Plant Physiology and Biochemistry 204 (2023) 108124

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

# Potential role of root-associated bacterial communities in adjustments of desert plant physiology to osmotic stress

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ARTICLE INFO

*Keywords:*
Desert plants
Osmotic stress
Plant physiology
Root-associated microbes

ABSTRACT

Plants possess the ability to adapt to osmotic stress by adjusting their physiology and morphology and by cooperating with their root-associated (rhizosphere and endosphere) microbial communities. However, the co-ordination of host self-regulation with root-associated microorganisms at the community level, especially for desert plants, remains unclear. This study investigated the morphophysiological responses of seedlings from the desert plant *Alhagi sparsifolia* Shap to osmotic stress, as well as the relationships between these adaptations and their root-associated bacterial communities. The results indicated that osmotic stress contributed to a reduction in height and increased levels of reactive oxygen species (ROS) and malondialdehyde (MDA). In response, *A. sparsifolia* exhibited a series of morphophysiological adjustments, including increased ratio of root to shoot biomass (R/S) and the number of root tip, enhanced vitality, high levels of peroxidase (POD), ascorbate peroxidase (APX), and glutathione (GSH), as well as osmolytes (proline, soluble protein, and soluble sugar) and modification in phytohormones (abscisic acid (ABA) and jasmonic acid (JA)). Additionally, osmotic stress resulted in alterations in the compositions and co-occurrence patterns of root-associated bacterial communities, but not α-diversity (Chao1). Specifically, the rhizosphere Actinobacteria phylum was significantly increased by osmotic stress. These shifts in root-associated bacterial communities were significantly correlated with the host's adaptation to osmotic stress. Overall, the findings revealed that osmotic stress, in addition to its impacts on plant physiology, resulted in a restructuring of root-associated microbial communities and suggested that the concomitant adjustment in plant microbiota may potentially contribute to the survival of desert plants under extreme environmental stress.

## Contributions

Zhihao Zhang, Xutian Chai, and Fanjiang Zeng conceived and designed the research. Zhihao Zhang and Xutian Chai cultured the plant and collected samples. Zhihao Zhang performed the statistical analyses. Zhihao Zhang and Xutian Chai co-wrote the manuscript. Yan Lu, Yanju Gao, Bo zhang, Akash Tariq, Fanjiang Zeng, and Xiangyi Li reviewed the manuscript. All authors have read and approved the final version of the manuscript.

## 1. Introduction

Water is a fundamental ingredient for life and is essential for execution of basic cellular functions. Water scarcity is an enormous global concern with significant implication for plant physiology and vegetation management (Lu et al., 2021; Miller et al., 2010). In arid and semi-arid regions, the evaporation of soil water and extraction of water

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https://doi.org/10.1016/j.plaphy.2023.108124
Received 4 May 2023; Received in revised form 15 September 2023; Accepted 17 October 2023
Available online 26 October 2023

**Abbreviations**

| | |
|---|---|
| ROS | Reactive oxygen species |
| MDA | Malondialdehyde |
| R/S | Ratio of root to shoot biomass |
| POD | Peroxidase (EC 1.11.1.7) |
| PPO | Polyphenol oxidase (EC 1.14.18.1) |
| APX | Ascorbate peroxidase (EC 1.11.1.11) |
| GSH | Glutathione |
| ABA | Abscisic acid |
| JA | Jasmonic acid. |

by plant roots lead to the accumulation of soluble salts in the surface soil (Bai et al., 2019). This results in osmotic stress, which is responsible for the reduced growth and productivity of crops and wild species (Zia et al., 2021). In response to osmotic stress, plants being sessile have evolved various adaptive mechanisms to maintain cell turgor and physiological functions. These include the accumulation of osmotically compatible solutes, biosynthesis of plant hormones, activation of antioxidant defense systems, and other related processes (Forni et al., 2017; Lu et al., 2021; Zhang et al., 2020). However, existing studies have largely ignored the role of microbes inhabiting the rhizosphere and within root in the adaptive strategies of plants to osmotic stress (Xu and Coleman-Derr, 2019; Zia et al., 2021). Currently, the potential impacts of osmotic stress on root-associated microbial communities of desert plants and the relationship between shifts in these communities and host physiological plasticity remain largely unclear.

The utilization of microbial communities to enhance plant stress resistance represents an environmentally friendly and cost-effective approach, which circumvents the potential risks posed by transgenic technology. Consequently, studies on plant-microbial interactions have garnered significant attention worldwide (Liu et al., 2020; Mathur and Roy, 2021). The rhizosphere, a narrow soil area (~2 mm) directly affected by root exudates, is populated by associated microbes and root endophytes, collectively referred to as the root-associated microbiome (Chen et al., 2019). Within this microbiome, certain plant growth-promoting bacteria (PGRB) and fungi (PGRF) can confer countless benefits to plants, bolstering their ability to withstand biotic and abiotic stresses. For instance, certain PGRB possess the capacity to synthesize phytohormones, such as indoleacetic acid (IAA), gibberellin (GA), and cytokinin (CTK), which can stimulate the initiation of lateral and adventitious roots (Cohen et al., 2008; Egamberdieva et al., 2017; Spaepen and Vanderleyden, 2011). Furthermore, a variety of PGPB has been found to enhance the host plant's accumulation of osmotically compatible solutes, such as proline, while also augmenting the activities of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) that eliminate reactive oxygen species (ROS) arising from osmotic stress (Nautiyal et al., 2013).

The root-associated microbiome is influenced by various factors associated with the host, microbe, and environment, leading to variations in composition and diversity (Dastogeer et al., 2020). Although both rhizosphere and root microbes enhance plant growth through the same mechanisms, only rhizosphere bacteria face additional challenges by both root secretions and environmental stresses due to the compartmentalization caused by the root cortex (Forni et al., 2017), which can result in various responses of these two communities to osmotic stress. Gram-positive bacteria, such as Actinobacteria and Firmicutes, exhibit strong tolerance to water stress, which can be attributed to their thicker cell walls and sporulation ability (Xu and Coleman-Derr, 2019). Moreover, the interactions among co-occurring organisms in an environment may impact the entire community's response to perturbations (de Vries et al., 2018). Although the positive (cooperative) interactions can benefit both parties, co-oscillation and positive feedback may occur under environmental disturbance, which is not favorable for community stability (Coyte et al., 2015). Plant roots secrete various exudates into the rhizosphere, allowing them to select specific root-associated microbial communities that match their physiological characteristics (Chen et al., 2019; Dastogeer et al., 2020; Hu et al., 2018; Liu et al., 2020). These findings suggest that any changes to the root-associated microbial community could affect host physiological processes, while this remains largely unknown, especially for desert plants.

