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Mitigating salt stress in *Zea mays*: Harnessing *Serratia nematodiphila*-biochar-based seed coating for plant growth promotion and rhizosphere microecology regulation

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ABSTRACT

Salt stress poses a significant challenge in contemporary agricultural practices. In response, using plant growth-promoting bacteria (PGPB) in conjunction with biochar coatings has emerged as an innovative biological strategy to mitigate salt stress in crops. Through a dual screening process based on gradient salt concentration and plant growth-promoting performance, a highly salt-tolerant PGPB, *Serratia nematodiphila*, was identified in this study. And a seed-coating formulation incorporating *S. nematodiphila* was developed using biochar as a carrier. With the help of potting experiments, this paper investigated the impact of this *S. nematodiphila*-biochar-based seed coating on *Zea mays* under salt stress, while also analyzing functional alterations in the core microbial community. These findings indicate that the *S. nematodiphila*-biochar-based seed coating selectively enriched beneficial microbial populations, such as Proteobacteria, Gammaproteobacteria, Verrucomicrobiae, and Bacteroidia, fostering a symbiotic association with the *Z. mays* root system. These beneficial consortia facilitated the accumulation of osmotic solutes, including soluble sugars, soluble proteins, and proline, thereby modulating leaf photosynthetic activity and alleviating salt stress (sodium and potassium homeostasis) in *Z. mays*. This investigation provides refined technical approaches for alleviating salt stress in *Z. mays*, contributing to developing sustainable agricultural practices.

1. Introduction

Ranked as the third most crucial food crop globally, *Zea mays* is known for its sensitivity to salt stress, which significantly affects its production (Liang et al., 2024). Currently, about 20 % of agricultural land and 33 % of irrigated farmland are afflicted by salt erosion, posing a significant challenge to global food production (Zhang et al., 2020). In the face of salinity stress, the deleterious effects of sodium (Na^+) toxicity on plants are profound, culminating in growth inhibition, oxidative stress, ionic perturbations, and metabolic dysregulation (Bai et al., 2023). Although conventional approaches, such as water conservation techniques and organic fertilizers, have been utilized to alleviate crop salt stress, their adoption is limited by challenges like nutrient leaching, high costs, and the risk of secondary contamination (Minhas et al., 2020). Considerable attention has been directed toward developing more efficient and ecologically sustainable biotechnologies to enhance crop salt tolerance (Bai et al., 2023). Salt-tolerant plant species have evolved an array of mechanisms to withstand such stress, encompassing the synthesis of osmoprotectants, modulation of endogenous hormone levels, and preservation of cellular redox equilibrium (Liang et al., 2024). While genetic factors predominantly underpin plant salt tolerance, the contributory role of plant-associated microorganisms in abiotic stress responses warrants thorough consideration (Zheng et al., 2024). In recent years, the ’cry for help’ hypothesis within plant-microbe interactions has garnered widespread recognition, positing that timely modulation of the plant core microbiota can alleviate abiotic stress (Wang and Song, 2022). For instance, under salt stress conditions, alterations in the rhizosphere microbial composition and structure in ryegrass have led to a notable enrichment of *Rheinheimera* and *Pseudanabaena*, both exhibiting significant pro-phytotic functions (Feng et al., 2023).

Plant growth-promoting bacteria (PGPB) are indispensable soil microorganisms crucial for enhancing plant growth and mitigating salt stress (Jiang et al., 2023). They achieve these goals either directly, by synthesizing beneficial compounds such as osmotic protectants, siderophores, and phytohormones, or indirectly, by inhibiting

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phytopathogens (Navarro-Torre et al., 2023). Various PGPB genera, including *Bacillus atrophaeus*, *Pantoea alli*, *Planococcus soli*, *Pseudomonas reactans*, and *Rhizophagus irregularis*, have been proven to alleviate the adverse effects of salt stress on *Z. mays* growth (Chen et al., 2022; Hou et al., 2022; Moreira et al., 2020). Recent investigations have further demonstrated that inter-root inoculation of plants with PGPB can recruit beneficial microorganisms, optimize the community structure of the rhizosphere microbiota through cross-feeding interactions, enhance the abundance of microbes conducive to salt tolerance, and exhibit significant potential for improving crop salt tolerance (Sun et al., 2022). However, the efficacy of PGPB alone is often compromised by indigenous soil microorganisms and protozoa (Jiang et al., 2023). To address these challenges, microbiological research has shifted its focus to developing salt-tolerant PGPB coating agents capable of functioning effectively in saline-alkali agricultural ecosystems (Jiang et al., 2023). Unlike conventional inoculation methods, microbial coating facilitates bacterial colonization under specific conditions and timelines, promoting stable and consistent plant growth (Vejan et al., 2019).

Mudrock and vermiculite are commonly used carriers for PGPB (Mayo-Prieto et al., 2020). However, the use of peat and vermiculite is constrained by the high cost of extraction and the scarcity of these materials in regions where they do not naturally occur. Therefore, there is a need for sustainable, widely available materials as alternative carriers for inocula (Ippolito et al., 2020). Biocarbon, a carbon-rich residue, serves as a metabolizable carbon energy source and offers the potential for sustainable production (Han et al., 2023; Ippolito et al., 2020). Recent studies increasingly demonstrate that applying biochar to saline soil mitigates salinity stress by reducing Na^+ uptake and promoting plant growth (He et al., 2020; Liang et al., 2021). Biochar, functioning as a carrier material, exhibits the capability to encapsulate strains from indigenous microbes, thereby enhancing the viability and efficacy of exogenous strains (Hill et al., 2019). Concurrently, biochar's porous structure and adsorption characteristics afford an advantageous habitat for microbes, fostering symbiotic relationships between microbes and crop roots. This augmentation bolsters the plant's resilience against salt stress, showcasing considerable potential in alleviating salt stress in crops (Hill et al., 2019; Ippolito et al., 2020; Vejan et al., 2019). However, research on improving crop salt tolerance using PGPB-biochar coating agents remains exploratory and developmental. Mature formulations have not been established, and biomarkers responsive to PGPB-biochar coating agents in saline soil environments have not been identified. A comprehensive examination of soil microflora is imperative to effectively mitigate salt stress in *Z. mays*. This includes a thorough consideration of the synergistic benefits conferred by biochar and PGPB in seed coatings, as well as an understanding of the physiological response mechanisms (e.g., osmoregulation and photosynthesis) of crops to microbial alterations (Liang et al., 2023).

Hence, this research hypothesizes that under salt stress conditions, plants coated with PGPB and biochar selectively recruit specific microorganisms, fostering symbiotic interactions with the root system to enhance crop salt tolerance. To test this hypothesis, this research will focus on i) developing a seed coating formulation with *Serratia nematodiphila* and biochar; ii) assessing the efficacy of this *S. nematodiphila*-biochar-based seed coating in mitigating salt stress in *Z. mays*; iii) examining its impact on the composition of the soil bacterial community; and iv) determining whether changes in the soil bacterial community composition affect salt stress tolerance in *Z. mays*.

2. Materials and methods

2.1. Isolation and screening of salt-tolerant PGPB

2.1.1. Isolation and screening of salt-tolerant strains

Salt-tolerant strains were isolated from common crops (e.g., *Ipomoea batatas*, *Arachis hypogaea*, and *Z. mays*) and farmland soils in two locations: Huangguang Village (Xikuangshan) and Yangjiaba, Shifeng District, Lengshuijiang City, Hunan Province, China. To initiate the isolation process, an initial screening was conducted using LB culture medium with 3 % NaCl. Subsequently, this study intensified the screening process by elevating the NaCl concentration in the isolation medium to 9 %, 12 %, and 15 % (Li et al., 2020).

2.1.2. Determination of plant growth-promoting traits of the salt-tolerant strains

To assess the strain's auxins production capability, this study followed the method outlined by Li et al. (2020), involving the addition of Salkowski's reagent to observe color changes and measure the OD_{530} value. The strain's capacity to produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase was evaluated using Dworkin and Foster minimal salt media supplemented with 5 mM ACC (Gupta et al., 2022). Concurrently, the strain's siderophore production ability was detected using a Chrome Azurol Sulfonate (CAS) culture medium (Penrose and Glick, 2003).

2.1.3. Germination assay of candidate salt-tolerant strains

Preparation of bacterial suspension involved inoculating 1 % of the bacterial solution into 250 mL conical flasks containing 100 mL of LB medium. The flasks were then incubated in a shaker at 180 rpm and 30 °C for 12 h, achieving an approximate concentration of 10^8 $CFU{\cdot}mL^{-1}$. Afterward, the cultures underwent centrifugation at 8000 rpm and 4 °C for 10 min. The bacterial cells were harvested, washed twice with sterile phosphate buffer, and suspended in 100 mL of sterile phosphate buffer at a concentration of 10^8 $CFU{\cdot}mL^{-1}$. This suspension was subsequently used for seed inoculation (Bai et al., 2023).

Candidate salt-tolerant strains were selected for *Z. mays* germination experiments to validate their practical PGP effects under normal and salt stress conditions. *Z. mays* seeds were immersed and disinfected with 10 % H_2O_2 for 30 minutes, then rinsed 2–3 times with sterile water. Subsequently, the cleaned *Z. mays* seeds were soaked in sterile water for 2 hours, continuously stirring throughout this duration. The seeds were then submerged in a bacterial suspension of selected strains for one hour and subsequently incubated in a flat petri dish at 28 °C for 7 days, with each process performed in triplicate. The germination rate was observed and critical indicators such as seed vitality and root length were measured (Li et al., 2020).

2.2. Study on biochar coating ratio and coating effect of target strain

2.2.1. Coating material characteristics

The maize straw biochar used has a moisture content of 10.26 %. It possesses an organic C content of 510.90 $g{\cdot}kg^{-1}$, a total nitrogen (N) content of 8.51 $g{\cdot}kg^{-1}$, a total phosphorus (P) content of 2.34 $g{\cdot}kg^{-1}$, and a total potassium (K) content of 15.76 $g{\cdot}kg^{-1}$.

2.2.2. Determination of the optimal biochar coating ratio

5 groups of biochar coating ratio schemes were set, T1 (seed weight: biochar weight = 8 %), T2 (seed weight:biochar weight = 12 %), T3 (seed weight:biochar weight = 16 %), T4 (seed weight:biochar weight = 20 %), T5 (seed weight:biochar weight = 24 %). According to the preparation scheme outlined below, the *Z. mays* seeds undergo a coating process, followed by a germination test aimed at determining the optimal microbial coating ratio. The seed germination test was referred to 2.1.

