

Adaptive responses of different halophytes to soil water stress regulate the composition, diversity, and functional differentiation of their phyllosphere microbial communities

Xinyu Ge

2857384525@qq.com

Chinese Academy of Agricultural Sciences Grassland Research Institute

Wenbo Zhang

Xiangjian Tu

Paul C. Struik

Ke Jin

Rula Sa

Zhen Wang

Research Article

Keywords: Phyllosphere Microbiome, Saline Environments, Halophytics, Plants soil water stress, Community assembly

Posted Date: May 13th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-6601546/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Plant and Soil on October 20th, 2025. See the published version at <https://doi.org/10.1007/s11104-025-07865-x>.

Abstract

Background

In saline ecosystems, halophytes reshape their phyllosphere microenvironment through unique salt-tolerance strategies, driving microbial community differentiation and functional adaptation. But under extreme conditions, a comprehensive understanding of how these microbes respond to environmental cues and subsequently influence their hosts remains elusive.

Methods

we collected and analyzed leaf physiological-biochemical traits and high-throughput amplicon sequencing data of phyllosphere microbiota from three representative halophytes—*Suaeda salsa* (SS), *Nitraria sibirica* (NS), and *Salicornia europaea* (SE)—along gradients of soil salinity and water content.

Results

soil water stress, induced by the combined effects of soil salinity and moisture, is a pivotal factor driving differences in plant physiological-biochemical traits. Under the influence of these trait variations, deterministic processes jointly governed the assembly of phyllosphere bacterial and fungal communities, yet their composition, diversity, and metabolic functions exhibited marked differences. Specifically, the key bacterial genus *Planococcus*, fungal taxa within *Ascomycota*, and metabolic functions associated with antioxidant stress responses were significantly enhanced in SS; the bacterial genus *Vibrio* and metabolic functions linked to microbial competition-defense mechanisms and oligotrophic traits were enhanced in SE. Varying degrees of increase in key fungal and bacterial taxa across the phyllosphere of all three species further influenced community diversity, but stochastic processes also contributed to fungal community assembly.

Conclusions

Findings reveal that soil water stress indirectly impacts phyllosphere microbial communities, with differences in the stress-tolerant physiological-biochemical traits of halophytes under varying water stress conditions significantly shaping microbial community composition. Moreover, the stress-resistance traits exhibited by phyllosphere microbiota may enhance plant adaptation to extreme environments.

Introduction

Amid the global trend of intensive land resource utilization, soil salinization and drought have emerged as primary abiotic stressors limiting crop productivity and quality (Zhang et al. 2012; Behera et al. 2022)

In China, saline soils span approximately 99 million hectares, with nearly 70% located in arid and semi-arid regions. Plants in these areas suffer from ion toxicity, osmotic stress, and oxidative damage, severely restricting the growth and development of economically important crops (Kefu et al. 2002; Yang and Guo 2018). Extensive evidence suggests that plant physiological responses to salinity and drought share certain similarities, as both induce osmotic stress: drought exacerbates the water potential gradient between roots and soil ($\Delta\Psi < 0$), while salinity reduces soil water availability, thereby constraining water uptake and triggering oxidative stress (Al-Yasi et al. 2020; Roşca et al. 2023). These stressors further disrupt plant metabolic processes, resulting in growth suppression, reduced photosynthesis, and impaired uptake of essential nutrients from the soil. Halophytes are defined as plants capable of completing their life cycles in naturally saline soils with NaCl concentrations exceeding 200 mM (Yuan et al. 2019). They are renowned for their efficient salt tolerance mechanisms and remarkable adaptability to saline-alkaline environments. According to the niche theory, a species' suitability is primarily determined by environmental conditions that define its fundamental niche, which encompasses the suite of biotic and abiotic factors enabling its persistence (Richards and Lavorel 2023). Consequently, the types of halophytes and their physiological-biochemical traits vary across gradients of salinity and water availability, equipping them to acquire resources, evade adversaries, and thrive under diverse adverse conditions.

