

# Microbial mechanisms underlying enhanced salt tolerance in soybean through sorghum–soybean intercropping

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## Research Article

**Keywords:** sorghum–soybean intercropping, saline stress, rhizosphere microorganisms, plant–soil interactions

**Posted Date:** August 27th, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-10643661/v1>

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# Abstract

## Background and Aims:

Intercropping is an effective cultivation practice for enhancing biodiversity in agroecosystems, which can improve soil environment and enhance crop adaptability by regulating rhizosphere microorganisms. However, the rhizosphere microbial regulatory mechanisms underlying the improved salt tolerance of soybean intercropped with salt-tolerant crops remain unclear.

## Methods

In this study, high-throughput sequencing technology was employed to compare the rhizosphere soil physicochemical properties and microbial community structure characteristics under monoculture and intercropping systems of sorghum and soybean under salt stress.

## Results

The results showed that, compared with monoculture, sorghum-soybean intercropping (IS/IG) significantly reduced soil pH and total salt content, while increasing soil organic matter content. The improvement in soil environment effectively promoted soybean growth, with aboveground biomass and net photosynthetic rate increasing by 23.5% and 18.9%, respectively. Meanwhile, intercropping alleviated oxidative damage induced by salt-alkali stress in soybean, with root ABA and H<sub>2</sub>O<sub>2</sub> accumulation decreasing by 9.53% and 72.29%, respectively. Principal coordinate analysis (PCoA) of rhizosphere microbial communities revealed that intercropping significantly altered the overall structure of the rhizosphere bacterial community. Differential genus screening showed that beneficial bacterial genera such as *Bacillus* and *Pseudomonas* were significantly enriched in the rhizosphere of intercropped soybean.

## Conclusions

Based on these findings, sorghum–soybean intercropping remodels rhizosphere bacterial communities and optimizes saline soil microenvironments, effectively alleviating salt-induced oxidative stress and improving soybean salt tolerance. This study reveals the core microbial mechanism by which intercropping enhances soybean salt tolerance, providing a practical strategy for soybean production in saline soils.

## 1. Introduction

Cereal and legume intercropping is a key cropping system in China. Reasonable cereal and legume intercropping practices can not only raise the utilization efficiency of farmland resources, but also slow

soil degradation and improve the stability of agroecosystems (Ruillé et al. 2026; Zhao et al. 2014). Given the global shortage of arable land, properly arranged intercropping is widely recognized as a sustainable land-efficient planting pattern worldwide. Developing intercropping modes including maize and soybean, maize and peanut, and sorghum and peanut can significantly boost land productivity and reduce conflicts over cultivated land between grain and oilseed crops (Li et al. 2026; Shi et al. 2023; Wang et al. 2026b). Furthermore, deploying intercropping on marginal saline land is critical to easing cultivated land shortage and restoring local ecological environments (Demie et al. 2022; Yang et al. 2024; Yang et al. 2026).

In intercropping systems, crops are exposed to salt stress while simultaneously responding to it through modifications of interspecific interactions. The type and intensity of these interactions are not independent but are closely interconnected and dynamically regulated by environmental conditions. The Stress Gradient Hypothesis (SGH) suggests that interspecific interactions shift from competition to facilitation as environmental stress increases (Bertness and Callaway 1994). This hypothesis has since been validated across a wide range of ecosystems. Under saline conditions, intercropping can improve the nutritional status of crop communities through interspecific nutrient complementarity, thereby exhibiting greater productivity than monoculture (Wang et al. 2026a). Furthermore, as soil salinity increases, the intensity of negative interactions between plants is significantly reduced, and the relationship between neighboring plants gradually shifts from competition to facilitation (Guo et al. 2023).

For decades, chemical amendment has been the primary approach for the reclamation of saline soils. However, owing to the unique and complex properties of saline soils, the application of exogenous amendments often fails to achieve maximum effectiveness, resulting in increased agricultural production costs and considerable environmental impacts (Reganold and Wachter 2016). Increasing evidence indicates that plant health and environmental adaptability are determined not only by the host genome but also by complex interactions with diverse biotic factors in the surrounding environment (Munns and Gilliam 2015; Vannier et al. 2015). Root-associated microbial communities are active partners in their interactions with host plants (Wen et al. 2025). These microorganisms have co-evolved with their hosts and continuously adjust their community composition in response to specific environmental stresses (Lau and Lennon 2012). Consequently, the belowground ecological interaction network involving plant roots, soil, and microorganisms plays a pivotal role in sustaining plant growth and enhancing plant tolerance to adverse environmental conditions.

However, intensive agricultural practices, including simplified cropping systems, soil fertility degradation, and the excessive application of chemical fertilizers and pesticides, have led to a continuous decline in the activity and diversity of soil microbial communities in agroecosystems. Numerous studies have demonstrated that cereal–oilseed (or legume) intercropping systems, such as maize–peanut, maize–soybean, and sorghum–peanut intercropping, can enhance interspecific complementarity, nutrient exchange, and edge effects through optimized crop combinations and cultivation practices, thereby providing an effective strategy for improving soil microbial diversity and optimizing the soil

microenvironment (Duan et al. 2026; Ma et al. 2024). Furthermore, interspecific root interactions and alterations in root architecture within cereal–oilseed (or legume) intercropping systems can regulate the spatial and temporal distribution of soil salinity, thereby creating a favorable rhizosphere microenvironment. This process is essential for alleviating soil stress and enhancing the stability and resilience of agroecosystems (Chen et al. 2025; Zhang et al. 2026b)

Although previous studies have demonstrated the ecological advantages of intercropping from the perspectives of crop configuration and microbial responses, it remains unclear whether the enhanced salt tolerance of soybean in sorghum–soybean intercropping systems is mediated by the restructuring of rhizosphere microbial communities and the strengthening of microbial interactions. To address this knowledge gap, the present study employed the salt-tolerant sorghum cultivar *Jinnuo 101* and the soybean cultivar *Jidou 12* under saline soil conditions, with monoculture and intercropping treatments. By integrating plant physiological analyses, high-throughput sequencing, and microbial co-occurrence network analysis, we systematically investigated the effects of intercropping on rhizosphere soil salinity, ion homeostasis, and antioxidant capacity in soybean, as well as the contribution of rhizosphere microbial community structure and interspecific microbial interactions to soybean salt tolerance.

Our results demonstrated that sorghum–soybean intercropping reduced rhizosphere salinity, enriched beneficial microorganisms, and strengthened microbial cooperative interaction networks, thereby improving ion homeostasis and antioxidant defense in soybean and ultimately enhancing its salt tolerance. These findings provide new insights into the mechanisms by which intercropping regulates the rhizosphere microbiome to improve crop salt tolerance and offer a theoretical basis for developing optimized intercropping systems for saline agriculture. Furthermore, the pot experiment confirmed that sorghum–soybean intercropping effectively alleviated the inhibitory effects of salt stress on soybean growth, providing a promising strategy for the sustainable production of soybean in saline–alkaline soils.

## 2. Materials and Methods

### 2.1 Experimental Design

The pot experiment was conducted at the Saline–Alkaline Land Improvement and Utilization Research Base in Huairan County, Shanxi Province, China (113°14'45" E, 39°54'43" N). The experimental site is located in the central Datong Basin and has a temperate continental monsoon climate characterized by four distinct seasons, with hot, rainy summers and cold, dry winters. The annual precipitation ranges from 360 to 400 mm, with most rainfall occurring in July and August, whereas the annual evaporation is approximately 1,500 mm. The mean annual temperature is 7.3°C, the average annual sunshine duration is approximately 2,850 h, the accumulated temperature above 10°C is approximately 3,100°C, and the frost-free period lasts 130–150 d.

