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Pseudomonas donghuensis S-1 Alleviates Saline-Alkali Stress in Wheat and Affects Rhizosphere Microbial Community

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Abstract

Saline and alkaline stresses are the major abiotic stress that limit crop growth and yield. The role of plant growth-promoting rhizobacteria (PGPR) in alleviating salt stress has been widely investigated. However, there are relatively few studies on *Pseudomonas* regulatory mechanisms on the stress tolerance of wheat under mixed saline-alkali stress. A strain of *Pseudomonas donghuensis* S-1, exhibiting siderophore production (48.90 mg/L), indole-3-acetic acid (IAA) synthesis (7.28 mg/L), and phosphate solubilization (21.50 mg/L), was isolated from saline soil. Different concentrations (1.5, 3.0 and 4.5 g/kg) of mixed salt solution (NaCl + $NaHCO_3$) was set to investigate the effect of S-1 on the growth, antioxidant capacity and osmotic regulation of wheat seedlings and soil physicochemical properties (e.g., pH, electrical conductivity, enzyme activity, and microbial diversity) under saline–alkali stress. The results showed that S-1 inoculation significantly enhanced wheat seedling growth by increasing plant height, stem diameter, leaf area, root length, and fresh and dry weights of both shoots and roots with the most significant effect under high-concentration saline-alkali stress treatment. Meanwhile, S-1 increased the activities of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) and the total antioxidant capacity (DPPH), while reducing malondialdehyde (MDA) content. and balancing the contents of osmotic regulatory substances (proline, soluble protein, and soluble sugars). Furthermore, S-1 improved soil physicochemical properties and enzyme activities. Soil pH and electrical conductivity were decreased significantly. S-1 also markedly enhanced the activities of soil enzymes (urease, alkaline phosphatase, sucrase, and β-glucosidase) and increased soil microbial biomass carbon (MBC) and nitrogen (MBN). Importantly, S-1 substantially affected the rhizosphere microbial community, elevating the relative abundance of the beneficial genus *Streptomyces* from 2.01% to 3.90% under severe stress. This study provides theoretical insights and demonstrates the potential application of screened saline-alkali tolerant PGPR strains for enhancing crop resilience in saline-alkali affected soils.

Introduction

Soil salinity and alkalinity, as one of the major obstacles to achieving global food security and sustainable agricultural development, is the critical environmental issue that constrain crop growth, yield, and the efficient utilization of land resources [1, 2]. According to statistics, the total area of saline-alkali land in the world is approximately 954.3 million ha, while in China, saline-alkali land accounts for 25% of the total arable land area [3]. As a vital global staple crop, increasing wheat yield is crucial for ensuring global food security [4]. Soil salinization is primarily caused by the accumulation of neutral and alkaline salts. Compared to single salt or alkali stress, combined saline-alkali stress poses a more severe threat to plants and soil, leading to osmotic stress, ion toxicity, oxidative stress, nutrient deficiency, and elevated pH [5, 6]. The primary factor inhibiting plant growth in saline-alkali soils is Na^+. Excessive Na^+ can disrupt chloroplast and cellular membrane structures, thereby weakening photosynthesis. Meanwhile, saline-alkali stress also inhibits the absorption of K^+ and Ca^{2+} in plants, affecting nutrient uptake and utilization. Additionally, under saline-alkali stress, plant cells generate reactive oxygen species (ROS). Excessive ROS production leads to oxidative damage in cells, resulting in the damage of proteins, lipids, and membranes, which ultimately causes growth retardation and yield decline [7–9]. Therefore, investigating the mechanisms by which wheat alleviates saline-alkali stress and identifying safe and effective strategies to enhance stress tolerance are crucial for improving crop resilience and advancing sustainable agricultural development.

Plants cope with saline-alkali stress by accumulating osmotic regulators, enhancing ROS scavenging capacity, and reducing cellular oxidative damage [10, 11]. Moreover, plants initiate a series of signal transduction pathways from the perception of salt stress to the expression of various salt stress-responsive genes. These pathways regulate processes such as ion transport, osmotic homeostasis, and detoxification [12]. Numerous studies have indicated that plant growth-promoting rhizobacteria (PGPR), composed of various bacteria, fungi, and other microorganisms, have gradually recognized as an effective approach to alleviate saline-alkali stress. Among these PGPR, some can enhance plant growth under abiotic stress conditions such as salinity and drought, and have given rise to inducing stress tolerance [13]. Different bacterial genera have been proposed to alleviate salt stress in plants, such as *Agrobacterium*, *Bacillus*, *Arthrobacter*,

Azotobacter, *Pseudomonas*, and *Rhizobium* [14]. PGPR not only enhances absorption of nutrients and water, but also produces indole-3-acetic acid (IAA), iron carriers and can solubilize inorganic phosphorus [15, 16]. For example, Fatma et al. demonstrated that the *Halotolerant Bacillus spizizenii* FMH45 enhanced the growth, physiological and antioxidant parameters of tomato plants under salt stress [17]. *Stutzerimonas stutzeri* A38 and *Bacillus pumilus* A49 significantly promoted root development in Medicago sativa and Medicago polymorpha plants exposed to osmotic stress[18]. *Enterobacter aerogenes* LJL-5 and *Pseudomonas aeruginosa* LJL-13 synergistically increased alfalfa biomass under saline-alkali conditions, and improved phosphorus content and the activities of antioxidant enzymes such as superoxide, dismutase, peroxidase and catalase [19]. A research has revealed the amelioration in plant health, yield and uptake of available zinc from rhizospheric soil to straw and grain after inoculation of *Pseudomonas protegens* CP17, along with enhanced dehydrogenase and phosphatase activities of rhizosphere soil under saline and non-saline conditions [20]. In addition, PGPR can contribute to the biocontrol of plant diseases by modulating plant defense systems, including salicylic acid (SA), ethylene (ET), and jasmonic acid (JA)-dependent defense signaling pathways [21]. Therefore, PGPR hold promise as biofertilizers to enhance crop tolerance to saline-alkali stress, thereby improving crop resilience and productivity.

Pseudomonas donghuensisis was first reported in 2015 with high-yields of siderophores [22]. Studies have demonstrated that *Pseudomonas donghuensisis* can inhibit the growth of fungal pathogens that would be powerful tools to act as a biocontrol agent [23]. However, the effect of *Pseudomonas donghuensisis* to alleviate abiotic stress has not been reported. In this study, a strain of *Pseudomonas donghuensis* S-1 was isolated from saline-alkali soil. Different concentrations of NaCl and $NaHCO_3$ solution (1.5 g/kg, 3 g/kg and 4.5 g/kg) were set to investigate the effects of S-1 on the growth of wheat seedlings, by detecting indicators including antioxidant enzyme activities, osmolyte content, soil physicochemical properties, soil enzyme activities and rhizosphere microbial diversity. This provides a theoretical basis for the application of *Pseudomonas donghuensis* S-1 under saline-alkali stress and for wheat cultivation in saline-alkali soils.

