced compared to CK, indicating an increase in the content of unsaturated bonds in soil macroaggregates. As for the absorption peak at 3617 $cm^{-1,}$ it was generated by the stretching vibration of hydroxyl functional groups. After applying vinegar residue, this absorption peak was significantly enhanced, especially at 100 and 200 t/ha.



(a) Infrared spectra of organic carbon functional groups in soil macroaggregates of 0.25-2mm and>2mm in saline alkali soil after application of earthworm manure sludge

(b) The effect of earthworm manure sludge application rate on the absorption peak intensity of soil organic functional groups

Figure 3. *Changes in organic carbon components in soil aggregates of 0.25-2 mm and > 2 mm after application of microbial regulators*



(a) Infrared spectroscopy 1

(b) Infrared spectroscopy 2

Figure 4. *Effect of vinegar residue application on soil macroaggregates' organic carbon characteristics*

Effects of microbial regulators on soil macroaggregates' physicochemical properties and carbon composition

Figure 5 shows the effect of microbial regulators on the carbon composition of soil aggregates. The application of microbial regulators significantly improved the

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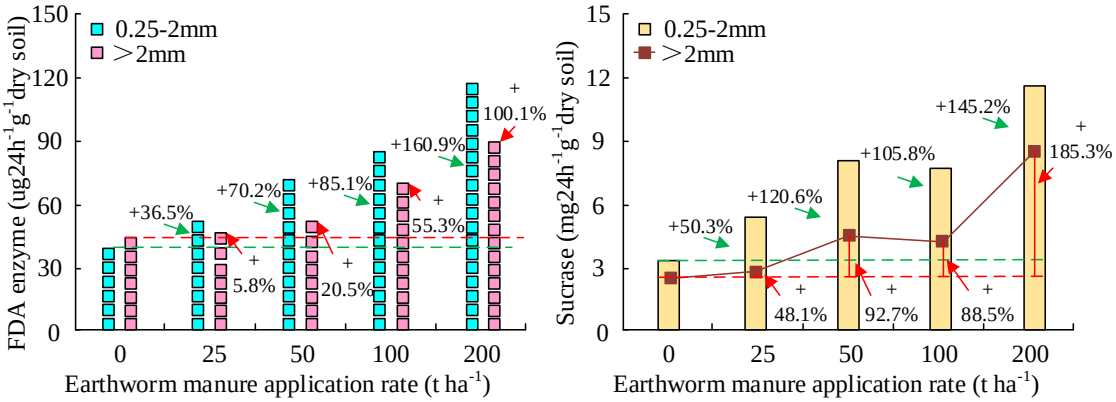
physicochemical properties of soil aggregates in saline alkali soil, especially affecting the accumulation of carbon components in soil aggregates positively. In *Figure 5a*, in the 0.25-2 mm soil aggregates, the FDA enzyme activity increased by 36.5%, 70.2%, 85.1%, and 160.9% compared to the control, respectively. This indicates that microbial regulators can significantly activate microbial activity in soil, thereby promoting the transformation of SOC. In soil aggregates larger than 2 mm, the increase in FDA enzyme activity was also significant, with increases of 5.8%, 20.5%, 55.3%, and 100.1%, respectively. These data indicate that microbial regulators not only affect the microenvironment of small aggregates, but also promote the internal structure and function of macroaggregates. In *Figure 5b*, the activity of SUC in 0.25-2 mm aggregates increased by 50.3%, 120.6%, 105.8%, and 145.2%, respectively. In aggregates larger than 2 mm, the increase in SUC activity was 48.1%, 92.7%, 88.5%, and 185.3%, respectively.



(a) The effect of microbial regulators on the physicochemical properties of aggregates

(b) The effect of sucrase activity on the physicochemical properties of aggregates

Figure 5. *Effect of microbial regulators on FDA enzyme activity and sucrase activity of soil macroaggregates*

Figure 6 shows the effect of microbial regulators on the URE activity and alkaline ALP activity of soil macroaggregates. In *Figure 6a*, the URE activity in the 0.25-2 mm aggregates increased from 0.35 mg/g dry soil in CK to 1.03 mg/g dry soil, with an increase of 189.9%. In macroaggregates (>2 mm), the URE activity increased from 0.16 mg/g dry soil in the control to 0.69 mg/g dry soil, with the highest increase reaching 319.6%. In *Figure 6b*, the alkaline ALP activity also increased with the increase of sludge and earthworm manure application. In the 0.25-2 mm aggregates, the activity increased from 0.83 mg/g dry soil in CK to 1.31 mg/g dry soil, with an increase of 57.1%. In aggregates larger than 2 mm, the activity increased from 0.64 mg/g dry soil in CK to 0.95 mg/g dry soil, with an increase of 49.0%.