*Alhagi sparsifolia* Shap, a widely distributed xerophytic plant in Central Asia, is crucial for maintaining oasis stability, animal husbandry, and Uygur medicine (Wei et al., 2021; Zhang et al., 2021a). At the seedling stage, this species exhibits a remarkable ability to tolerate environmental stress (i.e., drought and salinization), which is characterized by increased fine root biomass, accumulation of osmotically compatible solutes in leaves, enhanced antioxidant enzyme activity, and coordinated element allocation (Ullah et al., 2022; Wu et al., 2015; Zhang et al., 2020, 2022). However, the contribution of the root-associated microbial community to these adaptations remains unclear. In this study, we profiled the bacterial communities in the rhizosphere and root of *A. sparsifolia* seedlings under osmotic stress and correlated these microbial characteristics with a series of physiological processes involving biomass allocation, root traits, antioxidant defense system, osmolytes, and phytohormones. Based on previous studies, we proposed the following two hypotheses: 1) osmotic stress can induce changes in the diversity, community composition, and co-occurrence patterns of root-associated bacteria; 2) there can exist a strong coordination between shifts in root-associated bacteria and host physiological plasticity. By testing both the hypotheses, this study aimed to gain insights into the mechanisms underlying the osmotic stress tolerance of desert plants from a microbe-plant interaction perspective and provide valuable references for the restoration and management of desert vegetation.

## 2. Material and methods

### 2.1. Experimental design

To ensure consistent nutrient availability, appropriate drainage, and a consistent microbial seed bank in the growth environment, we utilized sand sterilized at 121 °C for 20 min as a culture substrate to investigate the response of *A. sparsifolia* seedling growth and root-associated microbiome to osmotic stress which was induced by polyethylene glycol (PEG). Seeds were collected in the desert-oasis transition zone on the southern edge of the Taklimakan Desert, subsequently disinfected with a 10% sodium hypochlorite solution, and rinsed with sterile distilled water. Ten seeds were sown in a 7.5 cm × 12.5 cm pot filled with 575 g of sterilized sand, following which osmotic stress treatments were initiated. Four levels of osmotic stresses, including CK (0 MPa), T1 (−0.09 MPa), T2 (−0.44 MPa), and T3 (−1.79 MPa) were induced by adding different concentrations (w/v, 0%, 7%, 18%, and 39%) of PEG-6000 to 1/2 Hoagland's nutrient solution, following the method outlined by (Michel and Kaufmann, 1973) (Fig. 1). The mixed solution was injected into the top, middle, and bottom of the pot once a week to maintain stable osmotic stresses and plant growth nutrient requirements. The Vapro® Pressure Osmometer (WESCOR Inc., United States) was employed to measure the solution osmolality during the experimental period. Each treatment was repeated four times. We placed these pots in an artificial climate chamber (day/night cycle, 18/6 h, 25/15 °C, photosynthetic photon flux density of 800 μmol $m^{-2}$. $s^{-1}$, and relative humidity of 55 ± 5%). After 35 days of osmotic-stress treatment, plant and soil samples were harvested, which resulted in total of 160 seedlings (=4 treatments × 10 seedlings × 4 replicates). The seedlings were categorized into two groups, one for measuring plant physiological parameters, and the other for collecting samples of root-associated microbes.

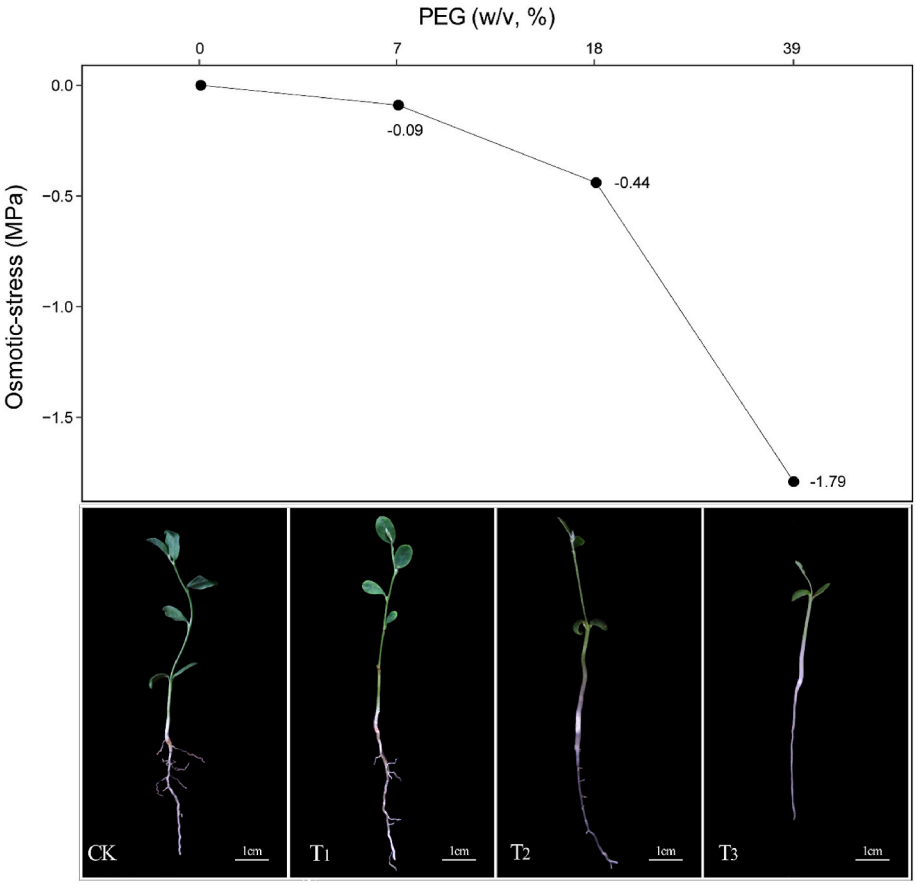


**Fig. 1.** PEG-induced osmotic stress gradients and corresponding plant growth statuses.

### 2.2. Measurements of host plant morphophysiological indexes

We adopted running water to rinse the sand away and obtain intact plants. A subset of these samples was used to determine plant height, root tip number (no.), and ratio of root to shoot biomass (R/S), while the remaining samples were immediately stored in liquid nitrogen for the determination of root physiological and biochemical indexes. The plant height was defined as the length of the aboveground portion. After recording the root tip number, the aboveground (shoot) and underground parts (root) of the plant were separated and placed in an oven at 70 °C for 24 h, respectively, to measure their respective biomasses and the ratios (R/S). The levels of proline, soluble sugar, soluble protein, ROS, and malondialdehyde (MDA), glutathione (GSH) content, root vitality, and activities of SOD, peroxidase (POD; EC 1.11.1.7), polyphenol oxidase (PPO; EC 1.14.18.1), and ascorbate peroxidase (APX; EC 1.11.1.11) in whole roots were determined using enzyme-linked immunosorbent assay (ELISA) kits purchased from Shanghai Xinyu Biotechnology Co., Ltd. The ELISA kits used were XY992020a (proline), XY992030a (soluble sugar), XY992031a (soluble protein), XY992061a (ROS), XY992021a (MDA), XY992080a (GSH), XY9896 (root vitality), XY992051a (POD), XY992060a (PPO), XY992040a (APX), XY992070a (abscisic acid, ABA), and XY992091a (jasmonic acid, JA).