Materials and Methods

Plant Materials and Treatments

To isolate rhizosphere microorganisms, 10 g of rhizosphere soil collected from native plants in saline-alkali farmland in Yuepuhu County, Xinjiang, China (39.21°N,77.01°E), was added to 90 mL of sterile water to prepare a soil suspension. The suspension was shaken for 30 min and then allowed to settle for 10 min. A serial dilution method was employed to dilute the suspension to appropriate concentrations. Subsequently, 100 µL aliquots from different dilution gradients were evenly spread on Luria-Bertani (LB) solid medium plates and incubated at 28°C for 2–3 days. Single colonies were selected and purified by repeated streaking (more than three times) to obtain axenic cultures. 16S rRNA sequencing was performed by Majorbio Bio-pharm Technology Co., Ltd. in Shanghai. for identification of S-1, which identified the bacterium as belonging to the genus *Pseudomonas*. The nearly full-length 16S rRNA gene sequence of strain S-1 (approximately 1400 bp) was determined and deposited in GenBank under accession number **PZ123209**. The taxonomic positions of S-1 strain was verified using the List of Prokaryotic names with Standing in Nomenclature (LPSN; https://lpsn.dsmz.de/) on 10 March 2026 [24]. The strain *Pseudomonas donghuensis* S-1 was deposited in the China Center for Type Culture Collection (CCTCC) under accession number **CCTCC M 2026455**. To better observe the cellular morphology and structure, strain S-1 was examined under scanning electron microscopy (SEM). Samples were fixed on a specimen stub, coated with gold using an ion sputter coater for 240 seconds, and subsequently observed by SEM.

The wheat variety Xin Chun 37 was chosen as the plant material to evaluate the growth-promoting effect of S-1. Uniform and plump wheat seeds were surface-sterilized with 1% sodium hypochlorite for 5 min, rinsed with sterile water, followed by disinfection with 75% ethanol for 1 min, and then rinsed again with sterile water. Each treatment was as follows:

Normal (control), T1 (1.5 g/kg), T2 (3.0 g/kg), and T3 (4.5 g/kg), which represented normal cultivation conditions, low-concentration salt-alkali treatment, Medium-concentration salt-alkali treatment and high-concentration salt-alkali treatment respectively. For the soil culture, NaCl and $NaHCO_3$ were mixed at a 2:1 molar ratio. Different concentrations of saline-alkali stress were simulated by adding mixtures of NaCl and $NaHCO_3$ to the soil at rates of 0 g/kg, 1.5 g/kg, 3 g/kg, and 4.5 g/kg. Wheat seeds were sown in seedling trays and irrigated weekly with a 100-fold dilution of the LB liquid medium (control) or the S-1 bacterial suspension (10^9 CFU/mL). The treatment was applied once every seven days, a total of three times. The whole plant that was treated for 21 days was used as material for subsequent experiments. Each treatment was set up with three biological replicates.

Evaluation of the Bacterium Traits

To assess inorganic phosphate solubilization, the strain was inoculated at 1% (v/v) into National Botanical Research Institute's Phosphate (NBRIP) liquid medium and incubated at 30°C with shaking for 7 days. The soluble phosphorus content in the bacterial culture was determined using the molybdenum-antimony colorimetric method [25]. The ability to produce indole-3-acetic acid (IAA) was quantified through the Salkowski colorimetric assay [26]. The strain was inoculated at 1% (v/v) into NB medium without tryptophan and cultured at 30°C with shaking for 48 h to calculate OD_{530} and IAA content. To assess siderophore production ability, the Chrome Azurol S (CAS) assay [27] was used to determine the siderophore concentration in the culture supernatant.

Determination of Physiological and Biochemical Parameters in Wheat Seedlings

0.2 g leaves were collected for detection enzyme activity. The activities of SOD (superoxide dismutase), POD (peroxidase), CAT (catalase), DDPH (total antioxidant capacity), as well as the contents of malondialdehyde (MDA), proline, soluble protein, and soluble sugars in wheat seedlings were measured using assay kits from Suzhou Ke Ming Biotechnology Co., Ltd. (Suzhou, China). Each treatment was set up with three biological replicates.

Analysis of Rhizosphere Soil Properties

Soil samples from wheat rhizosphere were collected for determination of physicochemical properties. Saturated soil extracts obtained by mixing soil and water at a ratio of 1:5 (w/v). The pH value was determined in soil–water suspension using a pH meter. Available phosphorus (AP) content was detected by the molybdenum-antimony colorimetric method. The water-soluble total salt content in soil is determined by the gravimetric method. The electrical conductivity (EC) was measured using conductivity meter.

The activities of soil urease (UE), alkaline phosphatase (ALP), sucrase (SC), and β-glucosidase (β-GC) were assayed using assay kits from Suzhou Ke Ming Biotechnology Co., Ltd. (Suzhou, China). The contents of microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were measured via the dichromate oxidation method (fumigation-extraction).

Analysis of Microbial Community Sequencing

The rhizosphere soil was the same as that used for 16S rRNA gene sequencing. A small-fragment library was constructed according to the characteristics of the amplified region. Paired-end sequencing of the library was performed on the Illumina NovaSeq platform. The raw data for each sample were demultiplexed based on their unique barcodes. To ensure the accuracy and reliability of subsequent analyses, the raw sequences underwent quality control and processing. Specifically, the Fastp software was used for quality control of the raw sequences, and the FLASH software was employed

for chimera detection and removal. Using default parameters, the DADA2 plugin within the QIIME2 pipeline was applied to denoise the quality-filtered and assembled sequences, yielding amplicon sequence variants (ASVs). All annotated chloroplast and mitochondrial sequences were removed from the dataset. For taxonomic classification, the Naive Bayes classifier in QIIME2 was utilized against the SILVA 16S rRNA gene database. Rarefaction curves were generated using the 'alpha-rarefaction' workflow in QIIME2, based on the observed Chao and Shannon index. Bacterial alpha diversity indices, were calculated using Usearch-alpha_div (v10.0.240). The raw sequencing reads of the rhizosphere microbial communities have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject ID **PRJNA1439164.**

Statistical Analysis

Statistical analyses were performed to assess the significance of the observed differences. One-way analysis of variance (ANOVA) was conducted using SPSS software (version 20) to evaluate the alpha diversity indices. For the physiological parameters of wheat and soil enzyme activities, one-way ANOVA was carried out, and corresponding graphical representations were generated using GraphPad Prism software (version 8).

Results

Identification of S-1

To identify the strain Sui-1 (S-1), the 16S rRNA fragment was amplified and sequenced. Through blasting the NCBI database and phylogenetic tree analysis, strain S-1 was identified as *Pseudomonas donghuensis* with 99.86% homology with the *Pseudomonas donghuensis* HYS^T (Fig. 1A). To observe the morphological structure of S-1, S-1 was observed under scanning electron microscopy (SEM). The cells appeared as short rods with individual cells measuring approximately 1–1.5 μm in length and 0.5–0.7 μm in width. The overall morphology was regular, with a smooth surface (Fig. 1B).

Growth-Promoting Traits of S-1

The siderophore production, potassium-solubilizing capacity and IAA production, of the S-1 strains were determined to initially explore their growth-promoting capacity. S-1 exhibited a phosphate-solubilizing activity of 21.50 mg/L after 7 days of cultivation in NBRIP medium, indicating its ability to solubilize phosphorus. Following incubation of S-1 on SMS medium, indicating siderophore production of S-1 was 48.90 mg/L. The ability of S-1 to produce indole-3-acetic acid (IAA) was quantified using the Salkowski colorimetric method, producing a concentration of 7.28 mg/L. These findings indicate that strain S-1 can provide available phosphorus, iron ions, and produce IAA. Collectively, S-1 exhibited plant growth-promoting traits in vitro.