Effects of microbial agents on soil macroaggregates' physicochemical properties and carbon composition

Figure 7 shows how the microbial agent affects soil macroaggregates' carbon composition. In *Figure 7a*, in the 0.25-2 mm soil aggregates, the FDA enzyme activity increased from 65 mg/g dry soil in CK to 90 mg/g dry soil. In aggregates larger than 2 mm, the activity increased from the control 50 mg/g dry soil to 75 mg/g dry soil. This

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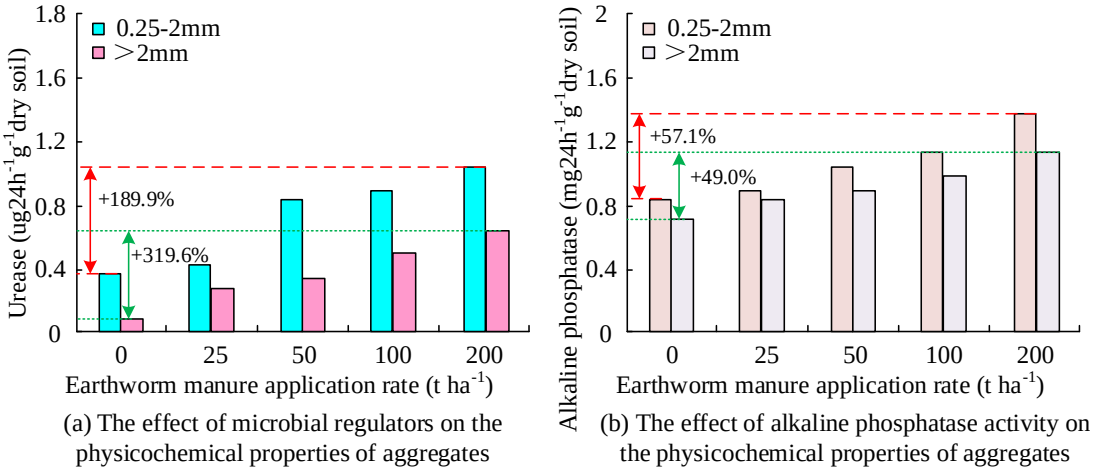
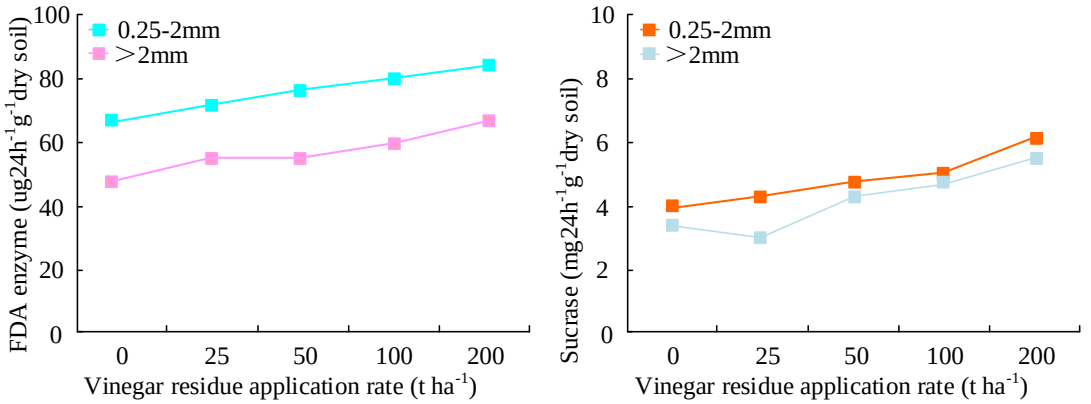
indicates that the application of microbial agents can significantly enhance lipase related activity in soil, which may promote the decomposition and transformation of carbon components in the soil. In *Figure 7b*, the activity slightly increased in the 0.25-2 mm aggregate, increasing from 4.20 mg/g dry soil in CK to 5.70 mg/g dry soil. In aggregates larger than 2 mm, the activity increased from 3.50 mg/g dry soil in the control to 5.00 mg/g dry soil. Although the increase in enzyme activity is not as significant as that of FDA, it also reflects the potential synergistic effect of microbial agents on soil carbohydrate degrading enzymes.