The phyllosphere is one of the most ubiquitous microbial habitats on Earth. Its microbiota harbor a rich reservoir of functional genes (Vorholt 2012; Bashir et al. 2022) and maintain a close synergistic relationship with rhizosphere microbes, collectively influencing plant health and ecosystem functionality. Nutrient accumulation and microbial colonization in the phyllosphere are not static processes; rather, they exhibit dynamic and discontinuous variations driven by environmental factors, rendering the determinants of microbial community composition highly complex. Consequently, integrating multi-omics data to elucidate the assembly mechanisms and driving factors of phyllosphere microbial communities has emerged as a research frontier. Phyllosphere microbes are subject to fluctuations in physical environmental conditions, such as temperature, relative humidity, wind speed, and radiation (Hirano and Upper 2000), with atmospheric conditions and host plant type widely recognized as the primary drivers of phyllosphere microbial community composition. Studies have shown that under extreme environmental conditions, plant leaves tend to stimulate beneficial and stress-tolerant bacterial taxa, which may enhance plant survival and adaptation (Li et al. 2022b). Concurrently, plants exert a significant selective influence on phyllosphere microbial community composition. For instance, in temperate grasslands, the composition of bacterial and fungal communities in the phyllosphere varies with plant species (Guo et al. 2024), and differences in plant traits further determine microbial community structure and the abundance of genes involved in nutrient cycling (Li et al. 2023). Moreover, phyllosphere microbial communities directly impact plants by secreting organic compounds, such as hormones or nitrogen-fixing enzymes, to regulate nutrient cycling (Abadi et al. 2020), or by producing surfactant-like compounds to enhance leaf wettability (Bunster et al. 1989), thereby supporting plant health and growth. A tight interplay exists between plant traits and phyllosphere microbiota: plant traits, shaped by responses to environmental stress, regulate the types of organic compounds secreted by

leaves; these traits, in turn, are constrained by the plant's carbon (C) and nitrogen (N) nutrient demands, with microbes involved in C and N cycling significantly influencing nutrient fluxes and, consequently, plant trait expression (Kembel et al. 2014; Legay et al. 2014).

However, beyond the filtering effects of the environment, biotic interactions and stochastic events such as birth, death, and dispersal of organisms also influence the assembly of phyllosphere microbial communities. In community ecology, community assembly is widely recognized as being co-regulated by deterministic processes based on niche theory and stochastic processes based on neutral theory (Li et al. 2019). Niche theory posits that each species occupies a distinct ecological niche, with deterministic processes—such as environmental filtering and biotic interactions (non-random ecological processes)—largely shaping the compositional patterns of community structure. In contrast, neutral theory assumes that all individuals are ecologically equivalent, and community composition is primarily governed by stochastic processes (e.g., birth, death, and dispersal) rather than differences in competitive ability (Rosindell et al., 2011; MacArthur and Wilson, 1967). In reality, these two theories are not contradictory but complementary, as deterministic and stochastic processes often operate simultaneously during community assembly. The integration of deterministic and stochastic frameworks provides a robust approach to elucidating the principles and mechanisms underlying community assembly, offering powerful tools for understanding the construction of phyllosphere microbial communities.

Previous studies have predominantly focused on the influence of plants on their phyllosphere microbial communities, while largely overlooking the effects of plant traits—shaped by distinct ecological niches within the same ecosystem—on these microbial communities. In this study, we analyzed the physiological and biochemical traits of three halophytic species along gradients of soil salinity and moisture in a saline ecosystem, coupled with sequencing data of their phyllosphere microbial communities. We aimed to investigate how soil water stress under different levels of salinity interact in influencing plant physiological and biochemical characteristics, how these stresses affect the phyllosphere microbiome and what the associations are between leaf traits and the composition of microbial communities. Accordingly, we propose the following hypotheses: (1) the adaptative responses of halophytes to soil water stress under different levels of salinity are key drivers of differences in phyllosphere microbial communities. (2) the physiological and biochemical traits of different halophytes regulate the composition, diversity, and functional differentiation of their phyllosphere microbial communities, and certain key taxa within these communities may further contribute to the host plants' adaptation to environmental stresses; (3) in microbial community assembly, deterministic processes predominantly govern the construction of both bacterial and fungal communities, although deterministic processes play a more pronounced role in shaping bacterial communities compared to fungal communities.

Materials and Methods

Site description

This study was conducted in a salt lake located near Baoligen Sumu, Xilinhot City, Xilingol League, Inner Mongolia Autonomous Region, China (43°57'34"N, 115°36'53"E). The region has an annual mean temperature ranging from 1.9°C to 6.8°C and an average annual precipitation of 236.5 mm. The soil is a predominantly grayish-white saline-alkaline soil, characterized by high salinity or alkalinity, poor structure, susceptibility to soil compaction, low organic matter content, and weak nutrient retention capacity. As soil salinity and water content increase progressively from the lake's periphery to its center, the types of halophytes growing in the area vary accordingly under natural conditions. Consequently, we selected sampling areas based on the habitats of three representative halophytes from the lake's edge to its center, classifying these zones into low, medium, and high salinity levels.

Experimental design

In three sampling regions characterized by a progressive increase in soil salinity, we selected one representative halophytic plant species from each region based on the salinity gradient: *Suaeda salsa* (SS), *Nitraria sibirica* (NS), and *Salicornia europaea* (SE). For each species, samples were collected from three distinct plots located at least 500 m apart within each sampling region. In each plot, four healthy individuals of the target species (four replicates), spaced at least 50 m, were randomly selected. Leaf samples were collected using sterile gardening scissors, which were disinfected with 75% ethanol and air-dried before use. Rhizosphere soil samples were simultaneously collected from the area surrounding the roots of each plant. All plant materials were placed in sterile paper bags, and both plant and soil samples were immediately transported to the laboratory under cooled conditions and stored at -20°C for subsequent analysis.