Table 1
Quantitative detection of IAA secretion and phosphorus dissolution

Strain	Qualitative result	Optical density	Productive rate (mg/L)
	Siderophiles (+)	**OD_{630}=0.35**	**48.90**
S-1	Phosphorus solubility (+)	OD_{880}=2.49	21.50
	IAA (+)	OD_{530}=0.23	7.28

S-1 Promotes the Growth of Wheat Seedlings under Saline-Alkali Stress

To elucidate the effect of S-1 on wheat seedlings, growth parameters were measured under normal condition and saline-alkali stress (Fig. 2). As shown in Table 2, compared to the non-inoculated groups (W), inoculation with S-1 (S) significantly enhanced the growth of wheat seedlings, where all parameters remained significantly higher than in the control under high-concentration salt-alkali treatment (T3). Under normal condition (N), inoculation with S-1 significantly improved growth characteristics relative to the non-inoculated control, plant height, stem diameter, leaf area, and root length increased by 60.4%, 96.7%, 149.8%, and 48.3%, respectively. Meanwhile, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight increased by 149.1%, 286.0%, 302.1%, and 236.5%, respectively. These results indicate that S-1 exhibits strong plant growth-promoting activity under normal condition. Interestingly, under low (T1) and Medium-concentration (T2) salt-alkali stress, the growth parameters of non-inoculated seedlings were higher than those in the normal condition (N-W). This phenomenon may be attributed to low-dose stress acting as an environmental signal that triggers plant defense and adaptation mechanisms. A decline in growth indicators was observed in T2 compared to T1, suggesting that increasing stress intensity begins to inhibit wheat growth, while inoculation with S-1 significantly improved growth under both T1 and T2 treatments. For the T1 treatment, inoculation resulted in increases of 23.1% in stem diameter, 22.6% in leaf area, 6.4% in shoot fresh weight, 26.9% in shoot dry weight, 43.1% in root fresh weight, and 44.6% in root dry weight. Under T2 treatment, inoculation led to improvements of 11.1% in plant height, 36.4% in stem diameter, 63.3% in leaf area, and 13.8% in root length, as well as 49.4% in shoot fresh weight, 58.0% in shoot dry weight, 53.8% in root fresh weight, and 68.3% in root dry weight. Compared to the normal group, high-concentration saline-alkali stress (T3) severely inhibited wheat seedling growth. Inoculation with S-1 significantly counteracted this inhibition. In the inoculated T3 group, plant height, stem diameter, leaf area, and root length increased by 56.9%, 114.6%, 202.5%, and 176.7%, respectively. Similarly, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight increased by 290.1%, 246.3%, 309.2%, and 318.2%, respectively. Collectively, these findings demonstrate that the growth-promoting effect of S-1 is most pronounced under severe stress conditions. The underlying mechanism appears to involve maintaining leaf area, promoting root development, and increasing biomass, thereby enhancing the saline-alkali tolerance of wheat seedlings.

Table 2
Effect of S-1 on the wheat growth

Groups	Treatments	Plant height (cm)	Stem thickness (mm)	Leaf area (cm^2)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Normal	W	20.77 ± 0.6489b	1.21 ± 0.0557b	6.12 ± 0.0173b	10.23 ± 0.3930b	0.399 ± 0.0447b	0.0321 ± 0.0166b	0.048 ± 0.0115b	0.0063 ± 0.0013b
	S	33.33 ± 0.4410a	2.38 ± 0.0203a	15.29 ± 0.6710a	15.17 ± 0.4410a	0.994 ± 0.1178a	0.1239 ± 0.0050a	0.193 ± 0.0352a	0.0212 ± 0.0033a
T1	W	34.17 ± 0.4410a	1.73 ± 0.1068b	10.12 ± 0.2869b	15.83 ± 0.0882a	0.741 ± 0.0185a	0.0914 ± 0.0069a	0.1200 ± 0.0089b	0.0139 ± 0.0012b
	S	29.17 ± 1.0138b	2.13 ± 0.0410a	12.41 ± 0.4233a	15.63 ± 0.4096a	0.787 ± 0.0581a	0.1160 ± 0.0079a	0.1717 ± 0.0156a	0.0201 ± 0.0019a
T2	W	28.50 ± 1.0408a	1.43 ± 0.1159b	6.59 ± 0.0985b	12.83 ± 0.2906b	0.623 ± 0.0368b	0.0838 ± 0.0056b	0.119 ± 0.0077a	0.0139 ± 0.0006a
	S	31.67 ± 0.8819a	1.95 ± 0.0555a	10.76 ± 0.2630a	14.60 ± 0.3055a	0.931 ± 0.0927a	0.1324 ± 0.0068a	0.183 ± 0.0400a	0.0254 ± 0.0050a
T3	W	17.00 ± 0.7638b	0.89 ± 0.0684b	3.15 ± 0.4684b	6.00 ± 0.2309b	0.182 ± 0.0116b	0.0298 ± 0.0056b	0.0317 ± 0.0041b	0.0044 ± 0.00022b
	S	26.67 ± 1.9221a	1.91 ± 0.1834a	9.53 ± 0.1146a	16.60 ± 0.3055a	0.710 ± 0.0549a	0.1032 ± 0.0072a	0.1297 ± 0.0049a	0.0184 ± 0.00115a

Note: Data are presented as the mean ± SEM of three biological replicate samples. Different letters for significant differences between treatments were assigned based on the Independent-Samples T Test; $p < 0.05$ was considered statistically significant.

S-1 Enhances Antioxidant Capacity and Osmotic Adjustment in Wheat Seedlings under Saline-Alkali Stress

Preliminary observations indicated that both T1 and T2 stimulated the growth of wheat compared to non-stressed conditions, while T3 showed an inhibitory effect. To focus the subsequent analysis on contrasting physiological responses, T1 and T3 treatments were measured. Antioxidant enzymes and osmoregulatory substances play crucial roles in modulating oxidative stress in crops. Antioxidant enzymes mitigate oxidative damage by scavenging free radicals, while osmoregulatory substances help maintain cellular osmotic balance by preventing water and biomolecule loss and by activating antioxidant systems. In this study, the activities of the antioxidant enzymes SOD, POD, and CAT in wheat seedlings were determined under normal and stress conditions. SOD activity was 1211.60 and 1489.31 U/g under normal conditions, 1452.45 and 1647.37 U/g under T1 treatment, and 1527.00 and 1659.71 U/g FW under T3 treatment for the non-inoculated (W) and S-1 inoculated (S) groups, respectively. Concurrently, S-1 inoculation significantly enhanced POD activity, with values of 419.95 and 478.34 U/g, 479.52and 552.02 U/g, and 435.34 vs. 535.36 U/g under normal, T1 and T3 treatments. CAT activity was also significantly increased by S-1 inoculation, recording 1173.60 and 1750.95 U/g, 967.68 and 1484.21 U/g, and 1289.79 and 2057.40 U/g for normal, T1 and T3 groups, respectively. Furthermore, ROS scavenging capacity (DPPH) was significantly higher in S-1 inoculated groups. The DPPH values were 2.08 and 2.73 mg Trolox/g, 2.25 and 3.37 mg Trolox/g, and 2.46 and 2.71 mg Trolox/g in N, T1 and T3. In addition, S-1 inoculation led to a decreasing trend