The experimental soil was classified as a sandy loam with **sodic saline soil** characteristics. A salt-tolerant sorghum (*Sorghum bicolor* L.) cultivar, **Jinnuo 101**, and a soybean (*Glycine max* L.) cultivar, **Jidou**

12, were used as the experimental materials. The pot experiment was carried out in 2025 in a greenhouse at the Institute of Eco-Environmental Industry Technology, Shanxi Agricultural University. Soil used in the experiment was collected from the cultivated layer (0–20 cm) of the Saline–Alkaline Land Improvement and Utilization Research Base.

The basic physicochemical properties of the soil were as follows: pH, 9.41; soil organic matter, 5.63 g kg<sup>-1</sup>; available phosphorus, 5.41 mg kg<sup>-1</sup>; total nitrogen, 0.42 g kg<sup>-1</sup>; available potassium, 77.91 mg kg<sup>-1</sup>; and electrical conductivity (EC), 493  $\mu$ S cm<sup>-1</sup>.

A completely randomized design was adopted. Three planting pattern treatments were established: soybean monoculture, sorghum monoculture, and sorghum–soybean intercropping. Each treatment included six replicates, resulting in a total of eighteen planting boxes. Each box measured 70 centimeters  $\times$  50 centimeters  $\times$  25 centimeters (length  $\times$  width  $\times$  height) and was filled with 84 kilograms of soil. In the monoculture treatments, eight soybean plants or eight sorghum plants were grown in each box. In the intercropping treatment, eight soybean plants and four sorghum plants were grown in each box (Fig. 1). In soybean monoculture and sorghum monoculture, plant spacing was 20 centimeters and row spacing was 40 centimeters. In the intercropping treatment, plant spacing for both soybean and sorghum was 20 centimeters, and row spacing was 20 centimeters. During the experiment, all treatments were managed uniformly. Soil moisture was maintained at 60 percent of field water-holding capacity. Water was supplied by drip irrigation to prevent salt leaching. Other management practices, including irrigation and pest control, were conducted according to local conventional methods.

## 2.2 Sample Collection and Pretreatment

Microbial samples from soil and plants were collected at the jointing stage of sorghum. All sampling tools were sterilized by autoclaving prior to use. Soil samples were collected using the root-shaking method. Soybean plants were carefully excavated to minimize root damage, and loosely attached soil was gently shaken off onto sterile kraft paper and collected as bulk soil. The intact root systems were immediately placed in an ice box and transported to the laboratory. Root samples were transferred into sterile Erlenmeyer flasks containing 50 mL of sterile phosphate-buffered saline (PBS, 10 mM) and shaken at 220 rpm on a constant-temperature orbital shaker to detach rhizosphere soil. This washing procedure was repeated until no visible soil particles remained attached to the root surface. The resulting soil suspension was centrifuged at 6,000  $\times$  g for 20 min at 4°C, and the pellet obtained after centrifugation was collected as the rhizosphere soil sample. The washed roots and intact nodules were then carefully separated under aseptic conditions using sterile forceps in a laminar-flow hood and stored at – 80°C until DNA extraction and 16S rRNA gene amplicon sequencing (Zhong et al. 2019).

A portion of the bulk soil samples was processed using the quartering method, air-dried naturally, ground, and passed through 2 mm and 100  $\mu$ m sieves for subsequent determination of soil physicochemical properties. Plant samples were oven-dried, ground into a fine powder, and used for physicochemical analyses. Plant tissues were digested with concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and the resulting digest was used for nutrient determination. Total

phosphorus (TP) and total nitrogen (TN) were determined using a continuous flow analyzer. Soil pH was measured using the potentiometric method. Soil organic matter (SOM) was determined using the external heating potassium dichromate oxidation–titration method. Alkali-hydrolyzable nitrogen (AN) was determined using the alkali diffusion method. Available phosphorus (AP) was measured using the molybdenum–antimony colorimetric method, and available potassium (AK) was determined by ammonium acetate extraction followed by flame photometry.

## 2.3 Determination of Agronomic Traits and Physiological Parameters

**Photosynthetic Parameters:** Photosynthetic parameters were measured between 09:00 and 11:00 a.m. on clear days using a portable photosynthesis system (LI-6400, LI-COR Inc., Lincoln, NE, USA). The net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) were determined on the youngest fully expanded soybean leaves.

### Dry Matter Accumulation

Plant samples were collected at the jointing stage of sorghum. Three representative soybean plants were randomly selected from each plot and separated into different organs. The fresh weight of each organ was recorded before the samples were initially dried at 105°C for 60 min and subsequently oven-dried at 85°C to a constant weight. The dry weight was then determined to evaluate dry matter accumulation.

### Determination of Ion Contents

Dried plant samples (0.1 g) were digested using the  $\text{H}_2\text{SO}_4\text{--H}_2\text{O}_2$  digestion method. The concentrations of  $\text{Na}^+$  and  $\text{K}^+$  were measured using a flame photometer (FP640, Shanghai, China), and the  $\text{K}^+/\text{Na}^+$  ratio was subsequently calculated.

**Determination of Oxidative Stress Indicators:** Fresh leaf tissue (0.2 g) was homogenized in 5 mL of 10% (w/v) trichloroacetic acid (TCA) under ice-cold conditions and centrifuged at  $10,000 \times g$  for 15 min. The activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were determined using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

## 2.4 DNA Extraction, PCR Amplification, and High-Throughput Sequencing of the 16S rRNA Gene

Total genomic DNA was extracted from rhizosphere soil, root endosphere, and root nodules using the FastDNA SPIN Kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. Prior to DNA extraction, root endosphere and root nodule samples were ground into a fine powder in liquid nitrogen. DNA quality and concentration were determined, and qualified DNA samples were diluted to  $10 \text{ ng } \mu\text{L}^{-1}$  for subsequent PCR amplification. The bacterial 16S rRNA gene was amplified using the

universal primer pair 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') (Rösch et al. 2002). PCR amplification was performed in a 20 µL reaction mixture containing 4 µL of 5× FastPfu buffer, 2 µL of dNTP mixture, 0.8 µL each of the forward and reverse primers, 0.4 µL of FastPfu DNA polymerase, 10 ng of template DNA, and nuclease-free water to the final volume. The PCR program consisted of an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s, with a final extension at 72°C for 10 min. PCR products were verified by 1% agarose gel electrophoresis and subsequently sequenced on the Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA) (Shi et al. 2026)

## 2.5 Bioinformatics Analysis

Raw paired-end sequencing reads were merged using FLASH software, and sequences were assigned to individual samples according to barcode information using the FASTX-Toolkit. Chimeric sequences were identified and removed using USEARCH v8.0, and frameshift errors were corrected using FrameBot (<https://github.com/rdpstaff/FrameBot>). High-quality sequences were denoised, and unique sequences differing by a single nucleotide were identified as amplicon sequence variants (ASVs). Taxonomic annotation of representative sequences was performed against the SILVA database. Sequences assigned to plant-derived organelles (chloroplasts and mitochondria) or those that could not be classified at the phylum level were removed from subsequent analyses. For comparison with previous studies, sequences sharing  $\geq 97\%$  similarity were clustered into the same operational taxonomic unit (OTU).