### 2.3. Sampling root-associated microbiome

Rhizosphere soil was collected within 2 mm of the root. The roots were rinsed with sterile water to collect the root microbiome based on the method described by (Chen et al., 2019), which did not distinguish bacterial communities between root endosphere and rhizoplane. All samples were stored at −20 °C for subsequent DNA extractions.

### 2.4. DNA extraction and 16S rRNA amplicon sequencing

Approximately 500 mg of samples were subjected to DNA extraction using the OMEGA Soil DNA Kit (M5635-02) (Omega Bio-Tek, Norcross, GA, USA), following the manufacturer's instructions. Subsequently, the quality and quantity of extracted DNA were assessed through 1% agarose gel and a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), respectively. Nearly full-length bacterial 16S rRNA genes were amplified using the primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR amplicons were purified, quantified, and homogenized to create a sequencing library (SMRT Bell). Qualified libraries were sequenced on a PacBio Sequel platform (Singer et al., 2016). PCR amplicons was purified with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, the quantified amplicons were pooled in equal amounts, and subjected to SMRT sequencing technology using the PacBio Sequel platform at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China). All raw sequences for 16S rRNA genes were deposited in the NCBI Sequence Read Archive (SRA) with the accession number PRJNA827267.

### 2.5. Sequence analysis

Microbiome bioinformatics analysis was performed with QIIME2 (release 2019.4) with minor modifications according to the official tutorials.[2] Briefly, the raw sequence data were demultiplexed using the

[2] https://docs.qiime2.org/2019.4/tutorials/.

demux plugin, followed by primers trimming with the cutadapt plugin (Martin, 2011). The sequences were then quality-filtered and dereplicated using functions of fastq_mergepairs, fastq_filter, and derep_fulength in Vsearch plugin. Subsequently, all the unique sequences were clustered at 98% similarity (via cluster_size) followed by chimera removal (via uchime_denovo). Finally, the non_chimera sequences were re_clustered to generate amplicon sequence variant (ASV) representative sequences and an ASV Table. A total of 91 taxa associated with "mitochondria" and "Chloroplast" were excluded from the ASV table. Bacterial α-diversity (Chao1) and β-diversity (differences in community composition between samples) were calculated at the rarefied depth of 1256 sequences per sample (minimum number of all samples) using the R package *microeco* (Liu et al., 2021). Taxonomy was assigned to ASVs using the classify-sklearn naive Bayes taxonomy classifier in the feature-classifier plugin against the SILVA Release 132 (Quast et al., 2012).

### 2.6. Statistical analysis

Data analysis and figure output were performed using R software (version 4.2.0).[3] The analysis of variance (ANOVA) was conducted to investigate whether there is a statistical difference in plant physiological parameters, as well as the relative abundance, α-diversity, and β-diversity of root-associated bacteria among various osmotic stresses treatments. Subsequently, multiple comparisons between samples were performed using the Least Significant Difference (LSD) test. The *t*-test was employed for the evaluation of the statistical difference in the relative abundance and α-diversity between two compartments (rhizosphere and root). Pearson's correlations between host plant physiological parameters and the abundance of dominant species (phylum and class levels). α-diversity were calculated and generated to heatmaps or linear regression. The permutational analysis of variance (PERMANOVA) with 999 permutation tests was adopted to quantify the independent or interactive effect of osmotic stresses and compartments on the Bray-Curtis distances of ASV, which were also visualized via Principal Coordinate Analysis (PCoA). The Mantel test with 999 permutations using the package *vegan* was conducted to explore the Spearman's correlation relationship between bacterial communities and host physiology. The reduced model with 999 permutations implemented in redundancy analysis (RDA) was used to identify the most robust indexes of plant physiology correlated with the variation of root-associated bacterial communities, which was quantified by the function *varpart* in the package *vegan*.

To construct highly reliable and precise co-occurrence networks, three criteria for the ASVs were applied. First, the cumulative relative abundance of all samples was greater than 0.5%. Second, the absolute value of paired Spearman's correlation coefficient values was greater than 0.7. Third, false discovery rates (FDR)-corrected *P* was less than 0.01. These co-occurrence networks were visualized by the Gephi platform.[4] The topology properties of networks, including the number of nodes and edges, edge density, average degree, and modularity, were calculated by the package *igraph*. The modularity of networks was calculated by a greedy algorithm (Deng et al., 2012). Pearson's correlation between these network properties and host physiological indexes was visualized with heatmaps.

## 3. Results

### 3.1. Host plant morphophysiological traits

The morphophysiology of *A. sparsifolia* was significantly impacted by PEG-induced osmotic stress (Fig. 1; Table 1). Specifically, the plant height was greatly decreased and ROS levels in roots were concomitantly increased in response to osmotic stress ($P < 0.05$) (Table 1). The number of root tips and soluble sugar level in roots initially increased and then decreased with osmotic-stress gradients. T2 marked an increase in the R/S. Compared with CK, ABA in T3 was significantly increased by 11.16%, while it was reduced in both T1 and T2. Osmotic stress improved root vitality and POD activity (except for T2), as well as levels of JA, soluble protein, and proline. T3 significantly decreased PPO activity by 10.24% but increased GSH activity by 13.95% and APX activity by 3.67%.