in malondialdehyde (MDA) content under stress conditions, with values of N (17.60 and 19.57 nmol/g), T1 (22.50 and 21.78 nmol/g) and (18.66 and 16.11 nmol/g). These results indicate that strain S-1 enhances the plant's antioxidant capacity, thereby effectively alleviating cellular oxidative damage. In addition, analysis of osmoregulatory substances revealed that S-1 increased the contents of total protein, proline, and soluble sugars (Fig. 3). Proline content was significantly elevated from 49.61 to 254.09 μg/g, 127.76 to 615.17 μg/g, and 59.95 to 157.30 μg/g in normal, T1 and T3 groups respectively. Total protein content increased from 12.45 to 18.34 mg/g, 15.46 to 18.50 mg/g, and 17.31 to 19.64 mg/g. Soluble sugar content increased from 2.08 to 2.73 mg/g, 2.25 to 3.37 mg/g, and 2.46 to 2.71 mg/g. Notably, while total protein content was significantly higher in the inoculated group under normal conditions, no significant differences were observed under T1 and T2 treatments. This may be attributed to the ability of S-1 to alleviate oxidative damage and osmotic imbalance, potentially reducing the plant's reliance on soluble proteins for osmotic adjustment. Notably, the total protein content was significantly higher in the inoculated group under non-stress conditions. However, under T1 and T2 stress levels, no significant differences in total protein content were detected between inoculated and non-inoculated groups. This may be attributed to the ability of Sui-1 to alleviate oxidative damage and osmotic imbalance, potentially reducing the plant's reliance on soluble proteins for osmotic adjustment.

S-1 Improves the Physicochemical Properties of Wheat Rhizosphere Soil under Saline-Alkali Stress

Soil quality is a direct determinant of crop yield, therefore, the physicochemical properties of the soil were analyzed in this study. As shown in Table 3, the severity of soil salinization increased with intensified saline-alkali stress. Specifically, the soil pH rose from 6.28 conditions to 8.14, indicating successful induction of saline-alkali stress. Concurrently, soluble salt content and electrical conductivity (EC) increased significantly, while available phosphorus (AP) content remained relatively stable. Inoculation with S-1 modulated these stress-induced changes. Compared to non-inoculated controls, pH decreased by 10.9% in the T1 treatment and by 14.1% in the T3 treatment, maintaining the pH within a range of approximately 6.5 to 7.0. Although the change in AP content was modest, its level in all inoculated treatments was higher than in the non-inoculated groups. Furthermore, S-1 significantly reduced the soluble salt content by 13.4% in the T1 group and by 26.3% in the T3 group. The electrical conductivity (EC) was significantly decreased both under normal and stress conditions, with reductions of 18.8%, 8.9%, and 18.4% in normal, T1 and T3 groups compared to the non-inoculated groups. Soluble salt content and EC are direct indicators of saline-alkali stress intensity, where higher values correlate with stronger osmotic stress and ionic toxicity. These results demonstrate that, in addition to directly promoting wheat growth, S-1 possesses soil amendment capabilities. This function is beneficial for mitigating soil salinization.

Table 3
Effect of S-1 on Soil physicochemical properties

Groups	Treatments	PH	Available phosphorus (mg/kg)	Soluble salt (g/kg)	Electrical conductivity (MS/CM)
Normal	W	6.28 ± 0.1447a	204.74 ± 6.30a	5.10 ± 0.0441a	5.42 ± 0.0950a
	S	6.46 ± 0.0794a	210.66 ± 5.55a	5.00 ± 0.0436a	4.40 ± 0.0473b
T1	W	7.51 ± 0.0750a	208.67 ± 6.64a	5.68 ± 0.0608a	4.95 ± 0.0513a
	S	6.69 ± 0.0872b	224.44 ± 6.33a	4.92 ± 0.0581b	4.51 ± 0.0176b
T3	W	8.14 ± 0.2182a	202.10 ± 6.03a	7.45 ± 0.0441a	6.51 ± 0.0712a
	S	6.99 ± 0.1833b	216.93 ± 9.22a	5.49 ± 0.0636b	5.31 ± 0.0463b

Note: Data are presented as the mean ± SEM of three biological replicate samples. Different letters for significant differences between treatments were assigned based on the Independent-Samples T Test; $p < 0.05$ was considered statistically significant.

Effects of S-1 on Soil Enzyme Activities and Microbial Biomass

Soil enzymes are important for nutrient cycling, with their activities being crucial for the material cycling and energy transformation to crop growth. Soil urease (UE) is a key enzyme in the nitrogen cycle. Inoculation with S-1 significantly enhanced UE activity under both normal (155.45 vs. 239.42 μg/g) and stress conditions (T1: 1191.21 and 1289.04μg/g, T3: 1198.55 and1297.00 μg/g), which is beneficial for providing plants with available nitrogen (Fig. 4). Alkaline phosphatase (ALP), a vital enzyme in the phosphorus cycle, also exhibited increased activity, with values of 18.92 and 20.12, 18.85 and 20.67, 18.25 and 20.01 μmol/g in normal, T1 and T3 groups respectively. Sucrase (SC) activity, which participates in the carbon cycle and reflects the capacity for organic carbon decomposition, was significantly increased, and the activities were 9.83 and 13.20, 42.31 and 61.99, 45.70 and 73.03 mg/g. Furthermore, S-1 significantly boosted the activity of β-glucosidase (β-GC), an enzyme involved in cellulose decomposition and soil organic matter accumulation, particularly under severe stress. The activities recorded were 269.47and 288.91, 259.68 and 273.30, and 201.58 and 266.45μmol/g. Soil microbial biomass carbon (MBC) and nitrogen (MBN), which reflect the total amount and activity of soil microorganisms and are key indicators of soil ecosystem vitality, were also influenced. S-1 significantly increased MBC content under both normal (126.00 and 139.67 mg/kg) and stress conditions (T1: 135.33 and 150.33mg/kg, T3: 117.29 and 177.63 mg/kg). The MBN content was significantly higher in inoculated groups under stress conditions (T1: 6.21 and 6.53mg/kg; T3: 6.13 and 6.85 mg/kg). In summary, S-1 enhances wheat tolerance to saline-alkali stress by modulating soil physicochemical properties and augmenting microbial biomass.

Analysis of Microbial Diversity

The impact of S-1 on the microbial diversity of the wheat rhizosphere was assessed via 16S rRNA gene sequencing. To validate the sufficiency of sequencing depth, the rarefaction curves were generated based on the Chao and the Shannon diversity index (Fig. 5A-B). When the sequencing data volume reached a sufficiently high threshold, the rarefaction curves manifested a gradual flattening trend, indicating that at this sequencing depth, the sample sequencing was relatively comprehensive and effectively represented their microbial community structure. The sequencing results revealed that S-1 had no significant effect on the alpha diversity indices (Shannon and Chao1) under saline-alkali stress. The Shannon and Chao1 indices represent community evenness and richness, respectively (Fig. 5C-D). Furthermore, beta diversity analysis