## 2.6 Statistical Analysis

Sequence data were rarefied to an equal sequencing depth using the vegan package in R. Based on the rarefied OTU table, alpha diversity indices were calculated using USEARCH (Edgar 2010). Bray–Curtis dissimilarity matrices were calculated using the `vegdist()` function in the vegan package, and principal coordinates analysis (PCoA) was performed using the `cmdscale()` function. The effects of different treatments on microbial community structure were evaluated using permutational multivariate analysis of variance (PERMANOVA) implemented in the `adonis()` function of the vegan package. Pairwise comparisons of microbial community structure among treatments were conducted using the `pairwiseAdonis` package (Sun et al. 2022).

Taxonomic composition was visualized as stacked bar plots using the `taxa` plugin in QIIME 2. Differentially abundant microbial biomarkers among treatments were identified using linear discriminant analysis effect size (LEfSe) through the Galaxy online platform (Segata et al. 2011). Microbial co-occurrence network analysis and Zi–Pi analysis were performed using the `microeco` package (Liu et al. 2021). Network files were exported in .gexf format and visualized using Gephi v0.10.1. All statistical analyses and graphical visualizations were performed using R v4.4.1 (R Foundation for Statistical Computing, Vienna, Austria), GraphPad Prism v8.0 (GraphPad Software, San Diego, CA, USA), and the `ggplot2` package.

## 2.7 Statistical analysis

Means and stand error values were calculated using SPSS software version 19. Permutation testing was used to analyze differences at the community level in ANOSIM. Duncan’s multiple comparison was used to calculate the significance among samples. GraphPad Prism version 7.0 (GraphPad Software Inc., San Diego, CA, USA; <https://www.graphpad.com>) and R (version: 4.3).

### 3. Results

#### 3.1 Phenotypic responses of sorghum-soybean intercropping under saline-alkaline stress

Saline-alkaline stress significantly affected the growth and biomass accumulation of sorghum and soybean under different cropping systems (Fig. 1). Phenotypic observations showed that both soybean and sorghum exhibited better overall growth performance under the intercropping system than under their respective monoculture treatments (Fig. 1A). Biomass analysis showed that, compared with soybean monoculture (MG), soybean grown in the sorghum–soybean intercropping system (IG) exhibited significantly greater aboveground and root dry biomass (Figs. 1B, C). Specifically, aboveground dry biomass increased by 14.86%, whereas root dry biomass increased by 22.37%. Similarly, compared with sorghum monoculture (MS), sorghum grown under intercropping (IS) also exhibited significantly higher aboveground and root dry biomass (Figs. 1D, E). Aboveground dry biomass increased by 13.79%, whereas root dry biomass increased by 35.80%, indicating a particularly strong promotion of root growth. Overall, sorghum–soybean intercropping significantly enhanced biomass accumulation in both crops under saline–alkaline stress, with the greatest improvements observed in the aboveground biomass of soybean and the root biomass of sorghum.

Compared with the monoculture systems, the sorghum–soybean intercropping system significantly altered the physicochemical properties and salinity characteristics of the rhizosphere soil under saline–alkaline conditions (Table 1). Specifically, intercropping significantly decreased rhizosphere soil pH and exchangeable sodium percentage (ESP), with soil pH decreasing from 9.52–9.95 under monoculture to 9.26, and ESP decreasing from 47.7%–60.7% to 41.2%. In addition, intercropping significantly increased the contents of alkali-hydrolyzable nitrogen (AN), available phosphorus (AP), available potassium (AK), and cation exchange capacity (CEC) in the rhizosphere soil by 7.1%–22.2%, 17.5%–35.8%, 9.6%–13.8%, and 5.3%–9.6%, respectively, compared with the monoculture treatments. Overall, sorghum–soybean intercropping markedly improved the physicochemical properties of the rhizosphere soil under saline–alkaline stress by reducing soil alkalinity and enhancing nutrient availability.

Table 1 Differences in rhizosphere soil physicochemical properties and saline-alkali characteristics under different planting patterns

| Crops           | pH           | Alkaline<br>hydrolyzable<br>nitrogen(mg/kg) | Olsen<br>phosphorus<br>(mg/kg) | Available<br>potassium<br>(mg/kg) | Exchangeable<br>Sodium<br>(cmol /kg) | Cation Exchange<br>Capacity<br>(cmol (+)/kg) | Exchangeable<br>Sodium<br>Percentage (%) |
|-----------------|--------------|---------------------------------------------|--------------------------------|-----------------------------------|--------------------------------------|----------------------------------------------|------------------------------------------|
| Sorghum         | 9.52±0.43 ab | 42.69±0.63 b                                | 12.79±0.17 b                   | 72.17±0.93 b                      | 2.54±0.12 bc                         | 5.33±0.03 b                                  | 47.7±2.31 b                              |
| Soybean         | 9.95±0.12 a  | 48.72±0.53 b                                | 14.78±0.59 b                   | 69.53±1.26 b                      | 3.11±0.35 a                          | 5.12±0.21 b                                  | 60.7±7.28 a                              |
| Sorghum/soybean | 9.26±0.37 b  | 52.17±0.96 a                                | 17.37±0.29 a                   | 79.11±2.04 a                      | 2.31±0.24 c                          | 5.61±0.09 a                                  | 41.2±4.33 b                              |

Under saline–alkaline stress, the sorghum–soybean intercropping system significantly regulated the physiological and metabolic characteristics of both crops, thereby effectively alleviating stress-induced physiological damage. Compared with monocropping, intercropping markedly enhanced the activities of antioxidant enzymes in the leaves of both sorghum and soybean. Specifically, CAT activity in sorghum leaves increased by approximately 17.19%, while SOD and CAT activities in soybean leaves increased by approximately 2.35% and 5.85%, respectively, under intercropping. These results indicate that intercropping substantially improved the antioxidant defense capacity of both crops against oxidative stress induced by saline–alkaline conditions.

Concomitant with the improvement of the rhizosphere microenvironment and the alleviation of overall stress in the intercropping system, the accumulation of stress-responsive metabolites in the roots of both crops declined significantly. Compared with monocropping, root ABA content decreased by approximately 8.41% in sorghum and 9.53% in soybean, indicating that the excessive stress-induced hormonal response was effectively mitigated. Meanwhile, root H<sub>2</sub>O<sub>2</sub> content decreased significantly by 23.38%-72.31%, thereby reducing oxidative damage caused by excessive reactive oxygen species (ROS) accumulation.

This phenomenon may be attributed to the contrasting stress environments experienced under different cropping systems. Under monocropping, crops are continuously exposed to severe saline–alkaline stress, which stimulates excessive ABA biosynthesis to activate stress-defense responses, consequently exacerbating cellular oxidative imbalance. In contrast, the complementary interactions between sorghum and soybean in the intercropping system alleviate the intensity of saline–alkaline stress, reduce the degree of stress experienced by plants, and thereby suppress the excessive synthesis and accumulation of ABA. At the same time, the enhanced activities of antioxidant enzymes efficiently scavenged excess H<sub>2</sub>O<sub>2</sub>, further alleviating membrane lipid peroxidation in the roots.

Overall, intercropping maintained physiological and metabolic homeostasis in both crops through a dual regulatory mechanism involving the enhancement of antioxidant enzyme activities and the reduction of stress hormone and reactive oxygen species accumulation, thereby significantly improving the adaptability of sorghum and soybean to saline–alkaline environments.