2.2.2.1. Seed coating agent preparation. Create a seed coating agent mixture by combining bacterial suspension, biochar (subjected to high-temperature and pressure sterilization), and arabic gum solution in a 2:2:1 (V/W/V) ratio (The bacterial suspension was referred to 2.1.). To safeguard cell viability during coating, glycerol is introduced into the bacterial suspension. Follow the seed coating procedure outlined by Scott et al. (1991), which entails drying and weighing the soaked seeds using clean kitchen paper. Place these seeds into a previously sterilized seed coating machine that has been cleaned with alcohol. Finally, blend the seeds with the seed coating agent.

2.2.3. *Verification of the coating effect of the target strain*

Based on the optimal coating ratio, the seed coating agent of the target strain was prepared, and the germination test was carried out to verify the coating effect of the target strain. The seed germination test was referred to 2.1.3.

2.3. *16S rRNA sequencing*

The bacterial strain was subsequently sent to BGI for sequencing. 16S rRNA amplification was performed using universal primers 27 F (5′-AGAGTTTGATCCTGG CTCAG-3) and 1492 R (5′-TACGGGCTACCTGTTACGATT-3′). The PCR amplification process involved an initial denaturation at 94 ℃ for 5 minutes, followed by 35 cycles comprising 20 seconds of denaturation at 96 ℃, 30 seconds of annealing at 55 ℃, and 30 seconds of extension at 72 ℃. The final extension was conducted at 72 ℃ for 10 minutes, followed by cooling to 4 ℃. Subsequently, this study compared the sequencing results with the NCBI BLAST database for further analysis (NCBI link: https://www.ncbi.nlm.nih.gov/) (Mirsam et al., 2022).

2.4. *Pot experiment*

The *Z. mays* pot experiment was conducted in June 2022 in the No.1 greenhouse at Southwest University. The potted soil used was purple soil collected from Xiema, Beibei District, Chongqing, China, situated in the parallel ridge of eastern Sichuan. The soil has a pH of 5.8, soil organic matter (SOM) of 9.11 g·kg^{-1}, alkali-hydrolyzed N of 60 mg·kg^{-1}, available P of 10.51 mg·kg^{-1}, and available K of 43.92 mg·kg^{-1}. The experiment employed a 2 * 4 random block design, with normal soil serving as the control. It introduced a salt concentration of 300 mM NaCl. Within each salt level, four treatments were established based on the application method of the bacterial solution: 1) CK: Control treatment with no additional application, 2) BCK: Sole application of biochar coating without bacterial inoculation, 3) ZH3_2: Conventional *S. nematodipila* inoculation via seed soaking, and 4) BZH3_2: *S. nematodiphila*-biochar-based seed coating. Each treatment had a total of 6 of biological replicates. To address the low SOM content in the tested soil, organic fertilizer was applied before the experiment commenced. Each pot was filled with 10 kg of soil, and seedlings were transplanted when the soil moisture content reached 70 %. After allowing the seedlings to stabilize for 4–5 days, 300 mM NaCl solution was applied daily, with 200 mL each time. To prevent salt shock effects, the initial NaCl solution used a lower concentration, gradually increasing to the established concentration while maintaining adequate moisture throughout the period (Liu et al., 2024).

2.5. *Determination of growth indicators*

Plant height of *Z. mays* was measured 20 d after transplanting. Leaf photosynthetic rate was recorded using a photosynthesis meter (3051D, Zhejiang, China). Root and shoot were separated, washed, and fresh and dry weights were measured. Additional root samples from *Z. mays* were thoroughly rinsed with water, then scanned and imaged using an HP ScanJet root scanner.

2.6. *Determination of plant and soil nutrients, physiological and biochemical indicators*

2.6.1. *Plant sodium (Na$^+$) and potassium (K$^+$) content*

Dried root and shoot samples were ground to 0.5 mm using a stainless steel mill (FZ102, Tianjin, China), digested with a concentrated HNO_3 and $HClO_4$ mixture (4:1 v/v/v), and analyzed via flame photometry (Liu et al., 2024).

2.6.2. *Leaf osmotic substances*

Fresh plant samples were used to measure physiological indices. Proline was quantified spectrophotometrically at 520 nm after reaction with ninhydrin under acidic conditions (Liu et al., 2024). Soluble sugars were determined colorimetrically at 620 nm using the sulfate-anthrone method (Huang et al., 2022), and soluble protein was measured colorimetrically at 595 nm with the Thomas brilliant blue method (Chen et al., 2023).

2.6.3. *Soil nutrients*

Fresh soil samples were extracted with 2 M KCl solution (soil:KCl = 1:5), and ammonium N (NH_4^+-N) and nitrate N (NO_3^--N) contents were determined using an elemental analyzer (Thermo-Element Flash EA 1112, USA) (Liu et al., 2024). Physicochemical properties were analyzed on air-dried, sieved soil samples. Soil alkali-hydrolyzed N content was determined using the alkaline diffusion method (Cheng et al., 2023), while soil water-soluble Na (Na$^+$) and potassium contents were determined via flame photometry (Liu et al., 2024).

2.7. *Analysis of soil bacterial community structure*

Z. mays roots were gently shaken to collect rhizosphere soils. Total microbial genomic DNA from 12 samples was extracted using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's instructions. The quality and concentration of DNA were assessed through 1.0 % agarose gel electrophoresis and a NanoDrop2000 spectrophotometer (Thermo Scientific, United States). The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified with primer pairs 515 F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806 R (5′- GGACTACNVGGGTWTCTAAT-3′) with a T100 Thermal Cycler PCR thermocycler (BIO-RAD, USA) (Zhou et al., 2024).

This study used Mothur software (http://www.mothur.org/wiki/Calculators, version 1.30.2) for Alpha diversity analysis, and used the rank sum test (Welch's (uncorrected) 0.95) for inter-group difference analysis of Alpha diversity. The similarity among microbial communities in different samples was assessed through principal component analysis (PCA) based on Bray-Curtis dissimilarity. The PERMANOVA test was employed to evaluate the percentage of variation explained by the treatment and its statistical significance. To identify significantly abundant taxa (phylum to genera) of bacteria among different groups (LDAscore > 2, $p < 0.05$), linear discriminant analysis (LDA) effect size (LEfSe) was performed. Furthermore, employing redundancy analysis (RDA) and correlation network diagrams, this study aims to explore the intricate interplay among environmental factors such as rhizosphere soil nutrient status, plant biomass, photosynthetic rate, and osmotic regulator substances, shedding light on the underlying mechanisms governing the phenotypic distinctions observed between samples (Goberna and Verdú, 2022).

2.8. *Statistical analysis*

IBM SPSS Statistics 25 was employed for data analysis. Single-factor analysis of variance was employed to analyze the effects of PGPB inoculation on soil nutrients, plant physiological indices, and plant growth indices under normal and salt stress conditions, followed by LSD tests ($p < 0.05$). After data analysis, a bar chart is created using Origin 2022. Partial least squares structural equation modeling (PLS-SEM) was employed to identify pathways through which an exogenous *S. nematodiphila*-biochar-based seed-coating agent mediated plant growth under salt stress conditions. The statistical analyses were conducted in R version 4.0.2 and executed using the PLS-SEM package (Jiang et al., 2021).

3. Results

3.1. Isolation and screening of salt-tolerant PGPB

In this study, more than 100 salt-tolerant strains, including both rhizosphere bacteria and endophytic bacteria, were screened from farmland soil and crop plants using an initial screening medium with 3 % NaCl. Subsequently, by incrementally increasing the salt concentration to levels such as 6 %, 9 %, 12 %, and 15 %, a total of 27 highly salt-tolerant strains were identified as potential candidates for further screening of salt-tolerant PGPB (Table S1). To further identify target functional strains with PGP properties, we assessed these strains for their ability to produce auxins and siderophore and utilize ACC deaminase. Qualitative results indicated that all 27 highly salt-tolerant strains exhibited certain PGP abilities (Table S2). Quantitative results for auxins revealed that strains M4 and ZH3_2 displayed the optimum auxins production capacity, with concentrations of 27.95 $\mu g \cdot L^{-1}$ and 26.19 $\mu g \cdot L^{-1}$, respectively (Table S3). Considering both the PGP indicators and the respective growth characteristics of these strains, we selected strains M1, M4, M7, and ZH3_2 as candidates for salt-tolerant PGPB. These four salt-tolerant PGPB strains were further evaluated for their actual PGP effects through *Z. mays* germination experiments (Fig. S1). The results of the *Z. mays* germination test revealed that, under normal conditions, the ZH3_2 strain exerted the most pronounced promoting effect on *Z. mays* germination, while the PGP effects of strains M1, M4, and M7 were relatively modest. However, when subjected to salt stress conditions, the PGP effect of strains M4 and ZH3_2 on root length and vitality index was significantly higher than that observed in the control and other strain treatments. These findings underscore the potential of strains ZH3_2 and M4 as principal candidates for mitigating salt stress in *Z. mays*.

The results of germination tests, conducted with different biochar coating ratios (Fig. S2a), demonstrated a significant improvement in both root and bud lengths. Notably, there was a 58 % increase in root length and a 73 % increase in bud length when the seed weight to biochar weight ratio was set at 16 %. This ratio was identified as optimal due to its pronounced promotion of growth. Additionally, germination tests focused on specific strains provided further confirmation, highlighting the substantial capacity of strains BM4 and BZH3_2 to enhance seed germination and subsequent seedling growth (Fig. S2b).

Following a thorough evaluation of the PGP capacities exhibited by strains M4 and ZH3_2, this study selected the ZH3_2 strain as the target strain. Utilizing an NCBI-BLAST comparison, strain ZH3_2 was identified as *S. nematodiphila*, with the accession number OQ561198.