based on Principal Coordinates Analysis (PcoA) showed that samples from non-stress and different stress conditions were significantly separated along the coordinate axes (Fig. 5E). A clear separation was particularly evident between non-stress and high-concentration saline-alkali stress treatment, indicating that different treatments altered the composition of the rhizosphere bacterial community. As shown in Fig. 5E, replicate samples from the same treatment did not show significant separation, whereas a distinct separation was observed between S-1 inoculated (S) and non-inoculated (W) groups along the axes, signifying a significant shift in microbial community structure due to inoculation. The number of bacterial operational taxonomic units (OTUs) in different samples, illustrated by a Venn diagram, indicated that S-1 increased the OTU count under both normal and severe stress conditions (Fig. 5F). The distribution of microbial communities at the phylum level is shown in Fig. 5G. At this taxonomic level, the dominant phyla in both non-inoculated and inoculated groups were *Pseudomonadota*, *Actinomycetota*, *Chloroflexota*, and *Bacteroidota*, among others. The relative abundance of *Pseudomonadota* exhibited a decreasing trend in Sui-1-treated groups. For instance, in non-inoculated groups, the relative abundances were N-W: 25.44%, T1-W: 24.41%, and T3-W: 24.35%. In contrast, in inoculated groups, the values were N-S: 24.29%, T1-S: 23.90%, and T3-S: 23.20%. The relative abundance of *Chloroflexota* was higher in inoculated groups (e.g., N-S: 19.49%, T1-S: 17.92%, T3-S: 17.85%) compared to non-inoculated groups (e.g., N-W: 16.74%, T1-W: 16.58%, T3-W: 15.16%). Additionally, S-1 increased the relative abundance of *Acidobacteriota* under mild and severe stress conditions. In non-inoculated groups, the abundances were N-W: 2.73%, T1-W: 1.76%, T3-W: 1.47%, whereas in inoculated groups, they were N-S: 1.28%, T1-S: 2.01%, T3-S: 1.82%. As shown in Fig. 5H, at the genus level, inoculation with S-1 significantly increased the abundance of Streptomyces under severe stress, elevating it from 2.01% in the non-inoculated group to 3.90%. The abundances of *Aggregatili*nea and norank_f__BIrii41 showed a decreasing trend under severe stress. For *Aggregatilinea*, the abundance was T3-W: 1.60% vs. T3-S: 0.80%, and for norank_f__BIrii41, it was T3-W: 2.90% vs. T3-S: 1.30%. These results demonstrate that while the dominant microbial phyla in the rhizosphere soil were similar between the W and S groups, significant differences existed in the relative abundances of certain key taxa.

Discussion

Saline–alkali stress is one of the major abiotic stresses limiting sustainable development of global agricultural, causing osmotic stress, ion toxicity, nutrient imbalance, and oxidative damage, which severely limits crop growth and yield [28]. As a critical food crop, enhancing the saline-alkali tolerance of wheat holds significant importance for ensuring food security. Utilizing plant growth-promoting rhizobacteria (PGPR) to improve crop stress resistance has emerged as an eco-friendly and promising strategy. Numerous studies have demonstrated that PGPR such as *Pseudomonas*, *Bacillus*, and *Enterobacter* can alleviate saline-alkali stress, promote seed germination, increase biomass, and thereby enhance crop growth and stress tolerance [29]. Sangeeta et al. showed that *Pseudomonas* sp. strain S3 was able to control the salt stress by the production of plant growth regulators such as indoleacetic acid and iron-chelating siderophores, and by solubilization of insoluble nutrient complexes including phosphate and zinc, thereby incresing plant biomass [30]. In this study, the effects of S-1 on the growth physiology of wheat seedlings under NaCl + $NaHCO_3$ stress were investigated. Firstly, strain S-1 was identified as *Pseudomonas donghuensis*. Functional assays revealed the ability of S-1 to solubilize inorganic phosphate, produce siderophores and indole-3-acetic acid (IAA). These are considered to be PGP traits [31]. Assessments of wheat seedling growth under saline-alkali stress showed that S-1 significantly promoted growth. Notably, under high-concentration saline-alkali stress treatment (4.5 g/kg), the growth promoting effect of S-1 was most significant. These findings indicate that S-1 promotes wheat growth under both normal and stress conditions, and its efficacy appears to be stress-intensity-dependent. This finding is consistent with studies reporting that PGPR increase biomass and enhance saline-alkali tolerance in crops such as pepper, soybean, and maize [32–34]. Although S-1 exhibited plant growth-promoting traits in vitro, further studies are needed to confirm whether these traits are directly responsible for the observed growth enhancement in wheat under saline-alkali stress

Saline-alkali stress induces oxidative damage in plants due to the accumulation of ROS and MDA, as well as an imbalance in osmoregulatory substances. Plants respond to oxidative damage by enhancing antioxidant capacity and osmotic

adjustment ability [35, 36]. Our results showed that inoculation of S-1 significantly increased the activities of SOD, POD, and CAT, and enhanced the antioxidant capacity (DPPH) in wheat. Meanwhile, the MDA content was significantly reduced. This suggests that S-1 can activate the antioxidant defense system and effectively scavenges ROS, thereby reducing membrane lipid peroxidation and ensuring the cellular structure and function under saline-alkali stress. Furthermore, S-1 modulated the accumulation of osmoregulatory substances, altering the content of proline, soluble sugars, and soluble proteins. These results were in agreement with Win et al. which *Pseudomonas* spp. conferring salinity tolerance in plants by stimulating the defensive activity of antioxidants [37]. Under stress, the balance of these substances helps maintain cellular osmotic pressure and stabilize protein structure and function, representing a crucial strategy for plants to cope with osmotic stress.

Soil physicochemical properties are related to nutrient transformation and soil fertility. PGPR enhance soil enzyme activities to promote nitrogen, carbon, and phosphorus cycles, and can improve soil physicochemical properties, thereby increasing nutrient availability and enhancing plant resistance to biotic and abiotic stresses [38, 39]. Our analysis of soil properties and enzyme activities revealed that S-1 decreased soil pH and maintains it around 6.5-7.0. The electrical conductivity (EC) and soluble salt content significantly decreased, while the available phosphorus content showed an increase in S-1 treatment group. Arora et.al demonstrated that *Pseudomonas atacamensis* KSS-6 improved pH and electrical conductivity (EC) under salinity stress [40]. Moreover, S-1 significantly enhanced the activities of UE, ALP, SC and β-GC. These enzymes are involved in the cycling and transformation of soil nutrients such as nitrogen, phosphorus, and carbon, and the enhancement of their activities indicates an improvement in soil nutrient availability. A similar study reported that the inoculation of *Bacillus cereus* Pb25 effectively improved the soil enzyme activities, including soil dehydrogenase and alkaline phosphatase activities, leading to the increased yield of mungbean (Vigna *radiate*) under salt stress [27]. Additionally, inoculation of S-1 increased soil microbial biomass carbon (MBC) and nitrogen (MBN) significantly, indicating enhanced overall soil microbial activity and abundance. These findings revealed S-1 might create a more favorable rhizosphere microenvironment for plant growth by improving soil physicochemical properties, soil enzyme activities, and promoting microbial biomass. This facilitates the recruitment of beneficial microorganisms, thereby influencing the diversity, abundance, and composition of the rhizosphere microbial community.