### 3.2 Analysis of Rhizosphere Microbial Community Structure and Diversity under Different Cropping Systems

The rhizosphere bacterial communities of soybean and sorghum were analyzed by sequencing the 16S rRNA gene using the Illumina MiSeq platform. After the removal of chimeric and low-quality sequences, approximately 30,123 high-quality reads were obtained from each sample. As shown in Figure 3, different cropping systems significantly altered the characteristics of rhizosphere bacterial communities in saline-alkaline soils.

The  $\alpha$ -diversity analysis showed that the Shannon index of rhizosphere bacterial communities in the intercropping interaction zone (IS/IG) was significantly higher than those in soybean monocropping (MG) and sorghum monocropping (MS), indicating a marked increase in community diversity. Principal coordinates analysis (PCoA) based on the Bray-Curtis distance further demonstrated clear clustering and separation among the three treatments, with significant differences in bacterial community composition ( $P = 0.041$ ), suggesting that cropping system was a major factor driving rhizosphere bacterial community differentiation. The Venn diagram analysis revealed that the three treatments shared 107 core bacterial operational taxonomic units (OTUs). Compared with the monocropping systems, the intercropping interaction zone retained most of the core bacterial taxa while enriching 15 unique OTUs. At the phylum level, Proteobacteria and *Actinobacteriota* were the dominant bacterial groups, with the relative abundance of *Actinobacteriota* being markedly higher in the intercropping interaction zone. Differential abundance analysis further showed that the intercropping interaction zone was significantly enriched in functional taxa associated with nutrient cycling and stress adaptation, such as the order *Rhizobiales*, whereas monocropping systems exhibited higher proportions of several salt-tolerant oligotrophic taxa. Overall, intercropping significantly increased the diversity of rhizosphere bacterial communities, reshaped their community structure, and selectively enriched beneficial functional microorganisms. These changes provide an important microbial basis for improving the rhizosphere environment and enhancing the adaptation of sorghum and soybean to saline-alkaline conditions.

Different cropping systems significantly altered the co-occurrence network structure and keystone node characteristics of rhizosphere bacterial communities in saline-alkaline soils (Fig. 4). The bacterial co-occurrence network in the intercropping interaction zone (IS/IG) exhibited substantially greater complexity and connectivity than those in the two monocropping systems. Specifically, the IS/IG network had the highest numbers of nodes and edges, as well as the greatest average degree, indicating a more densely connected network with stronger interactions among microbial taxa and more extensive intermodule associations. In contrast, the soybean monocropping (MG) network contained the fewest nodes and edges and exhibited the lowest connectivity, suggesting relatively simple microbial interactions.

The Zi-Pi node role analysis further demonstrated that the IS/IG and sorghum monocropping (MS) networks were dominated by peripheral nodes, with a small number of connectors, indicating a certain degree of modular organization and network stability. By contrast, no apparent hub nodes were identified in the MG network, which exhibited a looser network topology and weaker microbial associations. Overall, intercropping markedly enhanced the connectivity and interaction strength of the rhizosphere

bacterial co-occurrence network, resulting in a more complex and stable microbial interaction system. Such a network is expected to improve community resistance to saline–alkaline stress and increase functional redundancy, thereby providing a microbial interaction-based foundation for maintaining rhizosphere microenvironment stability and promoting crop growth.

The structural equation model (SEM) demonstrated that the cropping system regulated soil–microbe–crop interactions in the sorghum–soybean system under saline–alkaline conditions through multiple coordinated pathways (**Fig 5**). Intercropping significantly improved rhizosphere soil properties ( $\beta = -0.56^{***}$ ), which not only directly promoted the assembly of rhizosphere bacterial communities ( $\beta = 0.28^*$ ) but also indirectly reshaped the microbial community by improving soil physicochemical properties ( $\beta = 0.62^{**}$ ). The optimized microbial community subsequently exerted a significant positive effect on the physiological status associated with stress tolerance ( $\beta = 0.387^{**}$ ), and the maintenance of physiological homeostasis directly promoted crop growth ( $\beta = 0.546^{***}$ ). In addition, the microbial community had a significant direct positive effect on crop growth ( $\beta = 0.291^*$ ). In contrast, neither the direct effect of rhizosphere soil properties on crop growth ( $\beta = 0.124$ ) nor the direct effect of cropping system on plant physiological status ( $\beta = -0.15$ ) was significant. These findings indicate that the yield stability advantage of intercropping was primarily mediated through improvements in the structure of the rhizosphere bacterial community, which subsequently enhanced soybean salt tolerance and ultimately promoted crop growth.

Fig. 6 provides a schematic summary of the cascade regulatory pathway involving rhizosphere soil improvement, microbial community restructuring, oxidative stress alleviation, and growth promotion, thereby illustrating the integrated response mechanism of the sorghum–soybean intercropping system under saline–alkaline stress. Specifically, intercropping improved rhizosphere soil physicochemical properties, as reflected by reduced rhizosphere pH and improved ion homeostasis. These changes facilitated the enrichment of beneficial microbial taxa and increased the complexity of microbial co-occurrence networks, resulting in the restructuring of the rhizosphere bacterial community. The optimized microbial community subsequently alleviated oxidative stress by reducing ABA and  $H_2O_2$  accumulation while enhancing antioxidant enzyme activities. Consequently, the maintenance of physiological homeostasis and the improvement of photosynthetic performance jointly promoted biomass accumulation and overall crop growth.

Collectively, this cascade regulatory pathway demonstrates that the yield stability advantage of intercropping in saline–alkaline soils is not governed by a single factor but arises from the synergistic interactions among improvements in soil physicochemical properties, regulation of the rhizosphere microbial community, and enhancement of plant stress tolerance. These findings provide a systematic mechanistic framework for understanding the productivity-enhancing effects of cereal–legume intercropping in saline–alkaline soils and offer both theoretical support and practical guidance for the efficient utilization of saline–alkaline land resources, the maintenance of soil fertility, and the development of sustainable agricultural systems.

## 4. Discussion

### 4.1 Growth Performance and Biomass Accumulation of Sorghum and Soybean under Different Cropping Systems under Saline–Alkaline Stress

Saline–alkaline stress markedly inhibited biomass accumulation (shoot and root dry weight) and relative growth rate in both soybean monocropping (MG) and sorghum monocropping (MS), which is consistent with previous findings. For example, (Li et al. 2025) reported that treatment with 120 mM NaCl significantly suppressed the growth of both the salt-sensitive soybean cultivar *Dongnong 50* and the salt-tolerant cultivar *Qihuang 34*. Moreover, the inhibitory effect became more pronounced with increasing salinity. Compared with *Dongnong 50*, *Qihuang 34* exhibited superior growth performance, higher antioxidant enzyme activities, and lower malondialdehyde (MDA) accumulation under salt stress. Their study further demonstrated that salt stress activated flavonoid biosynthesis, glutathione metabolism, and ATP-binding cassette (ABC) transporter pathways, thereby enhancing the adaptive capacity of soybean to saline conditions.

Increasing farmland biodiversity has been recognized as an effective strategy for achieving sustainable agricultural intensification while mitigating resource limitations and environmental stresses. Previous studies have shown that cereal–legume intercropping systems can improve overall crop productivity by enhancing the utilization efficiency of light, water, and soil nutrients while reducing interspecific competition. Consistent with these findings, the present study demonstrated that, compared with soybean monocropping (MG), both soybean (IG) and sorghum (IS) grown under intercropping exhibited significantly greater shoot and root dry weights.