3.2. External S. nematodiphila-biochar-based seed coating agent alleviates salt stress in Z. mays

3.2.1. Rhizosphere nutrient content

Through an analysis of nutrient content in the *Z. mays* rhizosphere, we determined that, under normal (0 mM NaCl) and salt stress (300 mM NaCl) conditions, there were no significant differences in alkali-hydrolyzed N and available K contents across treatment groups (Fig. 1b, c). Notably, BZH3_2 and ZH3_2 significantly reduced Na^+ levels in both normal and salt-stressed soils, with reductions ranging from 23.04 % to 35.12 % under normal conditions and 32.51–42.77 % under salt stress (Fig. 1a). Regarding NH_4^+-N and NO_3^--N contents under normal conditions, the NH_4^+-N content in BZH3_2 rhizosphere soil significantly increased by 11.90 %, while the NO_3^--N contents in ZH3_2 and BZH3_2 rhizosphere soil showed significant increases of 62.02 % and 15.01 %, respectively, compared to the control (CK) (Fig. 1d, e). However, under salt stress, the NH_4^+-N contents of BZH3_2 and ZH3_2 rhizosphere soil increased by 5.63 % and 29.98 %, respectively, compared to the control, with only the increase in ZH3_2 reaching statistical significance. The NO_3^--N contents in BZH3_2 and ZH3_2 rhizosphere soil significantly increased by 11.72 % and 16.95 %, respectively, compared to the control. These findings collectively affirm that *S. nematodiphila* serves as a plant growth-promoting bacterium, regulating nutrient balance in both normal and saline soils without adversely affecting soil nutrient availability.

3.2.2. Growth and development status of Z. mays

Observations of *Z. mays* growth and development revealed that irrespective of the presence of salt stress, ZH3_2 and BZH3_2's plants exhibited increased vigor with more abundant root hairs and plant height when compared to the control groups (CK, BCK) (Fig. 2a, b). These enhanced root structures facilitate greater nutrient absorption. Furthermore, this research into plant photosynthetic rate also affirmed that, regardless of salt stress conditions, ZH3_2 and BZH3_2's plant also exhibited higher photosynthetic rates (Fig. 2c).

As evident from Fig. 2d-g, it was clear that salt stress consistently impacted both the aboveground (shoot) and underground (root) biomass of *Z. mays*. Under normal conditions, BZH3_2's shoot and root fresh weight were 19.71 g and 3.26 g, respectively, signifying a significant increase compared to the control. However, under salt stress, BZH3_2 exhibited aboveground fresh weight of 16.94 g and underground fresh weight of 2.64 g. These values represented remarkable increases of 74.46 % and 107.87 % compared to CK. When considering dry matter accumulation, it became apparent that under salt stress conditions, the total dry matter mass of BZH3_2 and ZH3_2 was 2.95 g and 2.01 g, respectively. These values represented remarkable increases of 130.47 % and 57.03 % compared to CK, showing that the promotion effect of BZ3_2 on plants was better than that of ZH3_2. These findings confirmed the PGP effects of *S. nematodiphila* and its ability to mitigate salt stress in *Z. mays*, also validating the superior effectiveness of biochar-based seed coating over biological soaking.

3.2.3. Sodium (Na^+) and potassium (K^+) homeostasis and osmotic regulation in Z. mays

Fig. 3a-f illustrate the effects of various treatments on Na^+ and K^+ homeostasis in the root and shoot of *Z. mays* under normal and salt stress conditions. Overall, inoculation with strain ZH3_2 positively influenced Na^+ and K^+ homeostasis. Specifically, both root and shoot systems of *Z. mays* inoculated with ZH3_2 and BZH3_2 exhibited higher K^+ levels, lower Na^+ levels, and improved Na^+/K^+ ratio under salt stress. Compared to CK, the ZH3_2 root system showed a 28.42 % reduction in Na^+ level, a 32.93 % increase in K^+ level, and a 46.21 % decrease in the Na^+/K^+ ratio. In contrast, the BZH3_2 root system demonstrated a 37.40 % reduction in Na^+ level, a 49.04 % increase in K^+ level, and 57.98 % decrease in the Na^+/K^+ ratio, all of which were statistically significant. These findings indicate that BZH3_2 was more effective than ZH3_2 in alleviating salt stress in *Z. mays*. Notably, under normal conditions, the BZH3_2 root and shoot systems also exhibited higher K^+ levels and lower Na^+ levels and Na^+/K^+ ratio. Specifically, the Na^+ level in the BZH3_2 root system was significantly reduced by 24.30 % compared with CK, and the Na^+/K^+ ratio was significantly decreased by 28.92 %. In the BZH3_2 shoot, the Na^+ level was significantly lower by 18.50 % compared to CK, with the Na^+/K^+ ratio reduced by 30.23 %. These results suggest that BZH3_2 promoted nutrient absorption and ion balance in *Z. mays*, demonstrating strong resistance and growth potential.

Osmotic regulators (e.g., soluble sugar, soluble protein, and proline) play a crucial role in adjusting crop osmotic pressure to enhance crop tolerance to salt stress. Overall, inoculation with strain ZH3_2 positively influenced the contents of soluble sugar, soluble protein, and proline in *Z. mays* leaf, both under salt stress and normal conditions (Fig. 3g-i). Specifically, compared to CK, the soluble sugar content in ZH3_2 leaves increased by 18.15 %, soluble protein content by 25.20 %, and proline content by 53.48 %. In contrast, leaves from BZH3_2 treatment showed a 22.97 % increase in soluble sugar, an 85.17 % increase in soluble protein, and a 96.09 % in proline. These results indicate that BZH3_2 exhibited a stronger osmotic adjustment capability under salt stress,

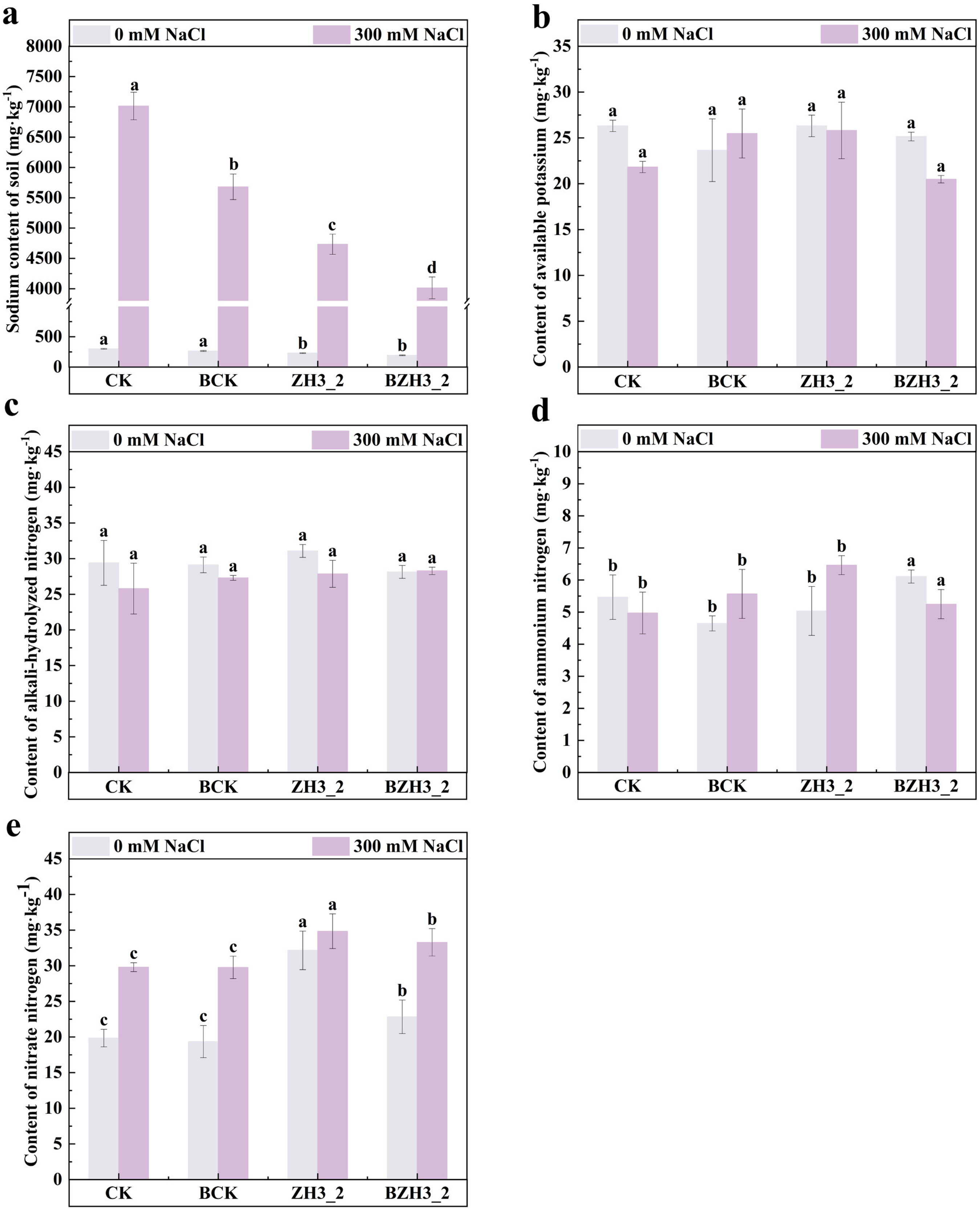

Fig. 1. Effects of *S. nematodiphila* inoculation directly and via biochar-based seed coating agent on the content of effective nutrients and sodium in the rhizosphere of *Zea mays* under normal and salt stress conditions, **Note:** 1) a, b, c, d and e respectively represent the content of sodium (Na^+), available potassium (K), alkali-hydrolyzed nitrogen (N), ammonium N (NH_4^+-N), and nitrate N (NO_3^--N) in the rhizosphere soil. 2) The letters above the bar chart indicate the significance of ANOVA ($p < 0.05$). The error bar represents the standard deviation of the mean.