**Table 1**
Growth indexes of host plant among osmotic-stress gradients. mean ± standard deviation (SD). Different lowercase letters in the same row indicate that there are significant differences among different osmotic stress treatments (ANOVA, $P < 0.05$). ROS, reactive oxygen species. MDA, malondialdehyde. R/S, ratio of root to shoot biomass. POD, peroxidase. PPO, polyphenol oxidase. APX, ascorbate peroxidase. GSH, glutathione. ABA, abscisic acid. JA, jasmonic acid.

| | CK | T1 | T2 | T3 |
|---|---|---|---|---|
| Height (cm) | 6.00 ± 0.24 a | 5.02 ± 0.08 b | 3.60 ± 0.18 c | 2.47 ± 0.11 d |
| Root tip number | 7.00 ± 0.82 b | 8.75 ± 0.96 a | 3.75 ± 0.96 c | 1.00 ± 0.00 d |
| Root vitality ($\mu g.mL^{-1}.g^{-1}.h^{-1}$) | 14.07 ± 1.66 b | 16.81 ± 1.25 a | 17.34 ± 0.09 a | 15.77 ± 0.40 ab |
| R/S | 0.48 ± 0.07 b | 1.30 ± 0.10 ab | 2.27 ± 1.28 a | 0.71 ± 0.34 b |
| MDA ($nmol.L^{-1}$) | 5.90 ± 0.06 a | 5.91 ± 0.07 a | 5.76 ± 0.07 b | 5.64 ± 0.04 c |
| ROS ($pg.mL^{-1}$) | 679.20 ± 9.16 c | 832.89 ± 38.97 a | 778.49 ± 6.70 b | 766.71 ± 11.44 b |
| ABA ($\mu g.L^{-1}$) | 292.79 ± 8.0 b | 280.32 ± 4.12 c | 281.18 ± 3.88 c | 325.47 ± 6.66 a |
| JA ($pmol\ L^{-1}$) | 871.84 ± 13.55 d | 892.96 ± 9.88 c | 940.95 ± 16.59 b | 1000.47 ± 10.11 a |
| POD ($mU.L^{-1}$) | 27.83 ± 0.11 c | 31.45 ± 0.16 a | 26.76 ± 0.71 d | 30.25 ± 0.27 b |
| PPO ($IU.L^{-1}$) | 127.10 ± 1.32 a | 126.01 ± 1.06 a | 128.99 ± 0.65 a | 114.08 ± 4.89 b |
| APX ($IU.L^{-1}$) | 213.41 ± 5.50 b | 207.85 ± 5.34 b | 213.86 ± 1.58 b | 221.23 ± 1.87 a |
| GSH ($U.L^{-1}$) | 51.97 ± 1.00 b | 52.07 ± 0.35 b | 51.24 ± 0.00 b | 59.22 ± 1.01 a |
| Proline ($ng.L^{-1}$) | 1985.28 ± 36.27 c | 2012.69 ± 18.56 c | 2574.70 ± 34.04 a | 2132.63 ± 13.13 b |
| Soluble protein ($\mu g.L^{-1}$) | 49.09 ± 0.71 c | 51.72 ± 0.91 b | 49.71 ± 0.41 c | 54.23 ± 0.33 a |
| Soluble sugar ($ng.L^{-1}$) | 17.18 ± 0.28 c | 17.71 ± 0.26 b | 18.73 ± 0.12 a | 16.52 ± 0.27 d |

### 3.2. Root-associated bacterial communities

After quality control, a total number of 305,313 sequences were obtained for analysis. To diminish the adverse impact of inconsistencies in the sequence number between samples on bacterial community structure, we randomly sampled 1256 sequences (the lowest sequences in all samples) for subsequent analysis, which yielded a total of 1872 ASVs. Of those, 401 ASVs (84.6% of sequences) were presented in both the rhizosphere and root compartments, while 1180 ASVs (12.0%) were unique to the rhizosphere community and 291 ASVs (3.4%) were unique to the root community (Fig. S1).

The overall taxonomic profile analyses revealed a total of 19 phyla present across the two compartments. Proteobacteria with an average relative abundance of 76% dominated all samples, followed by Bacteroidetes (15%) and Actinobacteria (2%) (Fig. 2a). A statistically significant enrichment of Proteobacteria was observed in the root, while Actinobacteria, Patescibacteria, Chloroflexi, Firmicutes, and Cyanobacteria were more abundant in the rhizosphere (*t*-test, $P < 0.05$). Among these phyla, only Actinobacteria exhibited a significant response to T3 (ANOVA, $P < 0.05$) (Fig. S2). Rhizosphere samples were enriched

[3] https://www.r-project.org/.
[4] https://gephi.org/.