(a) The effect of microbial regulators on the physicochemical properties of aggregates

(b) The effect of alkaline phosphatase activity on the physicochemical properties of aggregates

Figure 6. *The effect of microbial regulators on soil macroaggregate urease activity and alkaline phosphatase activity*



(a) The effect of microbial agents on lipase activity

(b) The synergistic effect of microbial agents on soil carbohydrate degrading enzymes

Figure 7. *The effect of microbial agent application on the carbon composition of soil macroaggregates*

Figure 8 shows how the microbial agent affects soil macroaggregates' URE activity and alkaline ALP activity. In *Figure 8a*, after treatment with microbial agents, the URE activity in 0.25-2 mm aggregates in saline alkali soil was significantly enhanced. Compared with CK (0.42 mg/g dry soil), the URE activity increased by 69% and 95% at the application rates of 25 t/ha and 200 t/ha, respectively. In aggregates larger than

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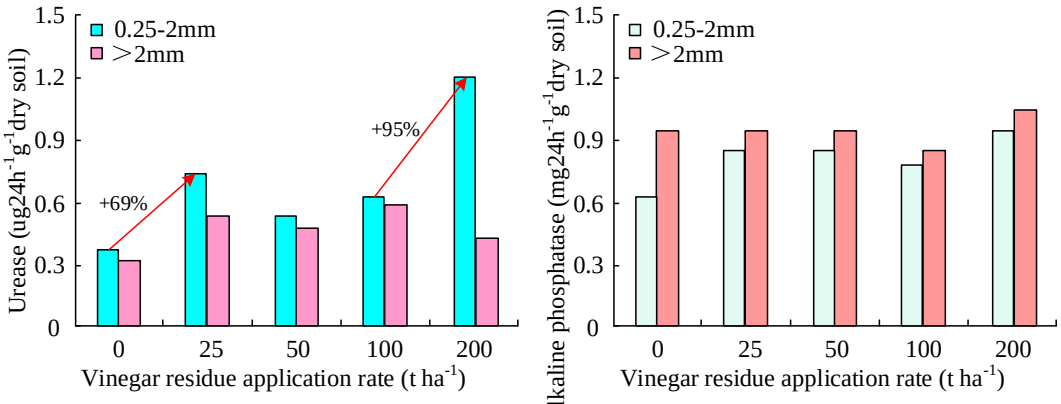
2 mm, there were corresponding increases of 77% and 107%, promoting the transformation of soil nitrogen. In *Figure 8b*, the activity of the 0.25-2 mm aggregates increased by an average of about 30% compared to CK (0.68 mg/g dry soil). In aggregates larger than 2 mm, the activity increased by an average of about 7%.



(a) The effect of microbial agent application on soil macroaggregate urease activity

(b)The effect of microbial agent application on alkaline phosphatase activity of soil macroaggregates

Figure 8. *The effect of microbial agent application on soil macroaggregate urease activity and alkaline phosphatase activity*

The effect of microbial agent application on microbial communities' composition and function in soil macroaggregates

Table 3 shows the effect of microbial agents on microbial diversity in soil macroaggregates (>2 mm). The application of microbial agents led to a Shannon diversity index of bacterial communities ranging from 7.30 to 7.50. Compared with untreated CK, a slight decrease was observed at a low dose of 25 t/ha, but bacterial diversity was enhanced at a high dose of 200 t/ha. The diversity index of fungal communities showed a similar trend, increasing from 3.80 to 4.50, indicating a positive effect of high-dose microbial agents on fungal diversity. After the application of microbial agents, the comparison of particle sizes between 0.25-2 mm and > 2 mm showed an increase in microbial diversity among larger aggregates.