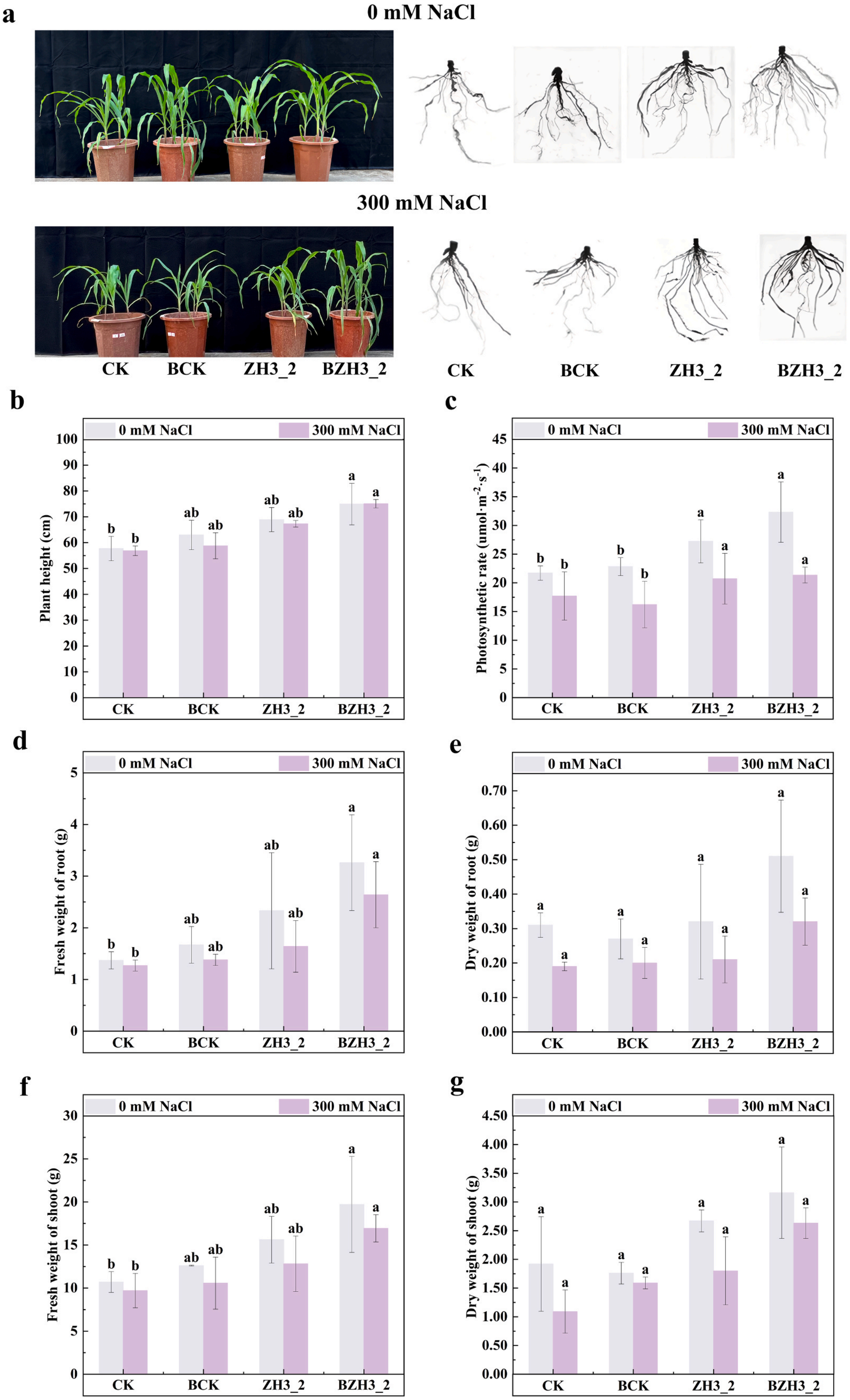

Fig. 2. Effect of *S. nematodiphila* inoculation directly and via biochar-based seed coating agent on plant growth and development of *Zea mays* under normal and salt stress conditions, **Note:** 1) a respectively represent *Z. mays* plant and root. 2) b, c, d, e, f, and g respectively represent the plant height, photosynthetic rate, fresh weight and dry weight of root system, fresh weight and dry weight of shoot system. 2) The letters above the bar chart indicate the significance of ANOVA ($p < 0.05$). The error bar represents the standard deviation of the mean.

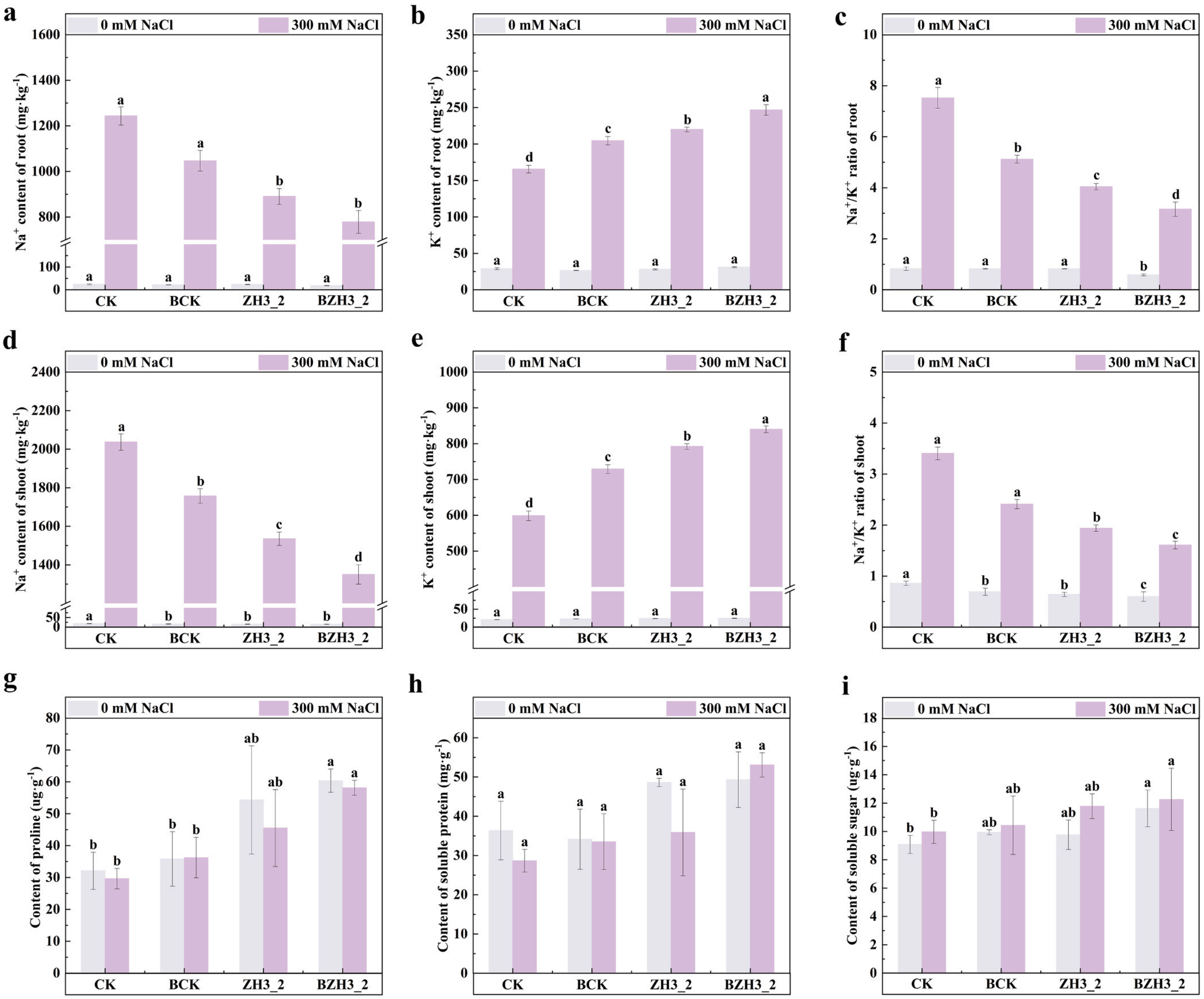

Fig. 3. Effect of *S. nematodiphila* inoculation directly and via biochar-based seed coating agent on Na^+, K^+, and osmotic regulating substances in *Z. mays* leaves under normal and salt stress conditions, **Note:** 1) a, b, and c respectively represent the Na^+-K^+ homeostasis of the root system. 2) d, e, and f respectively represent the Na^+-K^+ homeostasis of the shoot system. 3) g, h, and i respectively represent the proline, soluble protein, and soluble sugar content of the leaves. 2) The letters above the bar chart indicate the significance of ANOVA ($p < 0.05$). The error bar represents the standard deviation of the mean.

underscoring the efficacy of biochar seed coat in alleviating crop salt stress. Interestingly, BZH3_2 also significantly enhanced leaf soluble sugar content by 27.97 % under normal conditions, further confirming the beneficial effects of biochar seed coat on *Z. mays*.

3.2.4. *Correlation analysis*

The PEARSON correlation analysis results revealed that, under normal conditions, there was a significant positive correlation between the fresh weights of shoot and root and the levels of proline and soluble sugar (Fig. 4a). Additionally, the proline content in leaves exhibited a noteworthy positive correlation with the photosynthetic rate. Notably, there was a highly significant positive correlation between plant height and the contents of proline and soluble sugar. Regarding soil Na^+ level, soil Na^+ content exhibited a significant negative correlation with plant height, plant biomass (fresh weight of shoots and roots, dry weight of shoots), photosynthetic rate, osmotic substances (soluble sugar, soluble protein, and proline), while it was positively correlated with plant Na^+/K^+ ratio. These findings underscore how the *S. nematodiphila* strain (ZH3_2, BZH3_2) affects crop C metabolism levels, material accumulation, and promotes crop growth and development.