Rhizosphere microbial diversity analysis revealed the impact of S-1 on microbial community structure. At the phylum level, S-1 increased the relative abundances of *Chloroflexota* and *Acitomycetota*, while the abundance of *Pseudomonadota* slightly decreased. PGPR significantly alters microbial community structure, a finding supported by our results [41]. Studies have shown that *Bacillus subtilis* increased the biomass of chromium (Cr)-contaminated plants, reduced plant Cr concentration, and enhances the abundance of *Chloroflexota*, promoting microbial interactions and correlations [42]. The *Rhodopseudomonas palustris* PSB06 significantly promote rice growth, enhance stress resistance at the seedling stage, and increase the relative abundances of *Acitomycetota* and *Chloroflexota* [43]. Inoculation with halotolerant phosphate-solubilizing bacteria (PSB) can increase relative abundance of *Acitomycetota*, which correlates positively with soil available nitrogen and root dry weight. This helps to elucidate the microbial mechanisms by which PSB improve saline soil and promote plant growth [44]. At the genus level, the abundance *Streptomyces* was significantly increased under severe stress. *Streptomyces* produce secondary metabolites that can inhibit plant pathogens and confer significant benefits to plants by promoting growth and reducing disease incidence [45]. A deeper understanding of these mechanisms might enable the plants to recruit more beneficial microbial communities. For instance, a study confirmed the potential of combining *Streptomyces sp*. LAP3 with leonardite hydrolate as an environmentally friendly strategy to enhance crop productivity and enhance stress resistance [46]. These findings suggest that inoculation with S-1 may recruit or enrich functional microbial taxa involved in soil health and nutrient transformation, thereby optimizing the rhizosphere micro-ecosystem at the community functional level and indirectly supporting plant growth and stress resistance. It is important to note that the 16S rRNA gene amplicon sequencing used in this study does not permit direct quantification of the inoculated strain *Pseudomonas donghuensis* S-1 at the species or strain level. While we observed significant shifts in the

rhizosphere microbial community composition, including an increase in the relative abundance of *Streptomyces*, these changes only indirectly suggest an impact of S-1. To establish a direct link between inoculation and the abundance of S-1 in the rhizosphere, future investigations should employ metagenomic shotgun sequencing or strain-specific quantitative PCR (qPCR) to accurately track the colonization and persistence of S-1 under saline-alkali stress conditions.

Conclusion

In conclusion, the rhizobacterium *Pseudomonas donghuensis* S-1 effectively alleviates the growth inhibition of wheat under combined saline-alkali stress through synergistic actions, including the enhancement of plant antioxidant and osmoregulatory capacities, improvement of soil physicochemical properties, and elevation of soil enzyme activities and microbial functionality. This study systematically elucidates the effect of S-1 within the plant-soil-microbe interaction system, providing a solid theoretical foundation for its application as a bio-inoculant in wheat cultivation on saline-alkali lands.

Declarations

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Author Contribution

Zulihumaer Yakupu and Jiusheng Sun：Investigation, Methodology, Visualization, Writing– original draft, Writing– review. Jiawei Wang: Methodology, Validation, Writing– original draft. Dilbar Tursun: Resources, Supervision, Writing– review & editing. Zhenpu Liang: Project Administration, Funding acquisition, Resources, Supervision, Writing– review & editing. Huifang Bao: original draft, Writing– review & editing, Formal analysis. Hongmei Yang: Resources, Funding acquisition, Writing– review & editing. Yingwu Shi: Validation, Writing-review. Faqiang Zhan: Formal analysis, Investigation, Methodology, Resources, Writing– review & editing. Rong Yang: Data curation, Conceptualization, Writing – review. Min Chu: Data curation, Conceptualization, Writing – review & editing. Ning Wang and Qingqing Zhang: Conceptualization, Project administration, Funding acquisition, Supervision, Writing – review & editing.

Data Availability

The raw data supporting the conclusions of this article are available on request from the corresponding author. Strain *Pseudomonas donghuensis* S-1 has been deposited in the China Center for Type Culture Collection (CCTCC) under accession number **CCTCC M 2026455** . The nearly full-length 16S rRNA gene sequence of strain S-1 was determined and deposited in GenBank under accession number **PZ123209.** The raw sequencing reads of the rhizosphere microbial communities have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject ID **PRJNA1439164**

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Figures

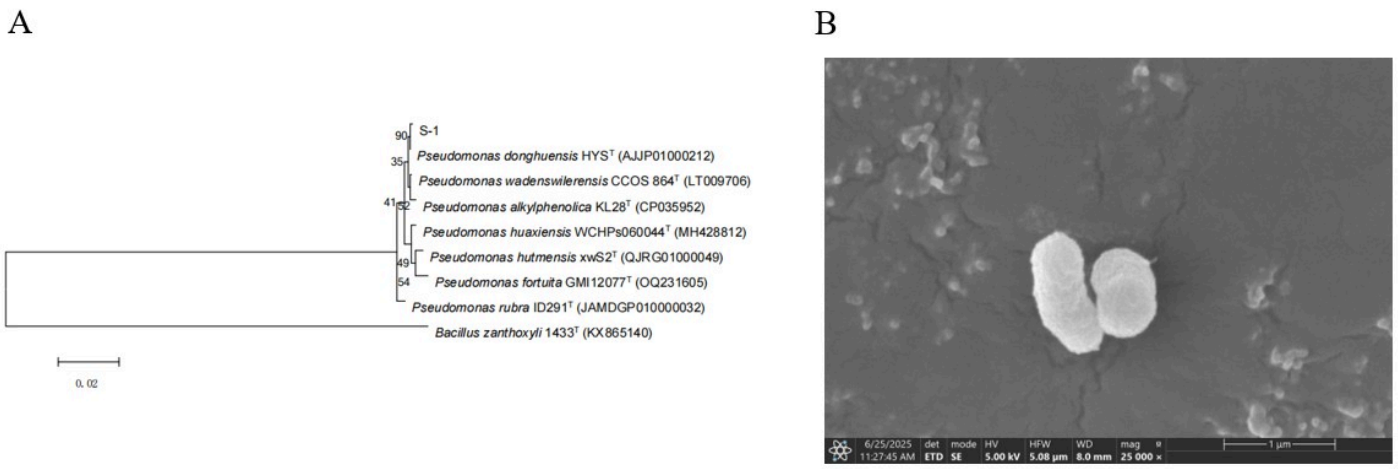


Figure 1

Identification of strain S-1 (A) Phylogenetic tree of strain S-1. (B) Microstructure of S-1 examined by scanning electron microscopy (SEM).