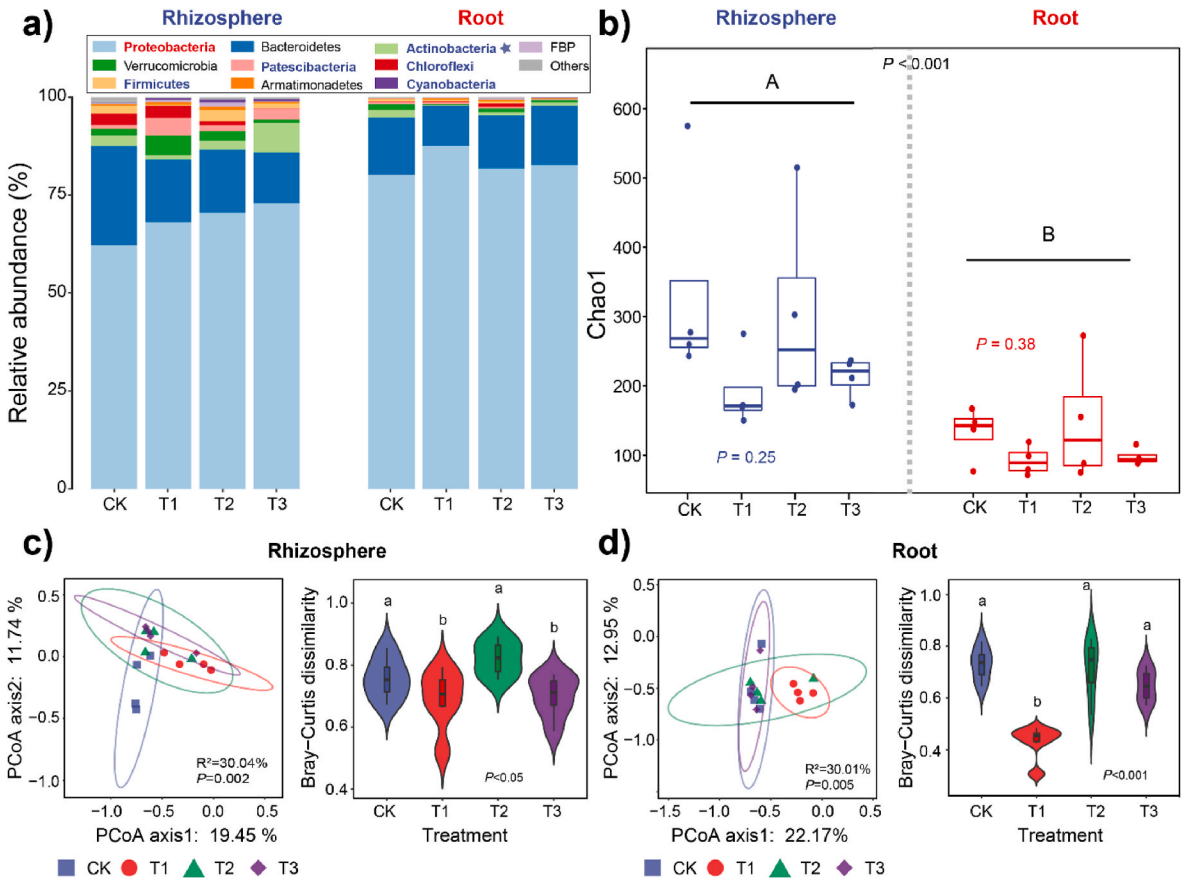


**Fig. 2.** (**a**) The abundance of dominant phyla (top 10) in the rhizosphere and root samples. "☆" colored in blue indicates a significant difference in rhizosphere samples among osmotic-stress treatments (ANOVA, $P<0.05$). The font of class names colored in blue and red indicate a significant enrichment in the rhizosphere and root samples (*t*-test, $P<0.05$), respectively. (**b**) The α-diversity was evaluated by Chao1 of root-associated samples. In each panel, the *P*-value indicates the difference between osmotic-stress treatments (ANOVA). Different capital letters represent a significantly different Chao1 between two compartments (*t*-test). Principal coordinate analysis (PCoA) and dissimilarity distance of bacterial communities in the rhizosphere (**c**) and root (**d**) samples. PCoA and dissimilarity distance was calculated using the Bray-Curtis distance at the ASV level. The ellipse is based on a 95% confidence interval (CI). The effects ($R^2$) of osmotic stress on bacterial communities were quantified by PERMANOVA with 999 permutations. Different lowercase letters in violin diagrams indicate significantly different bacterial communities among different osmotic stresses (ANOVA).

in certain classes, including Actinobacteria, Deltaproteobacteria, Bacilli, Saccharimonadia, and Parcubacteria, while Gammaproteobacteria was more abundant in root samples (*t*-test, $P < 0.05$) (Fig. S3). Osmotic stress exerted a significant influence on the relative abundance of Gammaproteobacteria and Parcubacteria in the root, as well as Actinobacteria and Gammaproteobacteria in the rhizosphere.

The richness of observed ASVs, indicated by the Chao1, was much lower in the root than in the rhizosphere (*t*-test, $P < 0.001$). However, there was no significant difference between osmotic stress treatments for both compartments (ANOVA, $P > 0.05$) (Fig. 2b). We evaluated the β-diversity of root-associated bacteria communities at ASV Bray-Curtis distance. PCoA revealed that axis 1 (16.73%) and axis 2 (9.13%) separated compartment (rhizosphere vs root) communities and osmotic stress treatments (T1 vs others), respectively (Fig. S4). PERMANOVA further confirmed that compartment ($R^2 = 7.75\%$, $P < 0.001$) and osmotic stress ($R^2 = 19.35\%$, $P < 0.001$) significantly affected these communities, while there was no interaction between the two factors ($R^2 = 8.35\%$, $P > 0.05$). Osmotic stress contributed 30.04% and 30.01% of the variation in the rhizosphere and root communities, respectively (Fig. 3c and d). Furthermore, divergent dissimilarity was observed between the controlled and the osmotic-stressed root-associated bacterial communities (ANOVA, $P < 0.05$).

### 3.3. Relationships between bacterial community and host plant physiology

The correlation between the relative abundance of dominant phyla and classes as well as host physiological indexes was found to be significant (Fig. S5). Among these taxa, those belonging to Actinobacteria were found to be most closely related to host traits. The POD activity was the only trait that exhibited a correlation with the Chao1 of the rhizosphere ($R = -0.50$, $P = 0.048$) and root ($R = -0.57$, $P = 0.021$) communities (Fig. 3a). Moreover, based on the ASV Bray-Curtis distance, the Mantel test indicated that there were significant correlations between the rhizosphere community and the plant height, POD activity, and ROS level (Fig. 3b). In contrast, the root community had a significant relationship with the plant height and root vitality ($P > 0.05$). Specifically, the RDA suggested that plant height and root ROS level were the most powerful factors in correlating with the rhizosphere community, explaining 8.2% of the variation in community structure (Fig. 3c). ROS level indicated a negative correlation with the rhizosphere community in CK, while it was positively correlated with the osmotic-stressed communities. The root community, on the other hand, was significantly correlated with the plant height, root tip number, as well as GSH and ROS levels, jointly contributing 18.4% of the variation in community structure (Fig. 3d).