Under salt stress conditions, a negative correlation was observed between the Na^+/K^+ ratios of the plant (shoot + root) and plant height, biomass (fresh weight of shoot and root, dry weight of shoot), soluble protein content, and proline content. Plant height and the fresh weights (shoot + root) of *Z. mays* demonstrated significant positive correlation with proline levels (Fig. 4b). Simultaneously, plant height, the dry weights (shoot + root), and the fresh weights (shoot + root) in *Z. mays* also exhibited a noteworthy positive correlation with the soluble protein content. These results confirm the close relationship between plant growth status, Na^+ and K^+ homeostasis, and osmoregulatory substances such as soluble protein and proline under salt stress. The *S. nematodiphila* strain alleviated crop salt stress by regulating these osmoregulation substances within the plant. In terms of soil nutrients, a positive correlation was observed between leaf photosynthetic rate and soil alkali-hydrolyzed N content. The plant height exhibited a noteworthy positive correlation with NO_3^--N content. Soil Na^+ content exhibited a positive correlation with plant height, biomass (fresh weight of shoot and root, dry weight of shoot), soluble protein content, and proline content. These indicated that the *S. nematodiphila* may also regulate the ecological function of saline soil, which may be related to the microbial

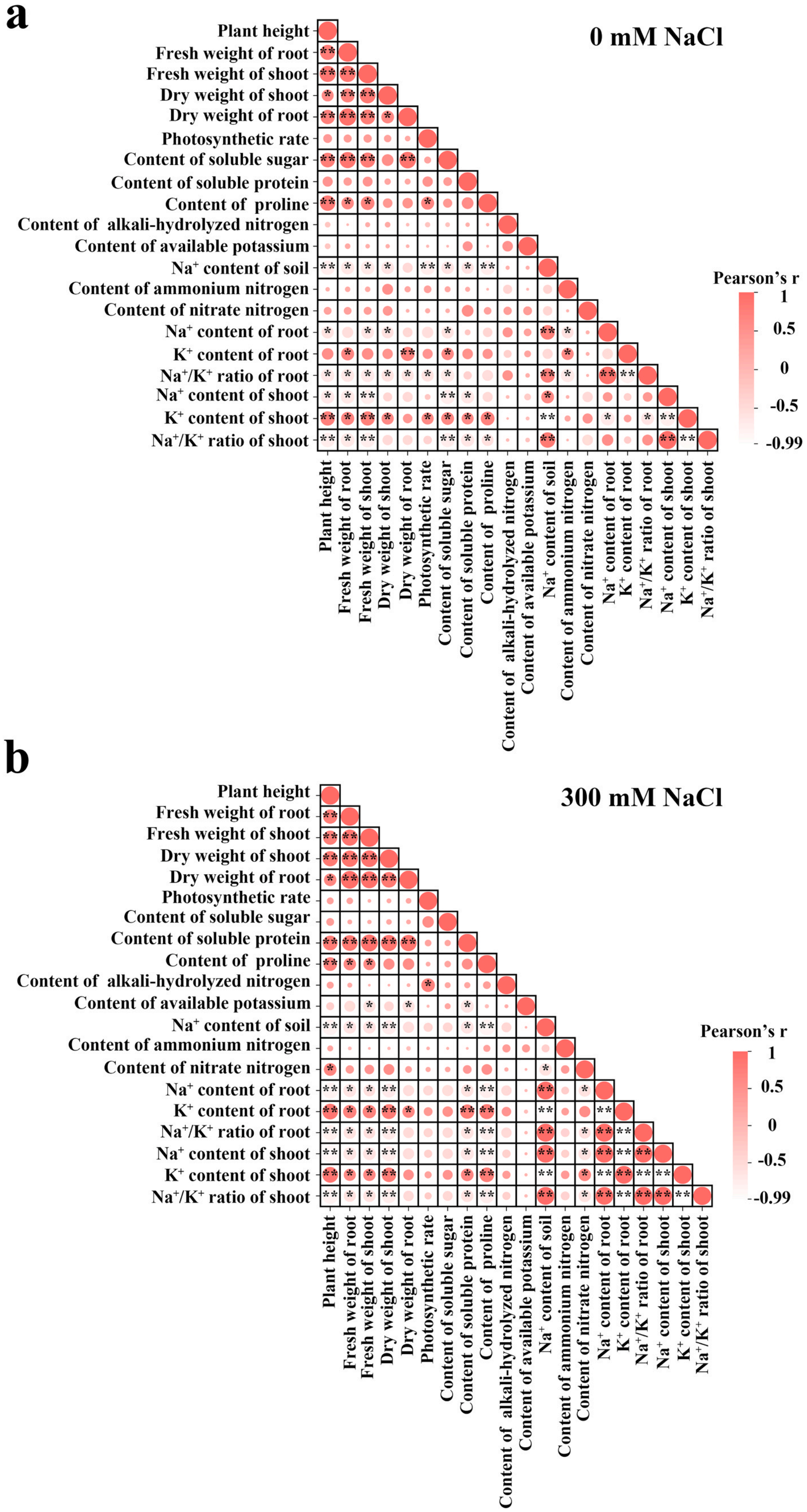

Fig. 4. Correlation analysis between rhizosphere nutrients and plant physiological indicators under normal and salt stress conditions, **Note:** 1) a and b respectively represent the correlation test results between rhizosphere nutrient contents and plant physiological indicators under non-salt stress and salt stress conditions. 2) The PEARSON correlation is depicted by a color spectrum ranging from red to white. 3) The symbol "* " indicates statistical significance.

community structure. Therefore, it is necessary to analyze the community structure of saline soil.

3.3. Recruitment of beneficial bacterial communities using exogenous S. nematodiphila-biochar-based seed-coating agent under salt stress

The results of ace, chao, coverage, shannon, simpson, and sobs indices showed that inoculation *S. nematodiphila* strain (ZH3_2, BZH3_2) did not significantly alter the Alpha diversity of the rhizosphere bacteria of *Z. mays* under salt stress conditions (Table S4). As for Beta diversity of rhizosphere samples, we used for principal component analysis (PCA) based on Bray_curtis distance (Fig. 5a). The results showed that *S. nematodiphila*-biochar-based seed coating agent caused changes in rhizosphere bacterial community structure, compared with the control group ($R = 0.4660$, $p = 0.002$).

For a more comprehensive understanding of bacterial community structure changes, we conducted an analysis of the network properties using Gephi (Fig. 5b). The outcomes revealed that among the top 100 species at the genus level, there were 364 positive and 263 negative correlations. The entire microbial network could be categorized into four modules (average degree = 11.98, network density = 0.121, modularity coefficient = 0.419). The results from the pie chart and Circos chart depicting community composition indicated that, at the phylum level, the top four microbial species were consistent across different samples, all being comprised of Proteobacteria, Actinobacteriota, Chloroflexi, and Firmicutes (Fig. 5c, e). Nevertheless, the relative abundance of these microbiota varied among the samples. In CK, the top four microbial species had the relative abundance of 20.56 %, 20.65 %, 10.11 %, and 7.68 %, respectively (Fig. S3a). In the case of BCK, the relative abundance of the top four microbial species was 20.98 %, 19.91 %, 9.84 %, and 9.20 %, respectively (Fig. S3b). For ZH3–2, the relative abundance of the top four microbial species was 24.76 %, 20.77 %, 10.27 %, and 8.55 %, respectively (Fig. S3c). Lastly, in the BZH3_2 treatment group, the relative abundance of the top four microbial species in BZH3_2 was 24.77 %, 16.71 %, 11.29 %, and 7.76 %, respectively (Fig. S3d).

The composition of bacterial communities at the class level mirrors the trends observed at the phylum level, with the top four microbial species encompassing Gammaproteobacteria, Alphaproteobacteria, Actinobacteria, and Bacilli (Fig. 5d, f). Nonetheless, there were variations in the relative abundance of these microbiota across different samples. In CK, the top microbial species exhibited relative abundances of 10.02 %, 10.53 %, 9.96 %, and 7.09 %, respectively (Fig. S4a). For the BCK group, the relative abundance of the top four microbial species stood at 9.98 %, 10.96 %, 10.09 %, and 8.23 % (Fig. S4b). In the ZH3_2 treatment group, the relative abundance of the top four microbial species was 14.35 %, 10.38 %, 11.35 %, and 7.69 % (Fig. S4c). Lastly, for the BZH3_2 treatment group, the relative abundance of the top four microbial species was 13.79 %, 10.94 %, 9.93 %, and 6.76 % (Fig. S4d).

3.4. Correlation analysis of environmental factors and rhizosphere bacterial communities

We employed redundancy (RDA) analysis (Fig. 6) and correlation network diagram (Fig. 7) to investigate the relationship between environmental factors (e.g., Na^+ and K^+ homeostasis, plant biomass, photosynthetic rate, osmotic regulator substances and rhizosphere soil nutrient status) and the structure of rhizosphere bacterial community. This analysis provides further insights into the mechanism underlying the formation of phenotypic differences among samples.

The correlation analysis between Na^+ and K^+ homeostasis and bacterial communities revealed that soil Na^+ exhibited the strongest correlation with rhizosphere bacterial communities, followed by root Na^+ and K^+ content, while the Na^+ and K^+ contents in shoots showed the weakest correlation with rhizosphere bacterial communities (Fig. 6a). In addition, soil and plant Na^+ content and $Na^+/\ K^+$ ratio were negatively correlated with the composition of the BZH3_2 bacterial community at the phylum level. Network diagram results indicated that soil and plant Na^+ content negatively correlated with Gammaproteobacteria, whereas K^+ content in plant positively correlated with Gammaproteobacteria (Fig. 7a).

The correlations between plant biomass and bacterial communities revealed that the strongest correlation existed between shoot dry weight and bacterial community, followed by root dry weight and shoot fresh weight (Fig. 6b). Notably, the correlation between root fresh weight and bacterial communities was comparatively weaker. Furthermore, the bacterial communities of BZH3_2 exhibited a positively correlation with plant biomass (fresh + dry). Specifically, fresh root weight demonstrated a positive correlation with Saccharimonadia and Bacteroidia at the class level (Fig. 7b). Additionally, Polyangia displayed positive correlations with dry root weight and fresh shoot weight.

Simultaneously, we conducted an analysis of the correlations between photosynthetic rate, osmotic regulatory substances and microbial communities (Fig. 6c). The findings indicated that proline content exhibited the strongest correlation with bacterial communities, followed by photosynthetic rate and soluble protein content. Conversely, the correlation between soluble sugar content and bacterial communities was the lest pronounced. Among the environmental factors, photosynthetic rate demonstrated a close relationship with soluble protein content, while proline content was closely associated with the soluble sugar content. In addition, the microbial communities of BZH3_2 displayed a positive correlation with both photosynthetic rate and soluble protein content. Furthermore, photosynthetic rate and proline content showed positive correlations with Gammaproteobacteria, while Verrucomicrobiae exhibited a positive correlation with photosynthetic rate, and Kapabacteria showed a positive correlation with soluble sugar content (Fig. 7c).