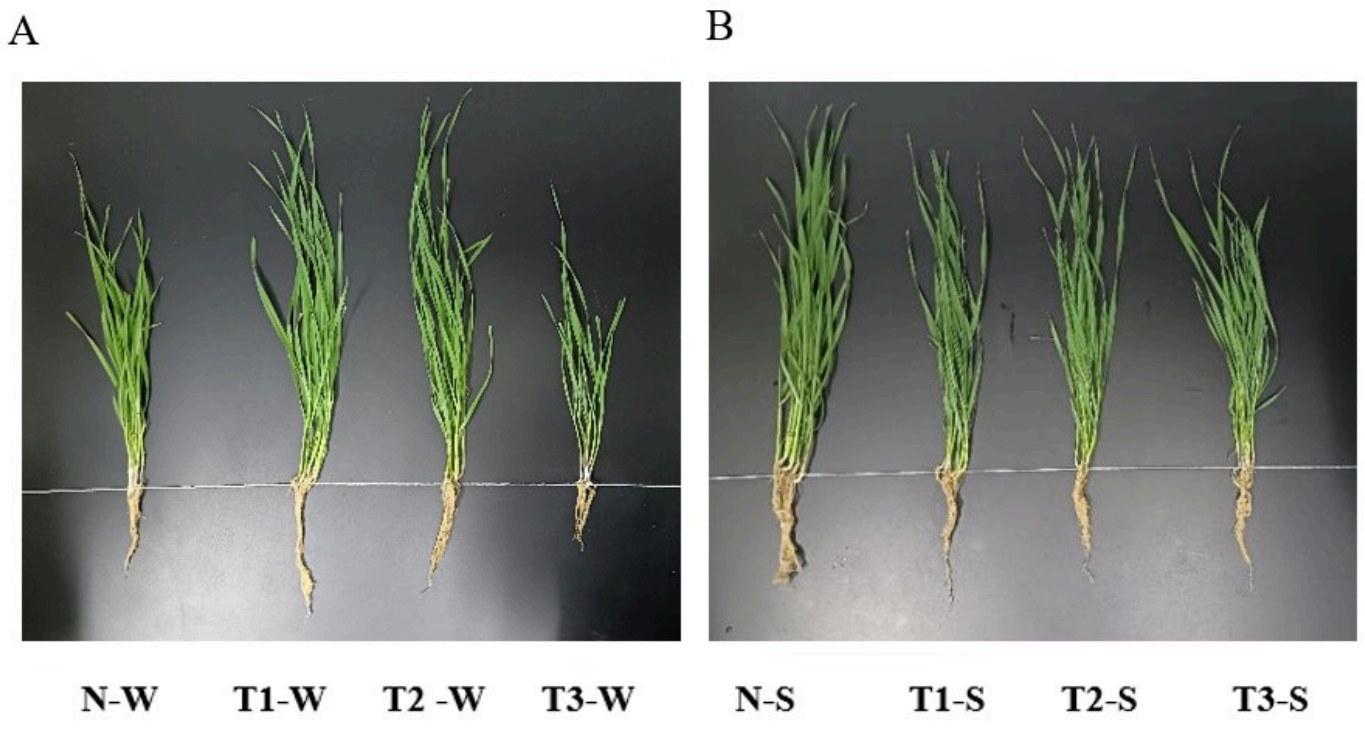


Figure 2

Effect of different treatments on wheat plant growth. (A) Uninoculated group. (B) Inoculated group. N, T1, T, and T3 represented for CK, 1.5g/kg, 3g/kg, and 4.5g/kg treatments respectively.

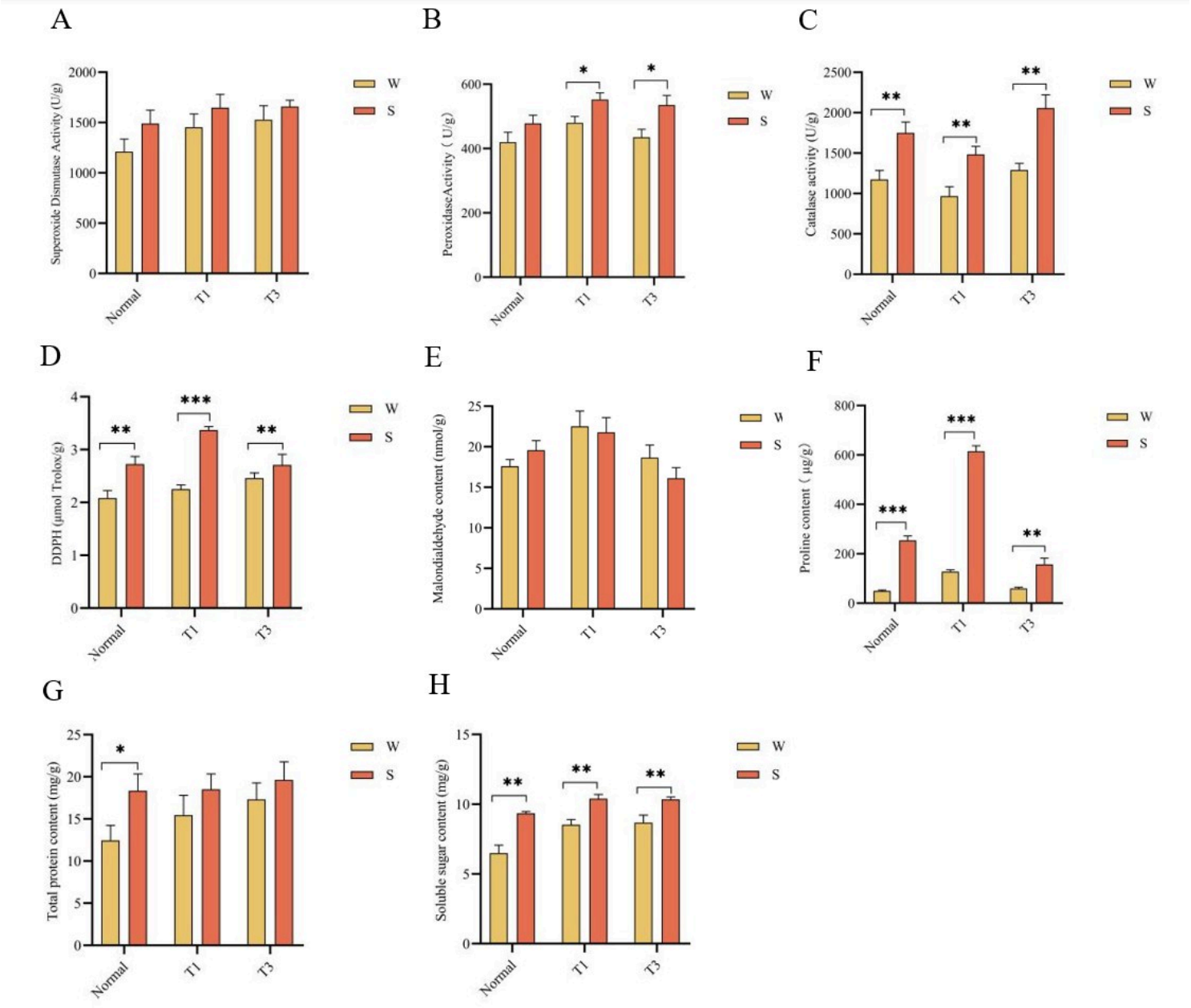


Figure 3

Antioxidant enzyme activity and content of osmotic regulators (A) Superoxide dismutase activity; (B)Peroxidase activity; (C) Catalase activity; (D) ROS scavenging capacity; (E) Malondialdehyde content; (F) Proline content; (G) Soluble protein content; (H) soluble sugar content. For the statistics, three biological replicates per sample were used. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Student's t−test.