Table 3. *Effects of microbial agents on microbial diversity in soil macroaggregates (>2 mm)*

Treatment (t/ha)	Aggregate size (mm)	Bacteria Shannon index	Fungus Shannon index
CK (0)	0.25-2	7.44 ± 0.061b	4.63 ± 0.188a
	> 2	7.46 ± 0.016a	4.15 ± 0.438a
25	0.25-2	7.30 ± 0.024c	3.80 ± 0.101b
	>2	7.32 ± 0.038b	4.20 ± 0.162b
200	0.25-2	7.50 ± 0.026a	4.50 ± 0.200a
	>2	7.50 ± 0.003a	4.50 ± 0.003a

Figure 9 shows the principal component analysis of the effects of microbial agent application on the community structure of bacteria and fungi in soil macroaggregates. The application of microbial agents increased the diversity and abundance of beneficial

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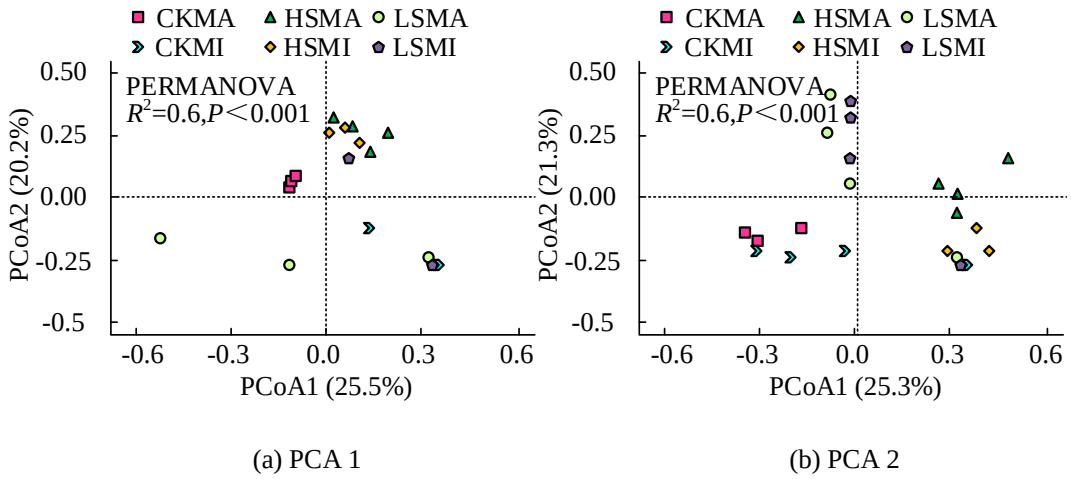
microorganisms in soil, thereby promoting the decomposition and carbon sequestration process of soil SOC, improving soil fertility and structural stability. The optimized data showed that after the application of microbial agents, the explanatory power of microbial communities in the first axis increased to 28.2%, and the explanatory power in the second axis was 23.9%. This indicated that microbial agents had a profound impact on soil microbial communities ($R^2 = 0.65$, $p < 0.001$), especially in the transformation and cycling of carbon components in soil.



(a) PCA 1 (b) PCA 2

Figure 9. *Principal component analysis of the effects of microbial agent on bacteria and fungi' community structure in soil macroaggregates*

Effects of microbial regulators on microbial communities' composition and function in soil macroaggregates

Figure 10 shows the principal component analysis of how microbial regulators affect bacteria and fungi' community structure in soil macroaggregates. In *Figure 10a*, the soil macroaggregates treated with microbial regulators showed significant adjustments in bacterial community structure. The principal component analysis showed that the interpretation rate for the first spindle was 27.6%, and for the second spindle it was 21.8%. Multivariate Statistical Analysis (PERMANOVA) showed significant changes in bacterial communities under different concentrations of microbial agents ($R^2 = 0.64$, $p < 0.001$), indicating that microbial agents played an important role in regulating the active carbon components in soil. In *Figure 10b*, the fungal community structure showed significant changes after the application of microbial regulators, with the first axis explaining 25.1% and the second axis explaining 19.9%. PERMANOVA further confirmed significant differences in community structure ($R^2 = 0.61$, $p < 0.001$), emphasizing the crucial role of microbial agents in promoting soil macroaggregate formation and enhancing soil SOC fixation.