The correlations between rhizosphere soil nutrient contents and bacterial communities revealed that alkali-hydrolyzed N and available K contents exhibited the strongest correlation with the rhizosphere bacterial communities, followed by NO_3^--N and NH_4^+-N contents (Fig. 6d). Additionally, a positive correlation was identified between the N content in the rhizosphere soils and the bacterial community composition of BZH3_2 at the phylum level. The network diagram results illustrated that the alkali-hydrolyzed N content was positively correlated with Verrucomicrobiae, NH_4^+-N content was positively correlated with c_S0134_terrestrial_group, and NO_3^--N was positively correlated with Gammaproteobacteria (Fig. 7d).

The PLS-SEM analysis identified pathways mediating plant growth by the exogenous *S. nematodiphila*-biochar-based seed-coating agent under salt stress (Fig. 8). The results revealed that the *S. nematodiphila*-biochar-based seed-coating agent had indirect positive effects on plant growth under salt stress. On one hand, *S. nematodiphila*-biochar-based seed-coating agent promoted plant growth under salt stress by recruiting beneficial bacterial communities that regulate plant osmotic balance. On the other hand, it assisted plants in coping with salt stress by enhancing Na^+ and K^+ homeostasis through the recruitment of beneficial bacterial communities. Notably, our findings also demonstrate that the *S. nematodiphila*-biochar-based seed-coating agent primarily exerted an indirect positive effect on the nutrient status of saline soil by fostering beneficial bacterial communities.

4. Discussion

4.1. External S. nematodiphila-biochar-based seed coating agent alleviated salt stress in Z. mays

Salt stress, an abiotic stress, severely inhibits plant growth and soil microbiology (Navarro-Torre et al., 2023). This study identified *S. nematodiphila*, a highly salt-tolerant and growth promoting bacterium, which demonstrated exceptional performance through dual screening based on gradient salt concentrations and growth promotion capacities.

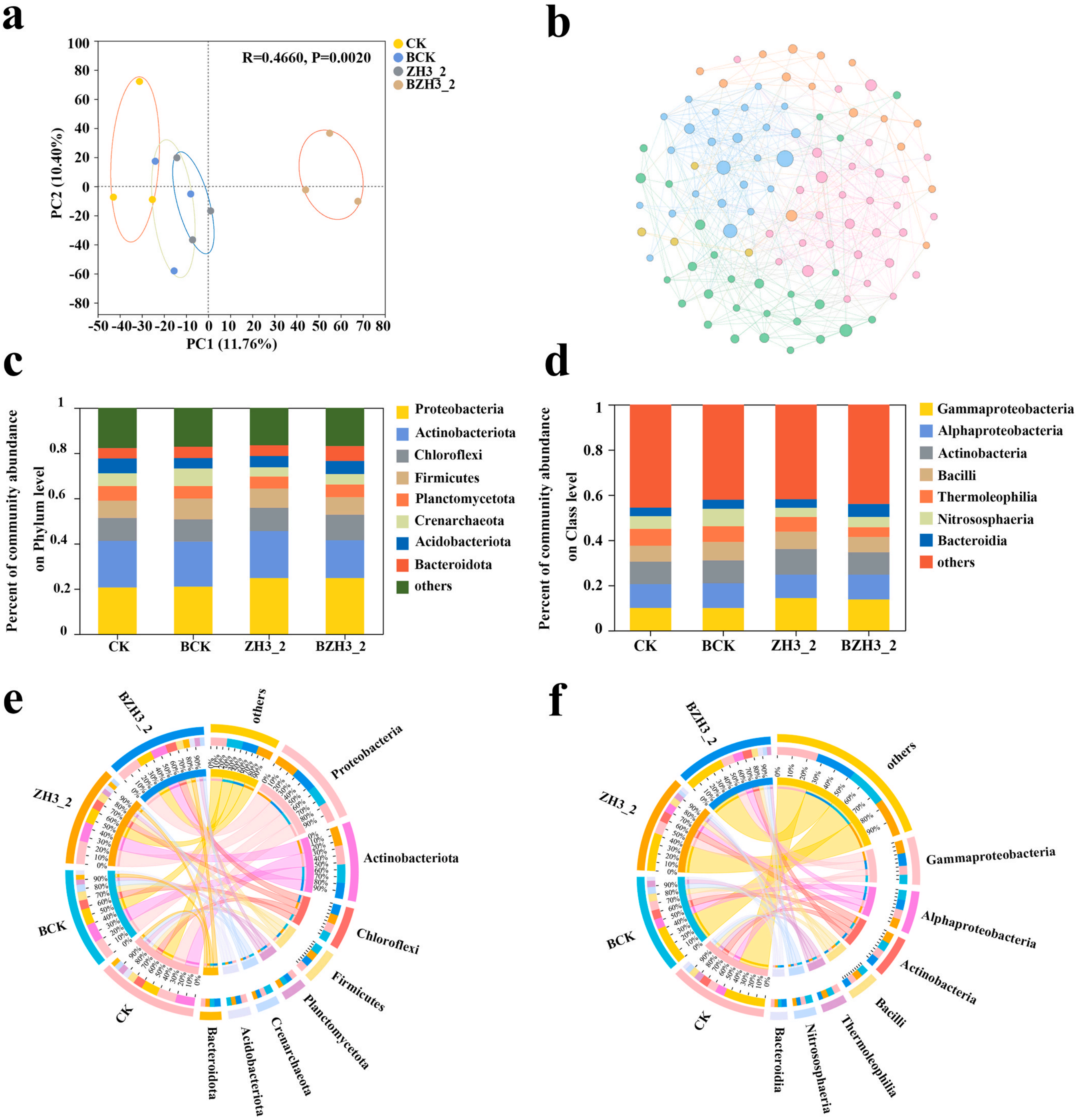

Fig. 5. Bacterial community composition and network analysis, **Note:** 1) a represents the Beta diversity of rhizosphere bacteria. The X axis and Y axis represent the two selected main coordinate axes, and the percentage represents the explanatory value of the main coordinate axis for the differences in sample composition. Points of different colors or shapes represent samples of different groups, and the closer the two sample points are, the more similar the species composition of the two samples is. 2) b is the species correlation network graph which mainly reflects the species correlation at each taxonomic level under certain environmental conditions. The size of nodes in the figure indicates the species abundance, and different colors indicate different modules. The color of the line is the module color. More lines indicate that the species is more closely related to other species. 3) c and d respectively represent the bacterial community composition at the phylum level and class level. Different colors represent different species. 4) e and f respectively show the relationship between samples and species at the phylum level and class level. The left half circle represents the species composition in the sample, the color of the outer colored band represents the grouping situation, the color of the inner colored band represents the species, and the length represents the relative abundance of the species in the corresponding sample. The right half circle represents the distribution proportion of species in different samples. The outer colored band represents the species, the inner colored band represents different groups, and the length represents the distribution proportion of the sample in a certain species.

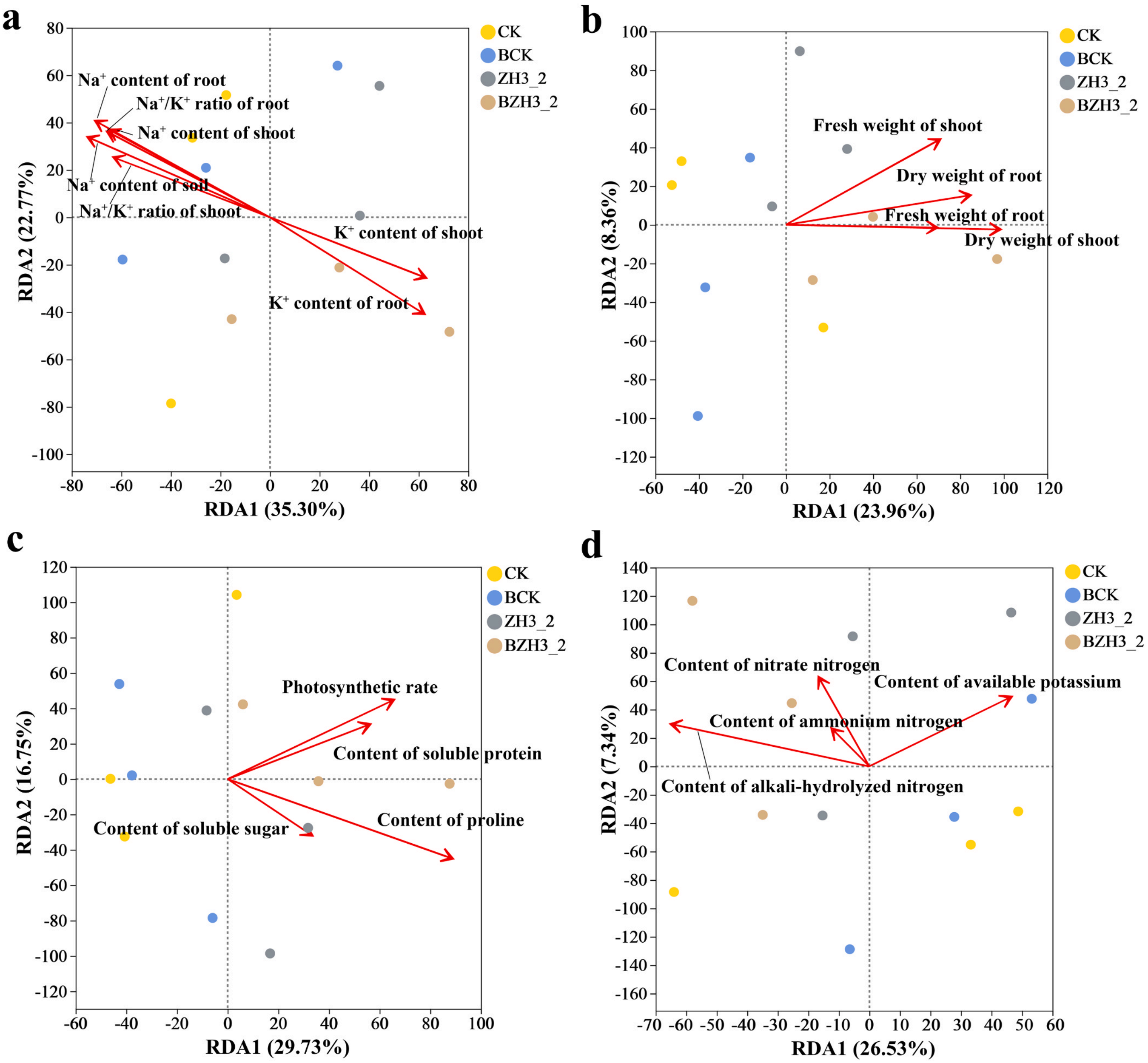

Fig. 6. RDA analysis of communities and different environmental factors, **Note:** 1) a, b, c and d respectively indicate the RDA analysis of Na^+ and K^+ homeostasis, plant biomass, osmotic matter, soil available nutrient and the structure of rhizosphere bacteria. 2) The angle between the arrows of environmental factors represents a positive and negative correlation (acute angle: positive correlation; obtuse angle: negative correlation; right angle: no correlation). Projection is made from the sample point to the arrow of quantitative environmental factors. The distance between the projection point and the origin represents the relative impact of environmental factors on the distribution of the sample community. Whether the direction of the point and arrow is consistent represents positive and negative correlations.