Overall, these results indicate that sorghum–soybean intercropping effectively promoted biomass accumulation under saline–alkaline stress, with particularly pronounced improvements in soybean shoot biomass and sorghum root biomass. These findings provide direct evidence that intercropping enhances crop growth and contributes to the yield stability of sorghum–soybean production systems in saline–alkaline soils.

### 4.2 Effects of Saline–Alkaline Stress on Rhizosphere Soil Properties under MG, MS, and IG/IS Cropping Systems

Saline–alkaline stress markedly affected the physicochemical properties and salinity–alkalinity characteristics of the rhizosphere soil under the MG, MS, and IG/IS cropping systems. Compared with the monocropping treatments (MG and MS), intercropping (IG/IS) significantly reduced rhizosphere soil pH and exchangeable sodium percentage (ESP). Specifically, soil pH decreased from 9.52–9.95 under monocropping to 9.26 under intercropping, whereas ESP declined from 47.7%–60.7% to 41.2%. These improvements may be attributed to enhanced root exudation of organic acids (e.g., oxalic acid and citric acid) by sorghum and soybean under intercropping. These organic acids not only directly neutralize soil alkalinity but also mobilize sparingly soluble calcium through chelation, thereby promoting the

displacement of exchangeable sodium from the soil matrix (Zhang et al. 2025). Intercropping also significantly increased the contents of alkaline-hydrolyzable nitrogen, available phosphorus, available potassium, and the cation exchange capacity (CEC) of the rhizosphere soil through complementary resource utilization between the two crop species. Compared with monocropping, these parameters increased by 7.1%–22.2%, 17.5%–35.8%, 9.6%–13.8%, and 5.3%–9.6%, respectively, indicating that intercropping enhanced soil nutrient availability and improved nutrient acquisition by both sorghum and soybean. Similar findings have been reported in maize–soybean intercropping systems. Soybean roots release substantially greater amounts of flavonoids, such as quercetin and genistein, than maize roots. These compounds act as signaling molecules that specifically recruit *Bradyrhizobium* spp. into the maize rhizosphere. The recruited rhizobia promote maize root elongation, increase root surface area and volume, enhance the availability of phosphorus fractions, including available and organic phosphorus, and strengthen the microbial functional potential associated with carbon, nitrogen, and phosphorus cycling, thereby creating a more fertile rhizosphere environment for maize growth (Zhang et al. 2026a).

Overall, our results suggest that intercropping effectively improved the rhizosphere environment under saline–alkaline stress by reducing rhizosphere alkalization, optimizing nutrient availability, and maintaining ion homeostasis. These improvements provided favorable soil conditions for biomass accumulation, enhanced photosynthetic performance, and the maintenance of antioxidant defense systems in both sorghum and soybean.

## 4.3 Rhizosphere Microbial Community Restructuring in the Sorghum–Soybean Intercropping System under Saline-Alkaline Stress

Saline stress is one of the major environmental constraints limiting global crop productivity (Hassani et al. 2021). Soybean, an important oilseed and protein crop, is highly sensitive to salinity, and soil salinization severely restricts its growth, nodulation, and yield (Lu et al. 2024). Although conventional breeding for salt-tolerant cultivars remains an effective strategy, its application is often constrained by the long breeding cycle and relatively low efficiency. Consequently, the use of plant growth-promoting rhizobacteria (PGPR) to enhance crop salt tolerance has emerged as a sustainable and environmentally friendly approach (Rath et al. 2019). In parallel, intercropping has been widely recognized as an effective agronomic practice for improving crop productivity and soil quality by reshaping rhizosphere microbial communities. However, the microbial mechanisms by which intercropping enhances soybean salt tolerance remain poorly understood. In the present study, the sorghum–soybean intercropping system markedly restructured the rhizosphere bacterial community under saline–alkaline conditions. Intercropping significantly enriched beneficial bacterial genera, including *Bacillus* and *Pseudomonas*, while increasing the complexity of the microbial co-occurrence network and the proportion of positive microbial interactions. Furthermore, the structural equation model (SEM) indicated that intercropping primarily enhanced soybean salt tolerance by regulating microbial interactions, which subsequently improved ion homeostasis, alleviated oxidative stress, and ultimately promoted plant physiological performance. Overall, our findings demonstrate that sorghum–soybean intercropping enhances soybean

salt tolerance through the coordinated improvement of the saline–alkaline rhizosphere environment and the restructuring of beneficial rhizosphere microbial communities. These results provide new mechanistic insights into the role of rhizosphere microorganisms in intercropping systems and offer a theoretical basis for optimizing soybean production and developing sustainable intercropping practices in saline–alkaline soils.

## Declarations

## Compliance with ethics guidelines

All the authors declare that they have no conflict of interest or financial conflicts to disclose.

## Acknowledgements

We thank Prof. Qing Yang in the Institute of Grain and Oil Crops, Hebei Academy of Agriculture and Forestry Sciences, for providing the soybean seeds (Glycine Max L., JiDou 12). This research was supported by the Shanxi Provincial Research Project Award for Doctoral Graduates and Postdoctoral Researchers Coming to Work in Shanxi Province(SXBYKY2026093); and the Shanxi Provincial Key Research and Development Program (202502140602068).

## Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

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## Figures

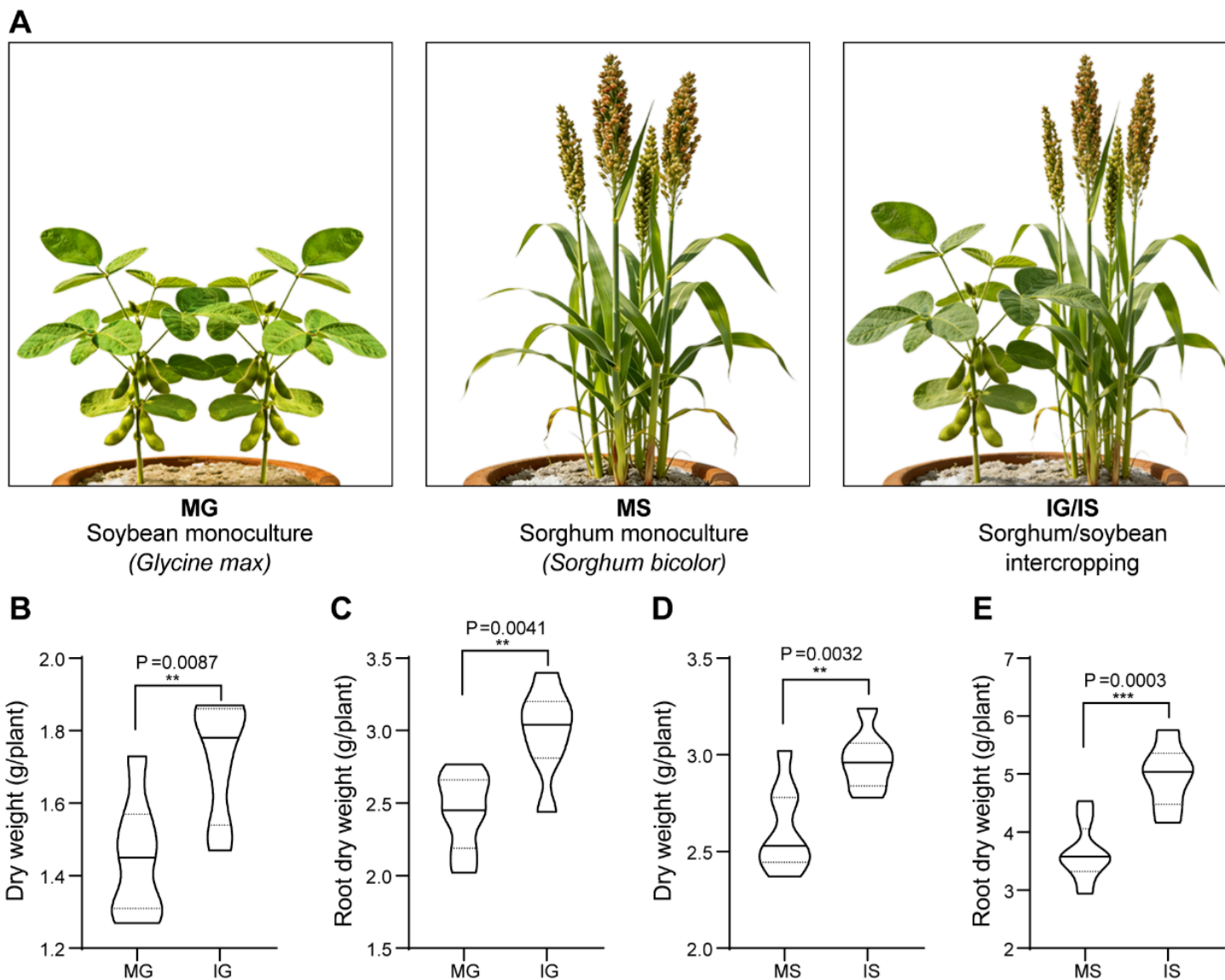


**Figure 1**

Plant morphology and biomass differences of sorghum and soybean under monocropping and intercropping patterns under saline-alkali stress

Note: MG: Glycine max monocropping; MS: Sorghum bicolor monocropping; IG: intercropped soybean; IS: intercropped sorghum. In the box plots, the boxes represent the interquartile range (IQR), the horizontal line inside the box is the median, and the whiskers indicate the range of the data distribution. Asterisks represented significant differences between IS and IG plants for the same trait in t-tests. \*\*:  $0.001 < P \leq 0.01$ ; \*\*\*:  $P \leq 0.001$ .

| Crops           | pH             | Alkaline<br>hydrolyzable<br>nitrogen(mg/kg) | Olsen<br>phosphorus<br>(mg/kg) | Available<br>potassium<br>(mg/kg) | Exchangeable<br>Sodium<br>(cmol /kg) | Cation Exchange<br>Capacity<br>(cmol (+)/kg) | Exchangeable<br>Sodium<br>Percentage (%) |
|-----------------|----------------|---------------------------------------------|--------------------------------|-----------------------------------|--------------------------------------|----------------------------------------------|------------------------------------------|
| Sorghum         | 9.52 ± 0.43 ab | 42.69 ± 0.63 b                              | 12.79 ± 0.17 b                 | 72.17 ± 0.93 b                    | 2.54 ± 0.12 bc                       | 5.33 ± 0.03 b                                | 47.7 ± 2.31 b                            |
| Soybean         | 9.95 ± 0.12 a  | 48.72 ± 0.53 b                              | 14.78 ± 0.59 b                 | 69.53 ± 1.26 b                    | 3.11 ± 0.35 a                        | 5.12 ± 0.21 b                                | 60.7 ± 7.28 a                            |
| Sorghum/soybean | 9.26 ± 0.37 b  | 52.17 ± 0.96 a                              | 17.37 ± 0.29 a                 | 79.11 ± 2.04 a                    | 2.31 ± 0.24 c                        | 5.61 ± 0.09 a                                | 41.2 ± 4.33 b                            |

**Figure 2**

Differences in antioxidant enzyme activities, ABA content, and H<sub>2</sub>O<sub>2</sub> levels between monocropping and intercropping systems of sorghum and soybean under saline-alkaline stress.

**Note:** SOD, superoxide dismutase; CAT, catalase; ABA, abscisic acid; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; FW, fresh weight. Data are presented as the mean ± standard error (SE). ns indicates no significant difference, whereas \*\* and \*\*\* indicate significant differences at P < 0.01 and P < 0.001, respectively.

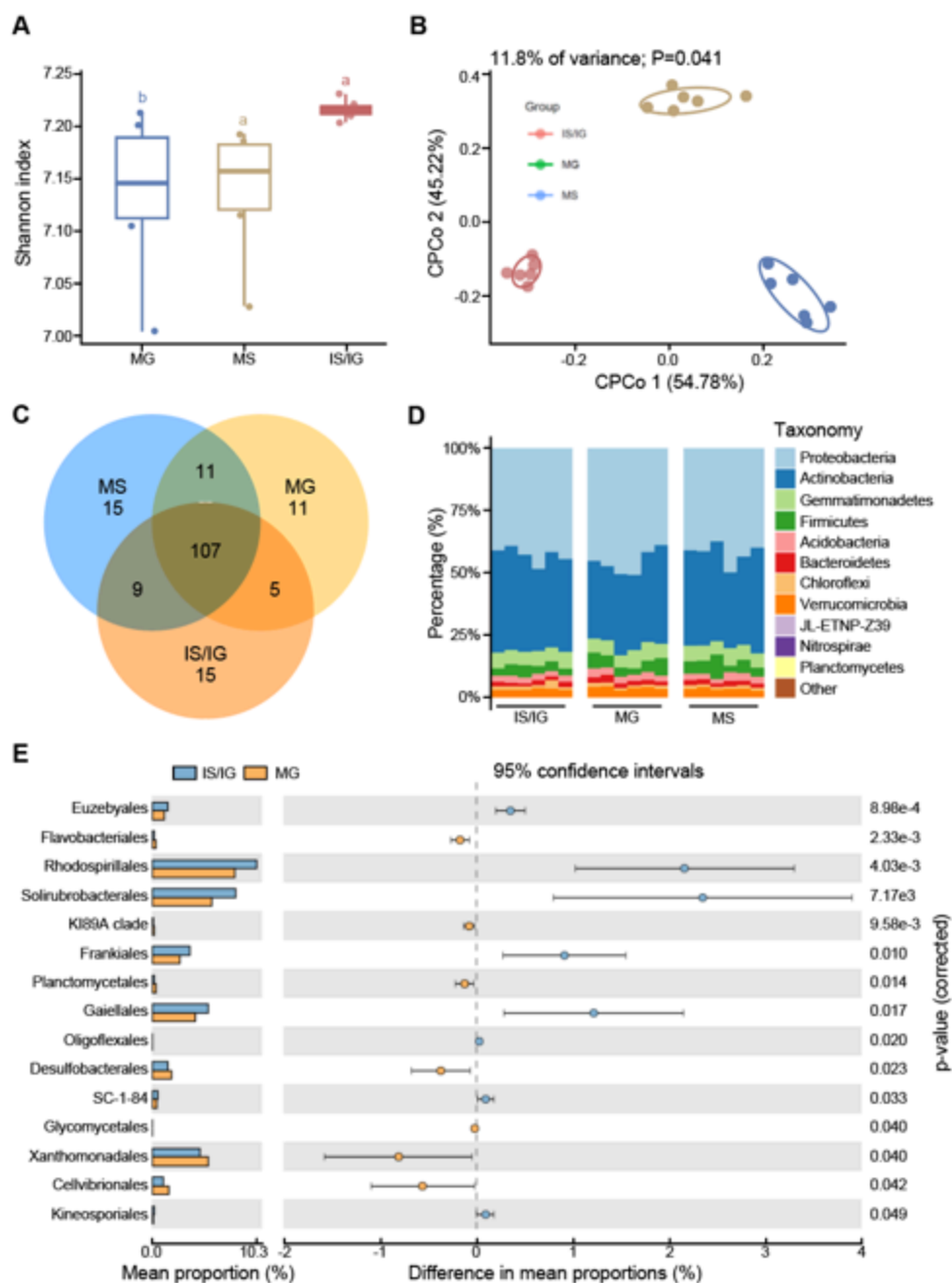


**Figure 3**

Diversity, composition, and differential characteristics of rhizosphere bacterial communities under different cropping systems.