Analysis of driving factors for carbon stabilization and the formation of soil macroaggregates after the application of microbial regulators

Figure 11 shows the SEM analysis of microbial regulators promoting soil aggregate formation and carbon stabilization. In *Figure 11a*, the application of microbial regulators significantly promoted the formation of soil macroaggregates and their

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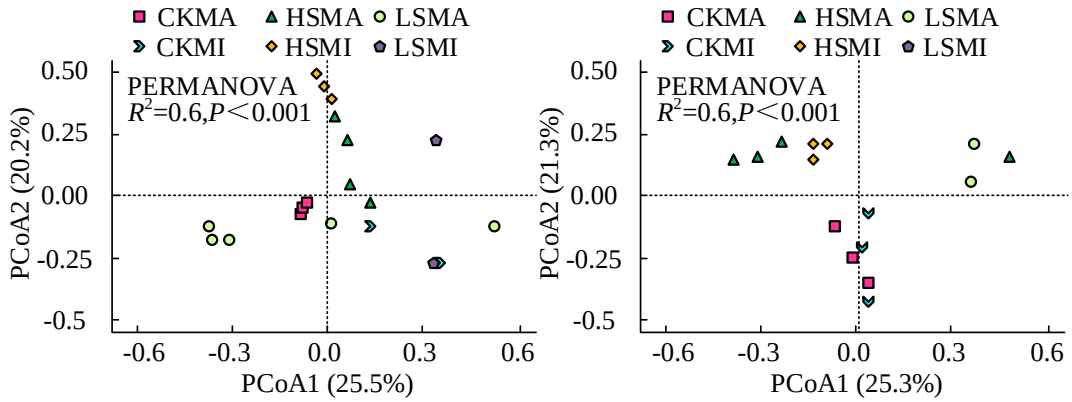
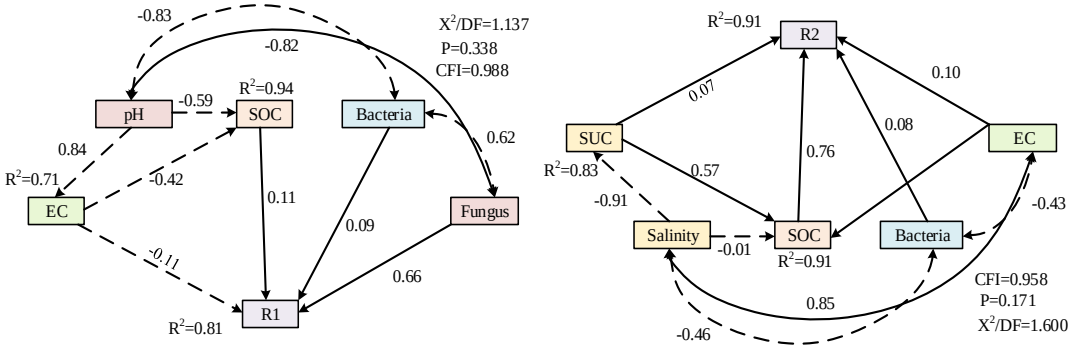
carbon stabilization (with high model fit, $\chi^2/DF = 0.39$, $P = 0.81$, $CFI = 0.998$). The correlation analysis between soil SOC, microbial community diversity and activity, and aggregate size (R1) showed that the direct effect of microbial regulators on SOC enhancement was 0.29. Its effect on the formation of macroaggregates was 0.14. This indicates that increasing SOC is a key pathway to promote soil structural stability through microbial regulators. In *Figure 11b*, the direct influence coefficient of SOC on R2 formation was 0.51, indicating its core role in the carbon stabilization of aggregates. The coefficient of influence of microbial regulators on R2 was 0.18, and the coefficient of influence on the improvement of soil enzyme activity was 0.15, emphasizing the positive effect of enhancing SOC content on soil physical structure stability.