Previous research has highlighted the PGP effects of *S. nematodiphila* and its ability to remediate soil stress induced by pollutants such as lead, nickel, and cadmium (Zulfiqar et al., 2022). However, there are no reports on its role in mitigating salt stress in *Z. mays*. In the present study, we confirmed the potential of *S. nematodiphila* to alleviate salt stress in *Z. mays* through germination and potting experiments. The results demonstrated that *S. nematodiphila* consistently promoted the germination and growth of *Z. mays* under salt stress (Fig. S1, S2b, Fig. 2). Furthermore, *Z. mays* plants inoculated with *S. nematodiphila* (ZH3_2 and BZH3_2) exhibited increased plant height, more robust root systems, and greater biomass accumulation (Fig. 2). These positive effects can be attributed to the various PGP properties of *S. nematodiphila*, including auxins and siderophore production, and ACC deaminase activity, all of which contribute to increased crop yields (Jagtap et al., 2023; Dastager et al., 2011).

This study highlights the growth-promoting effects of *S. nematodiphila*-biochar-based seed coating on *Z. mays* growth in saline soil. It is the first report demonstrating that this seed coating can alleviate salt stress in *Z. mays* by modulating plant physiological and biochemical processes, enhancing saline soil microbiology, and regulating Na^+ and K^+ homeostasis (Fig. 8). Salt stress often leads to excessive Na^+ accumulation and K^+ deficiency in plants, resulting in ionic imbalance and osmotic stress, which ultimately limits plant productivity (Navarro-Torre et al., 2023). Interestingly, in this study, the inoculation of *S. nematodiphila* under salt stress significantly reduced Na^+ content in both root and shoot, while increasing K^+ content and

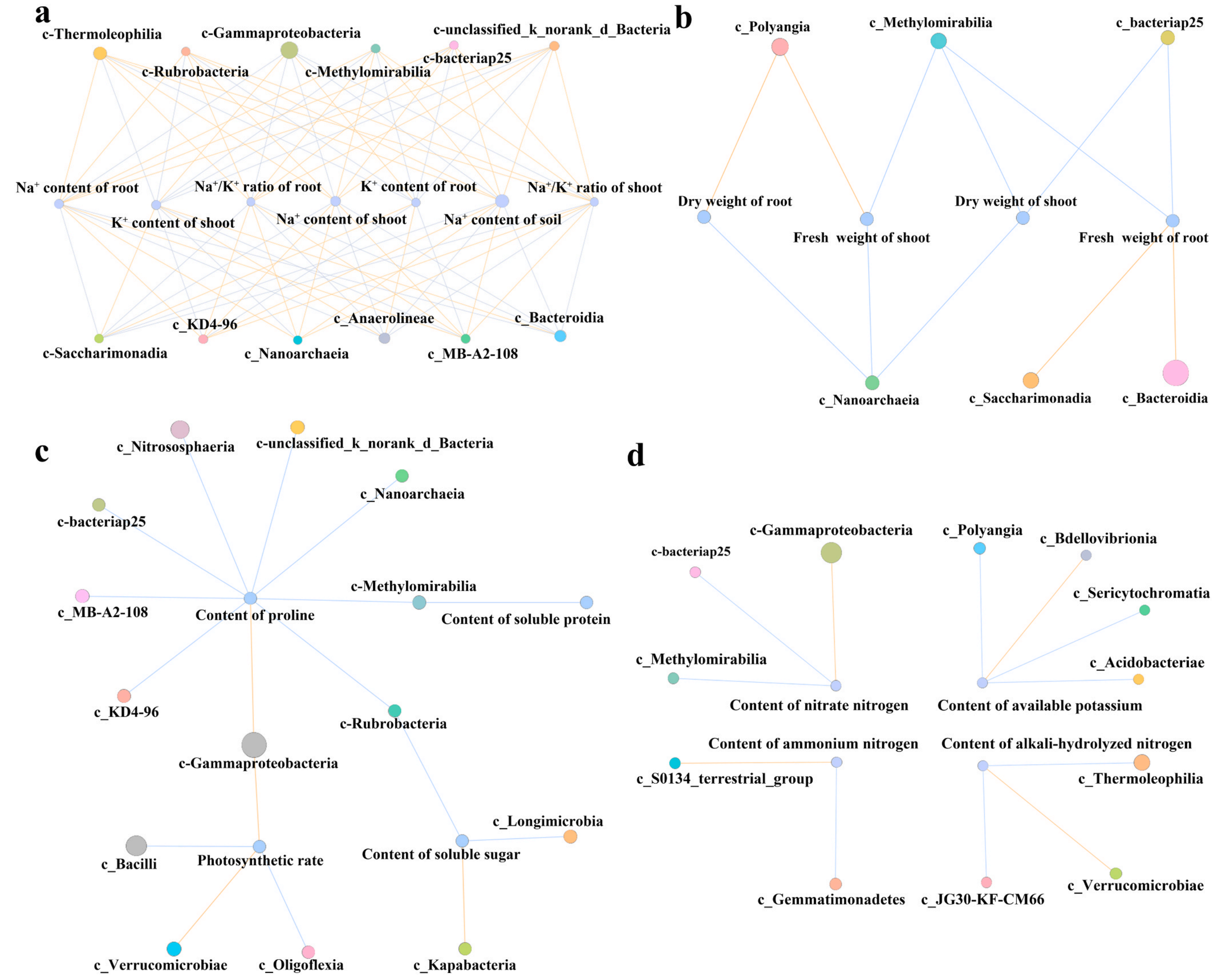

Fig. 7. Network diagram of community and different environmental factors; **Note**: 1) a, b, c and d respectively indicate a correlation network diagram of Na^+ and K^+ homeostasis, plant biomass, osmotic matter, soil available nutrient and the structure of rhizosphere bacteria. 2) The size of the nodes in the figure represents species abundance and environmental factors. The color of the lines indicates positive and negative correlation, with orange indicating positive correlation and blue indicating negative correlation. The thickness of the line indicates the size of the correlation coefficient, and the thickness of the line indicates the higher the correlation between species. The more lines there are, the tighter the connections between nodes.

lowering the Na^+/K^+ ratio. This reduction in Na^+/K^+ ratio indicates the alleviation of salt stress in *Z. mays* (Liu et al., 2024). Similar findings were reported by Liu et al. (2024), who demonstrated that the poly-γ-glutamic acid-producing bacterium *Bacillus amyloliquefaciens* W25 regulated ionic homeostasis and alleviated salt stress by lowering the Na^+/K^+ ratio of lettuce. Furthermore, our study revealed that the *S. nematodiphila*-biochar-based seed coating agent (BZH3_2) had a more pronounced effect compared to the inoculation of *S. nematodiphila* (ZH3_2) (Figs. 2, 3). This difference was attributed to the fact that biochar, as a carrier material, enhanced the colonization efficiency of the strains in the plants, thereby maximizing their PGP performance. Additionally, the porous structure and high specific surface area of biochar allowed for effective adsorption of Na^+ from saline soils, significantly reducing Na^+ uptake by the root system and mitigating the detrimental effects of salinity on the plants (Song et al., 2022).

Osmotic substances, characterized by their low molecular weight, play a crucial role in reducing cell osmotic potential, enabling plants to survive adverse conditions such as salt and drought stress (Kumar et al., 2020). Research has demonstrated that salt-tolerant PGPB stimulate the production of osmotic substances (e.g., soluble protein, soluble sugar and proline) in plants to counteract cell dehydration (Kumar et al., 2020). In this study, the inoculation of *S. nematodiphila* strain (ZH3_2 and BZH3_2) significantly increased proline content in leaves under salt stress, thereby harmonizing the water potential of vacuoles and cytoplasm (Fig. 3) (Liang et al., 2024). This enhancement leads to improved plant phenotypes and increased biomass, thus bolstering salt stress tolerance in *Z. mays* (Fig. 4b) (Liang et al., 2024). Similarly, Sánchez et al. (2023) observed the salt-tolerance and growth-promoting effect of PGPB on *Solanum lycopersicum*. Under salt stress, plants regulate Na and K uptake by accumulating osmotic substances (Liu et al., 2024). Elevated levels of osmotic substances can aid plants in controlling Na^+ uptake while promoting K^+ accumulation (Liu et al., 2024). Correlation analysis in this study further confirmed that the Na^+/K^+ ratio in *Z. mays* was significantly and negatively correlated with soluble protein and proline content (Fig. 4b). This effect may be attributed to the promotion of photosynthesis by *S. nematodiphila*. A robust photosynthetic rate facilitates the accumulation of assimilates such as amino acids and proteins (Zahra et al., 2022). Notably, it has been reported that the inhibition of photosynthesis under salt stress may result from non-stomatal limitations, such as RuBP carboxylase activity and