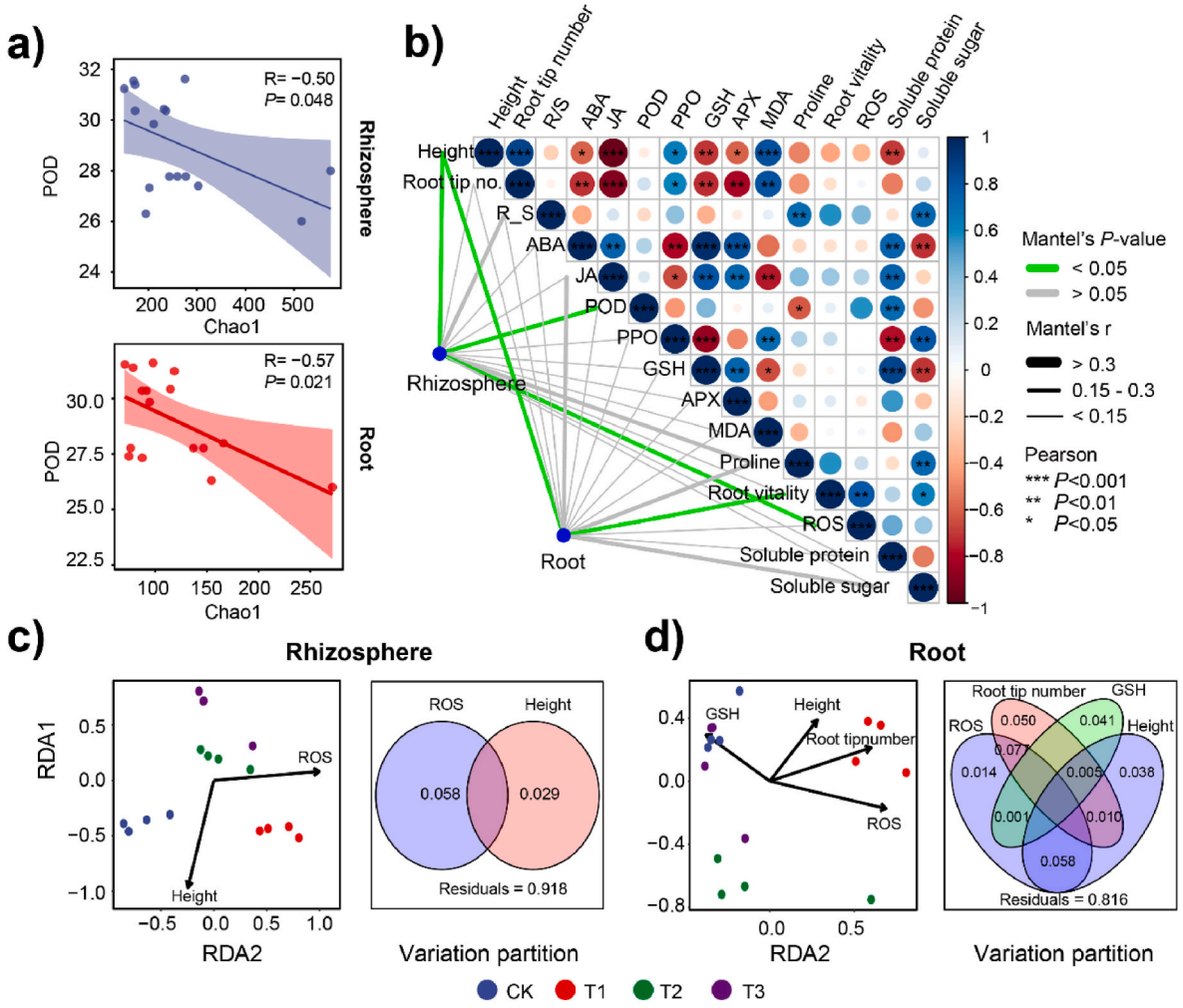


**Fig. 3.** (**a**) Relationships between host plant trait and the Chao1 of rhizosphere and root samples. Data are fitted using linear regression with 95% CI. (**b**) Mantel correlation between the growth indexes of host plant and its root-associated bacterial community structure (ASV Bray-Curtis distances) determined by Mantel test with 999 permutations. ROS, reactive oxygen species. MDA, malondialdehyde. R/S, ratio of root to shoot biomass. POD, peroxidase. PPO, polyphenol oxidase. APX, ascorbate peroxidase. GSH, glutathione. ABA, abscisic acid. JA, jasmonic acid.

### *3.4. Co-occurrence patterns*

The individual networks were constructed to explore the impact of osmotic stress on co-occurrence patterns of root-associated bacteria (Fig. 4; Table S1). For controlled networks, the size (number of nodes), connectivity (number of edges), complexity (mean degree), and modularity of the rhizosphere network were higher than those of the root one (Table S1). In contrast, the edge density of root networks was higher than that of rhizosphere networks. Under osmotic stress, the networks in both compartments had fewer nodes and edges, as well as a lower average degree; additionally, the proportion of negative interaction increased in both compartment networks, except for the root network under T2. Furthermore, except for the rhizosphere network under T1, osmotic-stressed networks presented a lower edge density. Along with the osmotic stress gradient, the modularity of the root network increased first and then decreased. However, in rhizosphere networks, T1 reduced modularity, and modularity increased with the increase in osmotic stress. We also investigated the correlation between the properties of these networks and host traits. Our analysis revealed that the edge density of the rhizosphere network was significantly negatively correlated with JA level, but significantly positively correlated with MDA level ($P < 0.05$) (Fig. 4b). In addition, the number of nodes in rhizosphere networks was significantly negatively correlated with root vitality ($P < 0.05$). The soluble sugar level suggested a significant correlation with the property of edge (positive or negative interaction) of root networks ($P < 0.05$). For root networks, significant negative correlations were observed in interactions between mean degree vs. root vitality, edge numbers vs. root vitality, modularity vs. APX activity, and mean degree vs. R/S ($P < 0.05$).

## 4. Discussion

The mechanism underlying the plant adaptation to osmotic stress has been extensively documented. However, there has been limited research on how these changes relate to root-associated microbial communities. In this study, through measuring the full length of the bacterial 16S rRNA gene, we characterized the community structure of rhizosphere and root bacteria under various osmotic stress conditions. The findings revealed that osmotic stress induced shifts in the composition and co-occurrence pattern of these root-associated bacteria, while exhibiting no significant impact on species diversity. These changes were significantly correlated with the biomass allocation, root trait adjustment, antioxidant defense system regulation, osmolytes accumulation, and phytohormone level of the host plant. Our results suggested that variations in the structure of the root-associated bacterial community under osmotic stress may potentially affect the stress-signaling networks within the host.

### 4.1. Physiological adjustments of the plant to osmotic stress

All plants respond to osmotic stress in somewhat similar ways, however, the degree of resistance is dictated by the efficacy of the response strategy (Mathur and Roy, 2021). Our study revealed a series of adaptive characteristics of *A. sparsifolia* seedlings in response to osmotic stress. At the early stage of vegetative growth, reduced water supply can inhibit cell expansion, resulting in internode shortening and plant height reduction (Zia et al., 2021). One of the apparent and earliest responses of plants to environmental stress was the production of ROS, which was activated and significantly adapted to the stress (Apel and Hirt, 2004). As a result, *A. sparsifolia* seedlings exhibited poor growth assessed by reduced height and high levels of ROS (Table 1). Stomatal closure mediated by ABA and modification of root architecture are crucial plant responses that enhance water and nutrient uptake to alleviate osmotic stress (Egamberdieva et al., 2017; Mathur and Roy, 2021). It is true in our findings that ABA content in roots and root tip number were significantly upregulated under osmotic stress (Table 1). The large accumulation of ROS in roots caused by stress can contribute to lipid peroxidation of membranes, and the damage degree can be evaluated by MDA level (Selote and Khanna-Chopra, 2010). Although ROS levels in roots of *A. sparsifolia* seedlings were significantly increased by osmotic