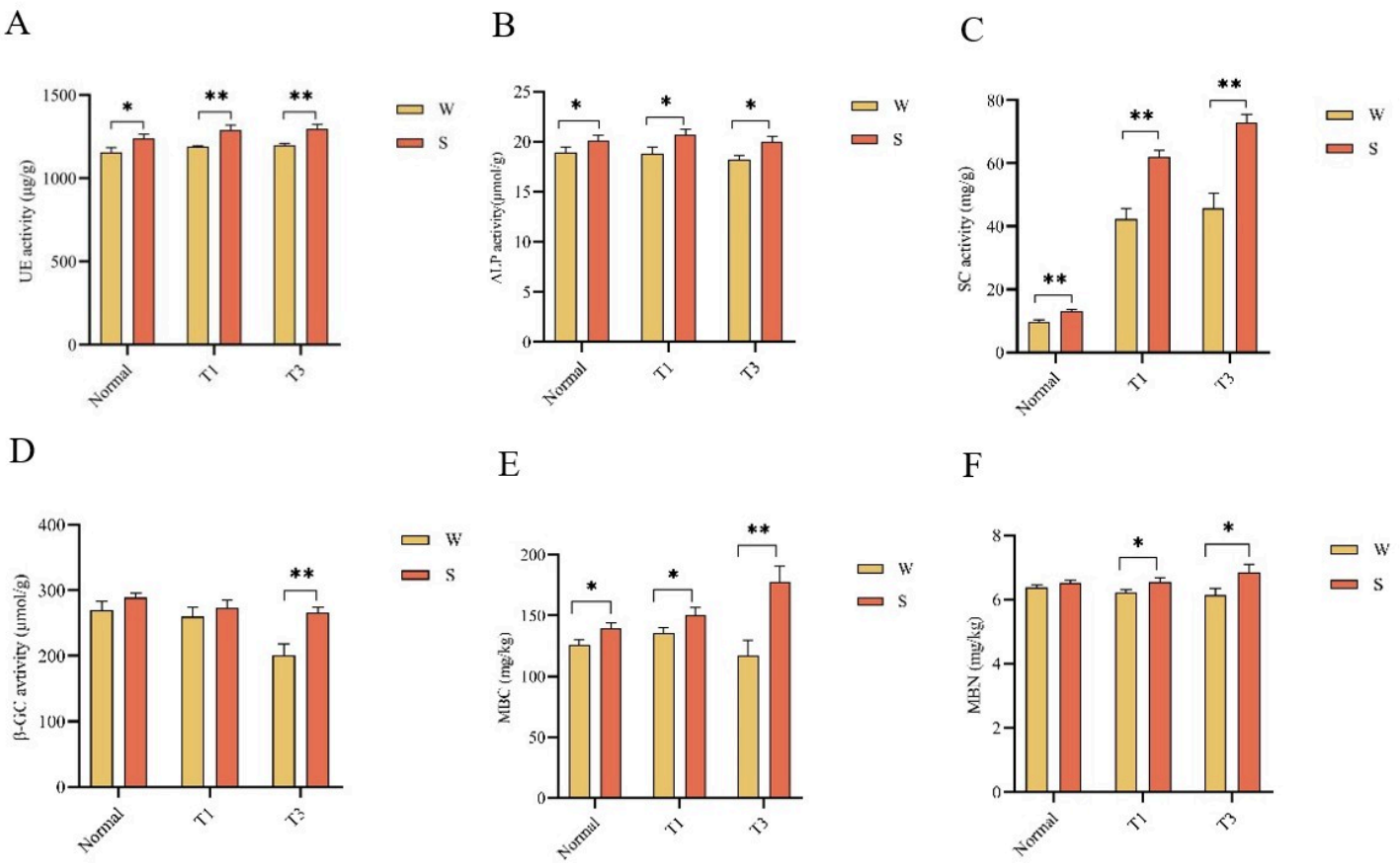


Figure 4

Soil enzyme activity and microbial biomass (A) Urease activity; (B) Alkaline phosphatase activity; (C) Sucrase activity; (D)β-glucosidase activity; (E) Microbial biomass carbon; (F) Microbial biomass nitrogen. For the statistics, three biological replicates per sample were used. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Student's t–test.

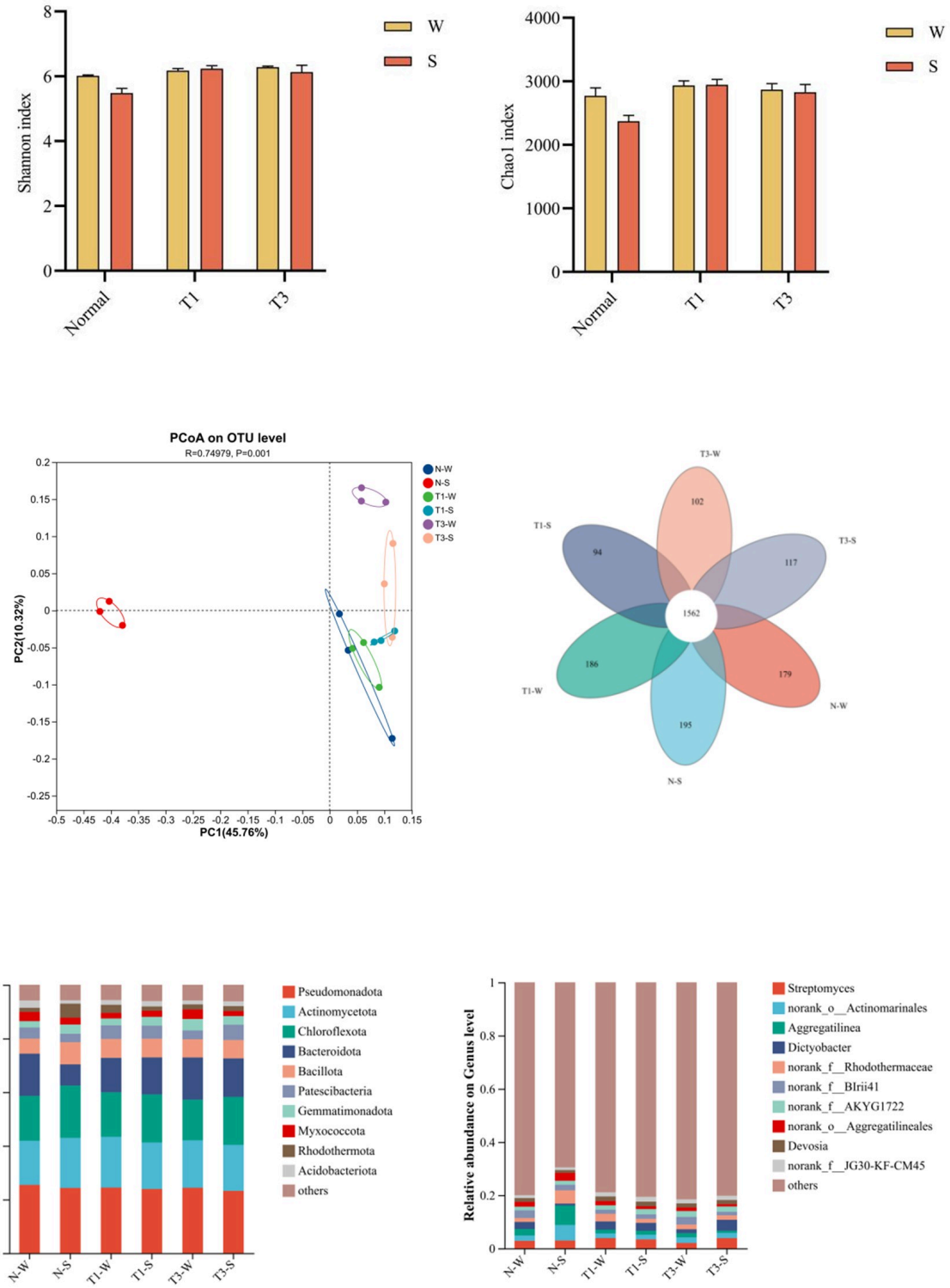


Figure 5

The effect of S-1 on the Rhizosphere microbial diversity under salt stress. (A-B) Alpha diversity index based on the Shannon index and Chao1 index; (C) PCoA plots based on OTUs; (D) Veen diagram based on OTUs; (E-F) Rhizosphere bacterial community structure at phylum level and genus level. For the statistics, three biological replicates per sample were used. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Student's t–test.