**Note:** MG, soybean monocropping rhizosphere; MS, sorghum monocropping rhizosphere; IS/IG, rhizosphere of the sorghum–soybean intercropping interaction zone. (A) Shannon diversity index ( $\alpha$ -diversity); different lowercase letters indicate significant differences among treatments ( $P < 0.05$ ). (B) Principal coordinates analysis (PCoA) based on the Bray–Curtis distance, showing differences in bacterial community structure among treatments. (C) Venn diagram showing the distribution of bacterial operational taxonomic units (OTUs) among treatments. (D) Relative abundance of bacterial communities

at the phylum level. **(E)**Differentially abundant bacterial taxa between the intercropping interaction zone (IS/IG) and the soybean monocropping rhizosphere (MG).

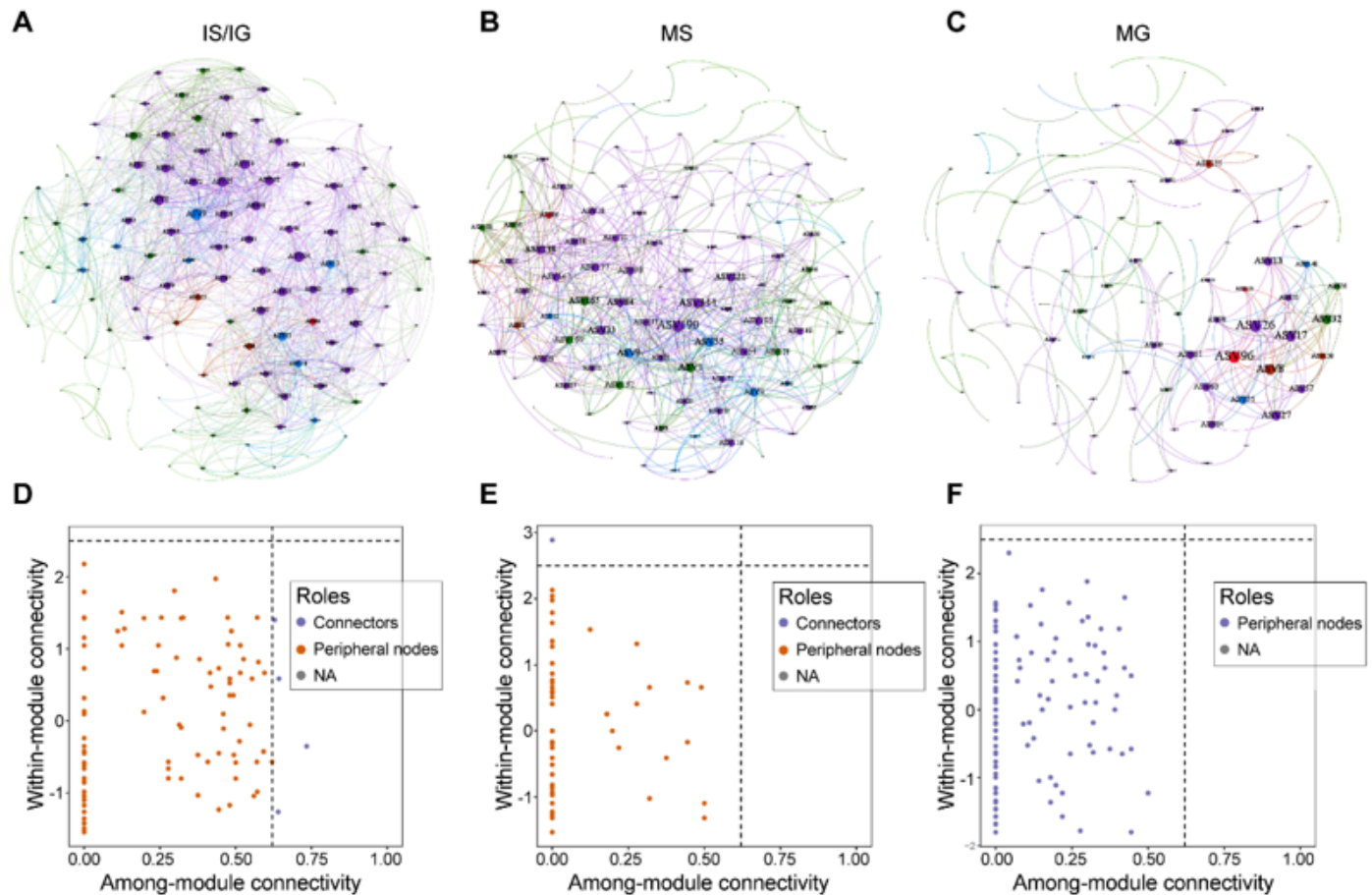


**Figure 4**

Co-occurrence network and keystone taxa characteristics of rhizosphere bacterial communities under different planting patterns

**Note:** IS/IG, rhizosphere of the sorghum–soybean intercropping interaction zone; MS, sorghum monocropping rhizosphere; MG, soybean monocropping rhizosphere. **(A–C)**Co-occurrence networks of rhizosphere bacterial communities under the IS/IG, MS, and MG treatments, respectively. Nodes represent amplicon sequence variants (ASVs), and edges represent significant correlations. Different colors indicate different network modules. Node color and size are not additionally annotated, whereas edge thickness reflects the strength of the correlation. **(D–F)**Node role classification based on Zi-Pi analysis for the IS/IG, MS, and MG treatments, respectively. Orange dots represent peripheral nodes, whereas blue dots represent connectors/module hubs.