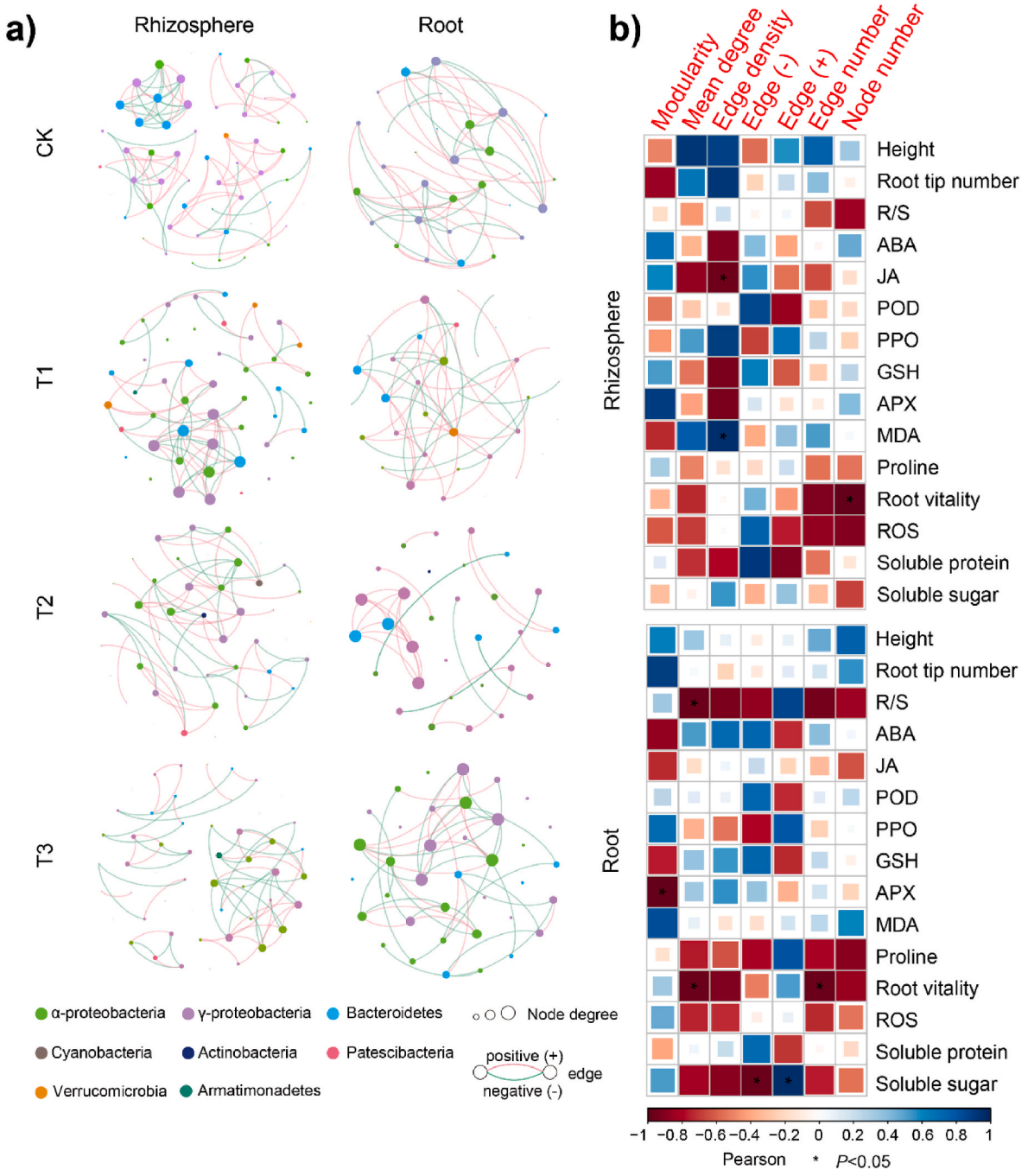


**Fig. 4.** (**a**) The sub-networks of different osmotic stress gradients and compartments. Nodes were colored according to the phylum or class they belong to. (**b**) Pearson's correlation between the host plant growth indexes and the topological properties. All *P*-values were FDR-corrected. ROS, reactive oxygen species. MDA, malondialdehyde. R/S, ratio of root to shoot biomass. POD, peroxidase. PPO, polyphenol oxidase. APX, ascorbate peroxidase. GSH, glutathione. ABA, abscisic acid. JA, jasmonic acid.

stress, MDA level was significantly down-regulated, which may be due to the intervention of antioxidant defense mechanisms that can counteract the adverse impacts of ROS (Baxter et al., 2014). Plants maintain a certain level of physiological activities to tolerate osmotic stress by regulating thousands of genes and various metabolic pathways (Zia et al., 2021). To achieve the best response and alleviate osmotic stress, *A. sparsifolia* seedlings adopted the strategy of coordinating a series of physiological traits, such as the upregulation of proline, soluble sugar, soluble protein, JA, POD, APX, and GSH in roots (Table 1). These adjustments ensure proper osmotic protection and regulation, as well as an active antioxidant defense system (Bao et al., 2009; Wu et al., 2015; Zhang et al., 2020; 2021b; Zhu et al., 2009).

### 4.2. Restructuring of root-associated bacterial communities under osmotic stress

The root-associated bacterial communities were influenced by both host selection and osmotic stress. The richness (Chao1) and composition (β-diversity) of the rhizosphere community demonstrate significant difference from those of the root communities (Fig. 2; Fig. S4), indicating a host selection, which was similar to that observed in rice (Edwards et al., 2018) and *Arabis alpina* (Dombrowski et al., 2017). Nevertheless, the comparable Chao1 and the distinct dissimilarity of bacterial communities across osmotic-stress gradients in our study indicated that osmotic stress restructured root-associated communities by changing species abundance rather than their richness, which supported our first hypothesis. These results may imply that plant's demand for the richness of root-associated bacteria remained relatively stable, without new taxa being introduced or lost. There might be another speculation here. The abundance-based data with low resolution to distinguish the survival strategies of bacteria may also explain this finding because certain taxa (e.g., gram-positive bacterium) existed but remained dormant under environmental stresses (Xu and Coleman-Derr, 2019). In similar species pools, differences in the tolerance of individual microbes to osmotic stress may ultimately result in various compositions of the entire community through fluctuations in relative abundance.