(a) Principal Component Analysis 1 (b) Principal Component Analysis 2

Figure 10. *Principal component analysis of the effects of microbial regulators on the structure of bacterial and fungal communities in soil macroaggregates*



(a) Driver Analysis 1 (b) Driver Analysis 2

Figure 11. *Structural equation modeling analysis of microbial regulators promoting soil aggregate formation and carbon stabilization*

Analysis of driving factors for carbon stabilization and the formation of soil macroaggregates after using microbial agents

Figure 12 shows the SEM analysis of microbial agents promoting soil aggregate formation and carbon stabilization. In *Figure 12a*, the application of microbial agents significantly improved the physical and chemical properties of the soil. This promoted

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the formation of macroaggregates (R1) and the stabilization of SOC (with good model fit, χ^2/DF = 1.05, P = 0.36, CFI = 0.990). The model showed that the direct path coefficients of soil EC, SOC, pH, Bacteria, and Fungus for R1 had been adjusted: EC = -0.10, SOC = 0.25, pH = 0.12, Bacteria = 0.62, and Fungus = 0.70. PH could influence R1 by changing EC and SOC. In *Figure 12b*, the updated model data showed that the direct path coefficients of SUC, SOC, Bacteria, and EC for R2 were: SUC = 0.10, SOC = 0.80, Bacteria = 0.15, EC = -0.08, respectively. Salinity indirectly affected R2 by influencing SUC and SOC, with a path coefficient of -0.42.

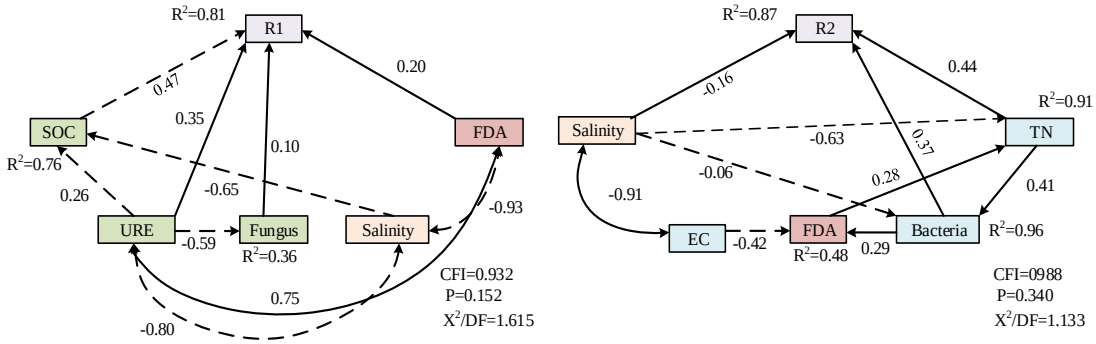

Figure 12. *Structural equation modeling analysis of microbial agents promoting soil aggregate formation and carbon stabilization*

Conclusion

The improvement of saline alkali soil is crucial for agricultural productivity and ecosystem function. The core of this research is to explore the effect of microbial agents on improving the structure and microbial activity of saline alkali soil. The experiment was conducted by applying different concentrations of vinegar residue (0, 25, 100, 200 t/ha) and using methods such as infrared spectroscopy analysis and soil enzyme activity measurement. The results confirmed that the application of vinegar residue significantly increased the absorption peak intensity of soil hydroxyl functional groups at 3617 $cm^{-1,}$ especially under 100 and 200 t/ha treatments. In the 0.25-2 mm aggregates, the URE activity increased by 189.9% compared to CK, reaching 1.03 mg/g dry soil. After bacterial treatment, the URE activity in soil aggregates with the same particle size was significantly increased. The activity increased by 69% and 95% respectively, especially at the application rates of 25 t/ha and 200 t/ha. Thanks to the optimization of microbial agents, the explanatory power of the first and second axes of soil microbial community structure had been increased to 28.2% and 23.9%, respectively. This indicated that the fungal community structure underwent significant changes after the application of regulatory agents. The research results indicate that the application of sludge has a significant positive effect on the formation of soil macroaggregates and carbon stabilization, which is statistically significant ($P < 0.05$). However, there is still insufficient research in field applications and long-term impact assessment. Future research will expand to field trials to evaluate the impact of long-term application of microbial agents on soil ecosystems, thereby providing strategies for the sustainable management and remediation of saline alkali soils.

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