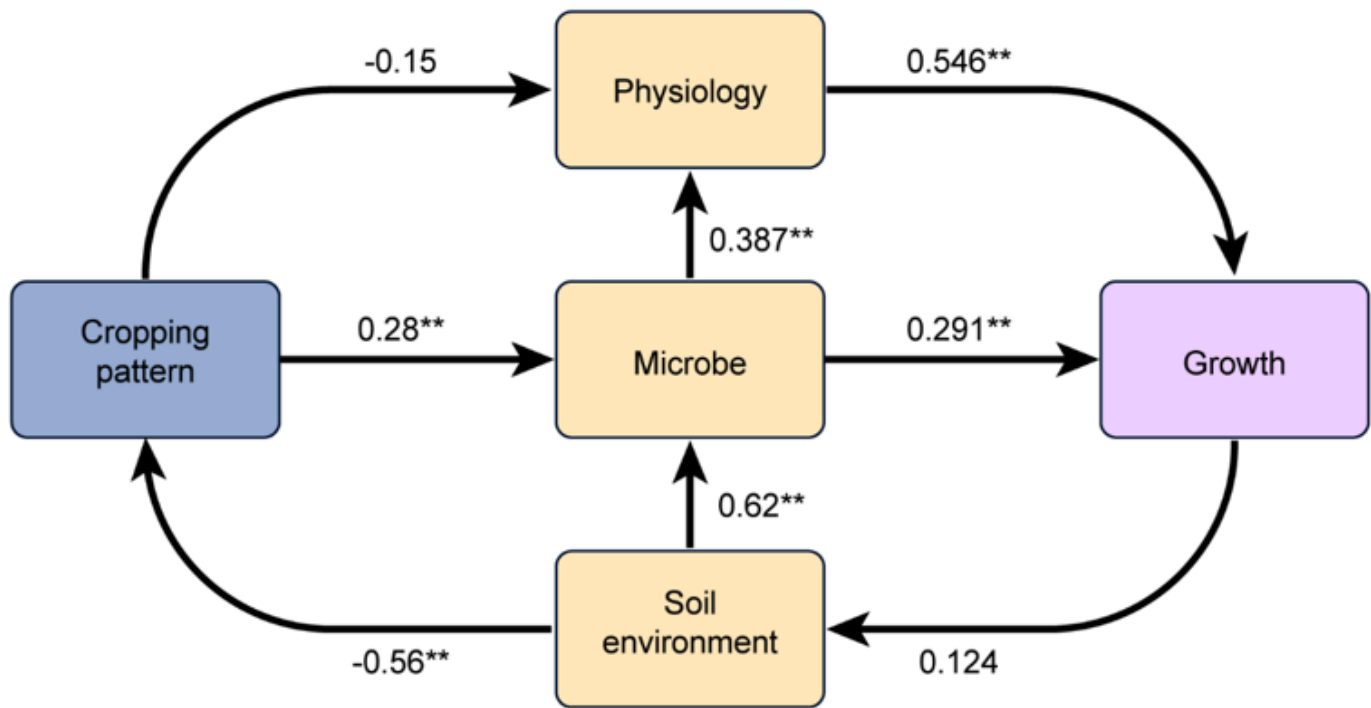


**Figure 5**

Structural equation model (SEM) depicting the effects of cropping pattern on soil environment, microbe, physiology, and growth.

**Note:** Rectangles represent latent variables. Numbers adjacent to the arrows indicate standardized path coefficients, and arrow thickness is proportional to the magnitude of the corresponding path coefficient. Significance levels are denoted as \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , and \*  $P < 0.05$ .

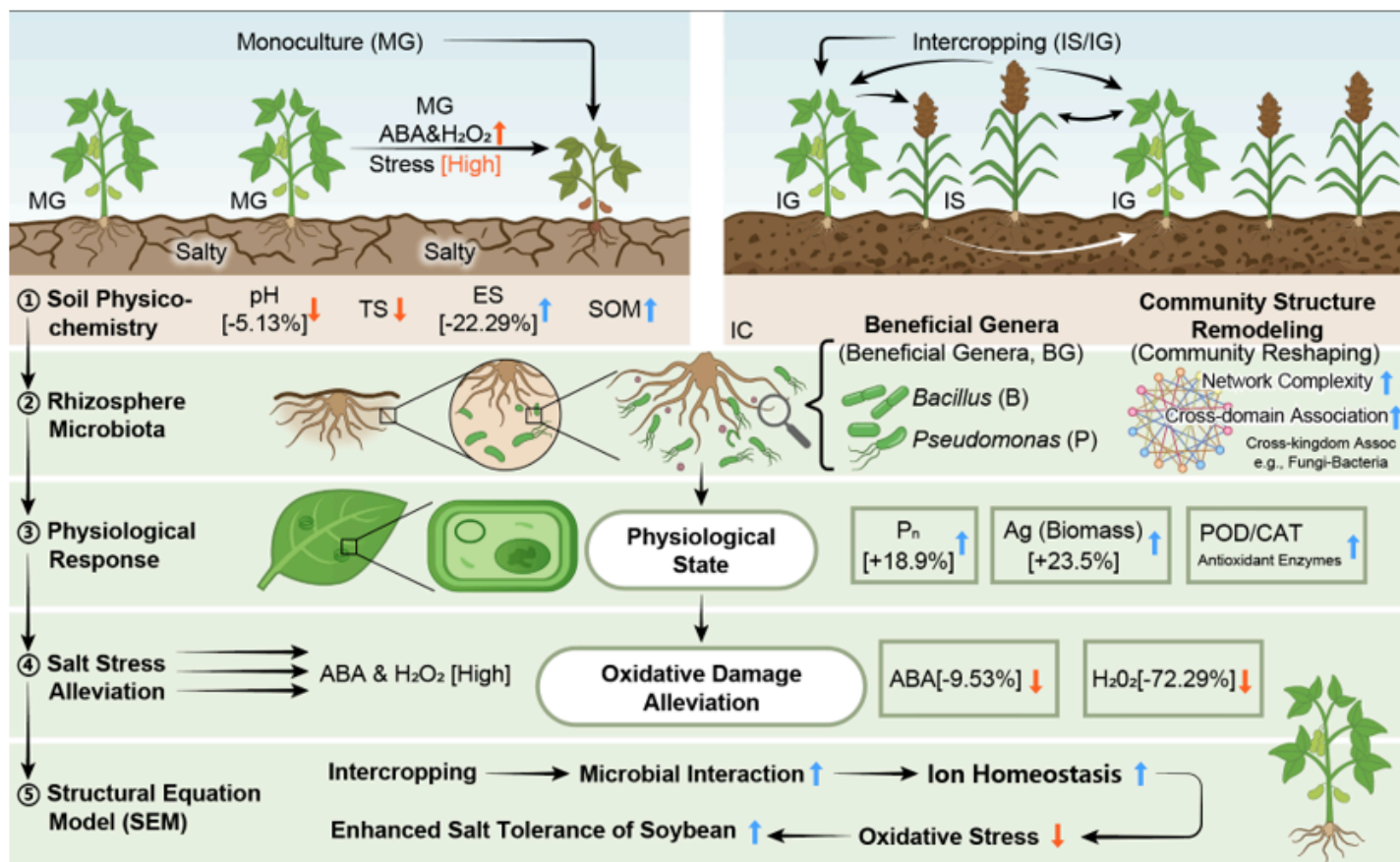


**Figure 6**

Schematic illustration of the multi-pathway synergistic mechanism by which intercropping alleviates saline–alkaline stress in the sorghum-soybean system.

**Note:** In the monoculture system, saline-alkali stress significantly inhibits soybean growth, which is characterized by elevated rhizosphere pH and exchangeable sodium content, reduced soil organic matter, excessive accumulation of endogenous ABA and  $H_2O_2$ , and suppressed antioxidant enzyme activities. Conversely, the intercropping system synergistically enhances the saline-alkali tolerance of soybean through multiple pathways:  $\square$ improving soil physicochemical properties, by decreasing pH, alkalinity, and exchangeable sodium, while increasing organic matter content;  $\square$ reshaping the rhizosphere microbial community, through enriching beneficial genera and increasing the complexity of the co-occurrence network;  $\square$  enhancing crop photosynthetic performance and the antioxidant system, thereby increasing the net photosynthetic rate, biomass, and antioxidant enzyme activities;  $\square$  alleviating oxidative damage, by significantly reducing endogenous ABA and  $H_2O_2$  contents.  $\square$  The structural equation modeling (SEM) further elucidates the cascade regulatory pathway, demonstrating that intercropping drives rhizosphere microbial interactions, improves ion homeostasis, and mitigates oxidative stress, thereby promoting the enhancement of soybean salt tolerance.