Co-occurrence networks offer a multidimensional view of microbial community responses to osmotic stress, beyond the conventional measures of richness and composition (Shi et al., 2016). Our results demonstrated that the rhizosphere assemblages formed larger (number of nodes), more connected (number of edges), and more complex (mean degree) networks than root communities. However, these characteristics of both communities were weakened by osmotic stress (Fig. 4b; Table S1). The organization of microbial communities has been shown to be impacted by the availability of resources in network studies (Coyte et al., 2015). Hence, we interpreted the decrease in the size, connectivity, and complexity of root-associated bacterial networks as a reduction in community organization, i.e., the decoupling of bacterial interactions and the degeneration of shared guilds or niche (positive edges), which represented a fundamental difference between controlled and osmotic-stressed conditions (Shi et al., 2016).

### 4.3. Potential role of root-associated bacteria in host physiological adaptations to osmotic stress

In addition to the self-regulation of *A. sparsifolia* seedling, the root-associated bacteria may play a crucial role in facilitating the host's adaptation to osmotic stress (Figs. 3 and 4, S5). Gram-positive bacteria (e.g., Actinobacteria) are known to possess enhanced tolerance to osmotic stress because of their thick cell walls and spore-forming strategy to avoid drought (Chapin III et al., 2000). In our study, Actinobacteria, especially in the rhizosphere (Fig. S2), exhibited a significant correlation with hosts' performance under osmotic stress, evidenced by the production of ABA, JA, and soluble protein (Fig. S5), implying a key role in contributing to plant growth when facing osmotic stress. Microbes that co-evolve in an environment have better adaptability and resilience to environmental stress (McCarty and Ledesma-Amaro, 2019). They can collaborate well as well as create a stable and persistent microbial community, which is more conducive to achieving the desired phenotypic outcomes (Armanhi et al., 2018). At the community level, changes in root-associated bacteria communities shaped by osmotic stress may align with the profile of potential antioxidant processes in the host. Previous studies have concluded that plant cells stressed by drought can generate toxic oxygen-free radicals (i.e., ROS), which induce POD activity (Miller et al., 2010; Zhang et al., 2020). In the current study, POD activity and ROS level in roots enhanced by osmotic stress demonstrated significant correlation with Chao1 and the composition of root and/or rhizosphere communities (Fig. 3). This suggested that the species richness and community structure of root-associated bacteria may induce the host's antioxidant defense system. Additionally, the community composition of root bacteria may play a crucial role in determining root vitality and tip number under osmotic stress. Root oxidability is an indicator of root vitality (Zhou et al., 1995). Therefore, these results suggested that the composition of the root-associated community may directly or indirectly affect the antioxidant defense system of plants.

A higher proportion of interaction among bacteria in the rhizosphere community may induce JA production and inhibit membrane lipid peroxidation, while the augmented proportion of negative (cooperative) interaction among bacteria in the root community potentially promoted the accumulation of osmolytes (Fig. 4b). These findings suggested that the interactions among root-associated bacteria may impact the osmotic stress tolerance of the host by adjusting phytohormone and membrane stability (Hassan and Mathesius, 2012). Root vitality and R/S evaluate the ability of roots to absorb mineral nutrients and water, as well as biomass allocation patterns. These parameters exhibited negative correlation with the number of nodes and edges, as well as the mean degree of root-associated networks (Fig. 4b). The growth of bacteria and their interactions depend on carbohydrate metabolism (Karolewski et al., 2010). As osmotic stress continued, the decrease in photosynthetic products may exacerbate competition for nutrients between the root function and root-associated bacteria. This could explain why there was negative feedback between root vitality and R/S and the observed co-occurrence patterns. Modules in microbial co-occurrence networks referred to clusters of phylogenetically closely related species, and modularity reflected habitat heterogeneity and different selection mechanisms (Shi et al., 2016). A negative correlation was found between the modularity of the root bacterial network and APX. This indirectly suggested that the lower clustering degree of the root bacterial network caused by osmotic stress may induce the activation of APX activity.

In light of our findings, it can be inferred that shifts in the relative abundance, composition, and co-occurrence patterns of root-associated bacteria may contribute to the host's physiology, supporting our second hypothesis.

### 4.4. Some notes of caution

There are several key limitations in linking the physiological functions of the host plant with certain valuable properties derived from deep amplicon sequencing data, such as the root-associated bacterial diversity, composition, and co-occurrence patterns. Although the present study strongly suggests a crucial role for root-associated bacterial communities in mitigating interaction between the desert plant and osmotic stress, direct evidence to support this claim is lacking. Firstly, root exudates are a valuable source of information for deciphering the interaction between plants and microbes (Cohen et al., 2008; Forni et al., 2017). Profiling root exudates can provide a direct link between microbial behavior and host metabolic networks. Secondly, this study encountered challenges in distinguishing the beneficial effects of root-associated bacteria from the host plant's self-regulation. This can be because of the overlap between the metabolites of microbes and plants. For example, various bacteria like plants have been also

presented to increase the production of plant metabolites, such as betaine, proline, and trehalose, as well as the synthesis of phytohormones (e.g., ABA) and enzymes (e.g., SOD and CAT) that detoxify ROS (Cohen et al., 2008; Forni et al., 2017). Future experiments with synthetic communities or single inoculations are necessary for the comprehension of the mechanisms underlying the patterns revealed in this study. Thirdly, speculations based on 16S rRNA genes cannot provide accurate functional information about the microbial community. Genomic analysis of individual groups or synthetic communities will shed light on the functional importance of root-associated microbiota (Bai et al., 2015). Despite these limitations, the associations found between the changes in community structure and co-occurrence patterns of root-associated bacteria and the host's osmotic stress tolerance in this study suggested that desert plant self-regulation interacts with its associated microbial community, which can be a potentially key mechanism for desert plant responses to environmental stress.

## 5. Conclusion

This study contributes to our comprehension of the variations in root-associated bacterial communities of desert plant under osmotic stress and the relationships between these shifts and the osmotic stress tolerance of the host. *A. sparsifolia* seedlings can adapt to the osmotic stress induced by PEG. This adaptation involves numerous modifications, including altered biomass allocation, shifts in the antioxidation defense system, fluctuations in plant hormones, accumulation of osmotic substances, and a restructuring of the root-associated microbiome. As these adaptations are interconnected, we conclude that the improved plant tolerance to osmotic stress by root-associated microbiome represents an osmotic stress-dependent trait. Further investigation is required to explore the underlying causes and consequences of osmotic stress-induced shifts in the desert plant-origin bacterial community, as well as to screen key beneficial strains that can support plant fitness when confronted with osmotic stress caused by drought or salinization.

## 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.

## Data availability

The authors do not have permission to share data.

## Acknowledgments

This work was supported by the Natural Science Foundation of Xinjiang Uygur Autonomous Region (2021D01D02), and the National Natural Science Foundation of China (41977050, 42271071).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2023.108124.

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