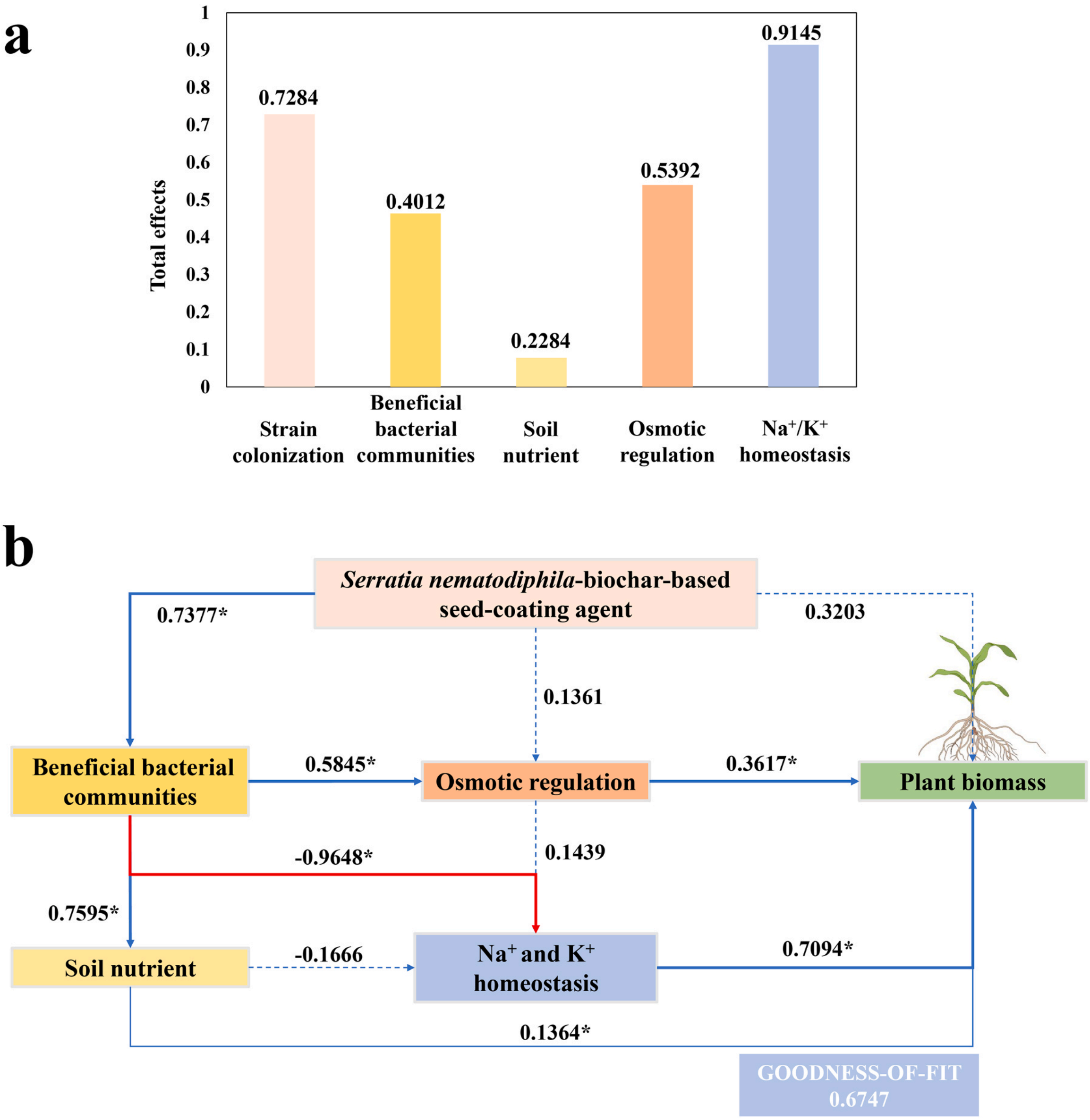

Fig. 8. The partial least squares structural equation model (PLS-SEM) for the effects of strain colonization, plant physiological traits, beneficial bacterial communities and soil nutrients on plant growth under salt stress; **Note:** Strain colonization, plant physiological traits, beneficial bacterial communities and soil nutrients are latent variables. Negative and positive effects are indicated by red and blue line, respectively. The line width is proportional to the effect strength. The numbers on the line represent the correlation between the indicators at both ends.

chlorophyll degradation (Zahra et al., 2022). Bai et al. (2024) also validated that PGPB can influence the biosynthesis of plant photosynthetic enzymes and pigments. However, some studies suggest that PGPB promote photosynthesis and alleviate the adverse effects of salt stress by enhancing the activity of electron transport proteins involved in photosynthesis (Rossi et al., 2021). In summary, this study confirms that *S. nematodiphila*-biochar-based seed coating enhances *Z. mays* tolerance to salt stress by increasing photosynthetic rate, regulating osmoprotectants, and influencing ionic homeostasis. Nonetheless, the detailed molecular mechanisms underlying salt tolerance mediated by this strain require further exploration.

4.2. Recruitment of beneficial microbial communities using exogenous S. nematodiphila-biochar-based seed coating agent

Rhizosphere microorganisms play a crucial role in the subterranean environment of plants, fostering plant growth and bolstering tolerance against salt stresses (Li et al., 2021). In a study by Wang et al. (2022), the inoculation with *Providencia vermicola* BR68 and *Sarocladium kiliense* FS18 significantly influenced the abundance of specific microbial flora. The correlation trends of these microorganisms with soil properties and *Z. mays* physiology under salt stress mirrored those observed for the inoculated strains. Hence, the mechanism of plant growth promotion by PGPB under salt stress includes the stress-induced recruitment of specific root-associated bacteria (Wang et al., 2022). This investigation noted a significant divergence in the community structure of the *S. nematodiphila*-biochar-based seed coating agent compared to other treatments (CK, BCK, ZH3_2) (Fig. 5a). Inoculation with the *S. nematodiphila*-biochar-based seed coating agent increased the relative abundance of Proteobacteria while reducing the relative abundance of Actinobacteriota (Fig. 5c, e, Fig. S3). This finding aligns with research by Trabelsi et al. (2011), demonstrating that inoculation with *S. meliloti* and *Rhizobium gallicum* increased overall bacterial community richness in the soil, particularly in the abundance of α- and γ-Proteobacteria.

Proteobacteria and Actinobacteriota collectively dominate the microbial niche in the rhizosphere (Shi et al., 2020). Previous studies have indicated that various members of Proteobacteria and Actinobacteriota respond to soil salinization, with their mechanism of action attributed to ecological strategies (Shi et al., 2020). These microbial groups interact with plant roots, altering root configuration to adapt to saline soils, as confirmed in this study (Figs. 6b, 7b). It has also been proposed that PGPR utilize biofilm to share metabolic substances with recruited beneficial bacteria, influencing soil metabolic function and mitigating crop salt stress (Cacho et al., 2021; Lee et al., 2023).

This study identified key microbial species at the class level, including Gammaproteobacteria, Verrucomicrobiae, Bacteroidia, Saccharimonadia, Kapabacteria, which responded significantly to the *S. nematodiphila*-biochar-based seed coating agent under salt stress (Figs. 6, 7). These microbial communities have been recognized for their pivotal role in promoting plant growth and their close association with soil nutrient status and plant biomass (Bünger et al., 2020; Huang et al., 2023; Li et al., 2022). This is likely due to these beneficial microorganisms, recruited by PGPB, being major producers of auxins, abscisic acid, cytokinin, carotenoids, and rhodopsin, all of which contribute to plant growth (Bünger et al., 2020). Notably, Gammaproteobacteria and Bacteroidia are typical halophiles with a preference for high-salinity niches, making them potential biomarkers for high-salinity-tolerant communities (Li et al., 2021; Liu et al., 2023). These microorganisms employ two strategies to maintain osmotic equilibrium: 1) aggregation of inorganic salt ions, such as KCl, and 2) accumulation of organic 'compatible' solutes, including glycine betaine, amino acid derivatives, sugars and sugar alcohols (Minhas et al., 2020). These substances also contribute to maintaining the ecological balance of the soil. This study also found that, compared with ZH3_2, BZH3_2 enriched a large number of Bacteroidia ($LDA = 3.85$), Cyanobacteriia ($LDA = 3.56$), Gemmatimonadetes ($LDA = 3.39$), Sericytochromatia ($LDA = 3.29$), Nitrospiria ($LDA = 2.94$), c_unclassified_p_Chloroflexi ($LDA = 2.85$), Acidobacteriae ($LDA = 2.71$), Berkelbacteria ($LDA=2.57$), Thermoplasmata ($LDA=2.28$) at class level (Fig. S5). This may explain the enhanced effectiveness of the *S. nematodiphila*-biochar-based seed coating agent compared to ZH3_2 (Han et al., 2023; Ippolito et al., 2020).

In nature, microorganisms predominantly exist within complex communities rather than in isolation, a fact recognized since the time of van Leeuwenhoek (Gest, 2004). While most previous studies have concentrated on the ability of individual microorganisms to alleviate plant salt stress, the present study reveals more positive correlations among microbial communities associated with *S. nematodiphila* at the inter-root level, implying that this flora exhibits a more cooperative than competitive behavior (Li et al., 2021). Synergistic interactions among microbes have been identified as a crucial mechanism for their adaptation to challenging environment, such as drought and salt stress (Li et al., 2021; Liang et al., 2024). Notably, the study by Li et al. (2021) found that salt-induced interactions among root-derived flora provide lasting resistance to salt stress. Furthermore, this study indicates that the microbial flora is closely linked to plant physiological processes (osmotic adjustment, Na^+ and K^+ homeostasis, and photosynthesis) as well as soil quality, confirming that exogenous PGPB promote nutrient turnover and improve salt tolerance in *Z. mays* by recruiting specific soil microbiota (Fig. 8) (Liu et al., 2023; Romano et al., 2021; Wang et al., 2023). This suggests that the strategic recruitment of beneficial and functional bacterial communities tailored to specific species enables plants to effectively cope with salt stress.

5. Concusion

To summarize, post-inoculation, the *S. nematodiphila*-biochar-based seed coating triggered structural alterations in rhizosphere bacterial communities within saline soils. It positively impacted the abundance of beneficial microbial groups, such as *Proteobacteria*, *Gammaproteobacteria*, *Verrucomicrobiae*, and *Bacteroidia*, thereby enhancing the resilience of *Z. mays* in saline soil conditions. This enhancement was evidenced by elevated photosynthetic rates, balanced osmotic pressure, greater biomass, and a reduced Na^+/K^+ ratio. This method emerges as the optimal strategy for enhancing crop yields in saline soils and holds significant potential for broader improvements in saline soil conditions.

CRediT authorship contribution statement

Yingying Cheng: Formal analysis, Data curation, Writing – original draft. **Mengyuan Cao**: Methodology, Investigation. **Xiaojun Shi**: Writing – review & editing. **Xinping Chen**: Writing – review & editing. **Zhenlun Li**: Writing – review & editing. **Ying Ma**: Conceptualization, Methodology, Validation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2024.120164.

Data availability

Data will be made available on request.

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