Determination of Soil Physicochemical Properties and Plant Leaf Physiological-Biochemical Traits

Soil and plant leaf organic carbon contents were determined using the potassium dichromate oxidation method (Nelson and Sommers 2018). Soil nitrate-N and ammonium-N levels were determined using a continuous flow analyzer. Total phosphorus in both soil and plant leaf samples was measured following acid digestion, employing the molybdenum blue spectrophotometric technique. Soil electrical conductivity (EC) was evaluated using a conductivity meter, with a 1:5 (w/v) soil-to-deionized water suspension. Soil pH was measured with a pH meter using a 1:2.5 (w/v) soil-to-water suspension. Water-soluble sodium (Na⁺) content was assessed using the ammonium acetate-ammonium hydroxide extraction method combined with flame photometry. Soil chloride (Cl⁻) concentration was determined through titration with standard silver nitrate solution, using potassium chromate as an indicator. Gravimetric analysis was employed to quantify soil moisture content. Total nitrogen (TN) content in plant samples was measured by the Kjeldahl method, with digested samples analyzed using a Kjeldahl nitrogen analyzer. Total potassium (TK) concentration was determined via atomic absorption spectrophotometry at 766.5 nm. Leaf malondialdehyde (MDA) content was evaluated using the thiobarbituric acid (TBA) reaction method, following the protocol by Heath and Packer (2022). Superoxide dismutase (SOD) activity was quantified based on the inhibition of nitroblue tetrazolium (NBT) photoreduction, while peroxidase (POD) activity was assessed via the guaiacol oxidation

method (Zieslin and Ben-Zaken 1991). Proline (Pro) content in plant tissues was analyzed using the ninhydrin-based colorimetric assay (Carillo and Gibon, 2011.). Soluble sugar (SUG) and soluble protein (SP) concentrations were determined using the anthrone colorimetric method and the Coomassie Brilliant Blue assay, respectively (Kong et al., 2011; Bradford, 1976.). Catalase (CAT) activity in leaves was determined by homogenizing plant tissue in precooled trichloroacetic acid (TCA), followed by centrifugation. One milliliter of the supernatant was mixed with 1 mL of 100 mmol·L⁻¹ phosphate-buffered saline (PBS, pH 7.0) and 2 mL of 1 mol·L⁻¹ potassium iodide (KI). After thorough mixing and a 10-minute incubation, absorbance was recorded at 390 nm. And in this study, EC was used to evaluate soil salinity. (McGeorge 1954)

DNA Extraction and Sequencing of Phyllosphere Microorganisms

To collect phyllosphere microorganisms, 1 g of fresh leaves from each of four samples of a specific plant species from the same plot was aseptically placed into four sterile 50 mL centrifuge tubes. Each tube was filled with 50 mL of 0.1 M potassium phosphate buffer (pH 8.0). The samples were then subjected to ultrasonication for 60 s followed by vortexing for 10 s, with this process repeated twice. After washing, the leaf material was transferred to new sterile centrifuge tubes, and the washing procedure was repeated. The suspensions from both washes were combined and centrifuged at 13,000 g for 10 min to pellet the precipitate. The resulting pellet was stored at -80°C for subsequent DNA extraction (Bodenhausen et al. 2013).

Sequencing for this study was performed by Personalbio Co., Ltd. using the Illumina MiSeq high-throughput sequencing platform. Bacterial 16S rRNA gene sequences (V3-V4 region) and fungal 18S rRNA and ITS rDNA sequences (ITS2 region) from the plant microbiome samples were targeted. After demultiplexing the paired-end (PE) reads obtained from Illumina sequencing, quality control and filtering of the PE reads were conducted based on sequencing quality. Reads were then assembled using the overlap between PE sequences to generate optimized data post-quality control. The optimized data were processed using sequence denoising methods (e.g., DADA2 or Deblur) to obtain amplicon sequence variants (ASVs) along with their representative sequences and abundance information (Benjamini and Hochberg 1995; Rognes et al. 2016). Based on ASV representative sequences and abundance data, a series of analyses were performed, including taxonomic classification, community diversity assessment, differential species analysis, correlation analysis, phylogenetic analysis, and functional prediction. These analyses were supported by statistical and visualization techniques tailored to the dataset.

Statistical Analysis

All statistical analyses were performed using R software (version 4.4.0). Linear regression analysis of the plant MDA content with soil physicochemical properties and other physiological and biochemical indicators of the plant was conducted using the “geom_smooth()” function from the “ggplot2” package to fit the data model. The multivariate regression tree (MRT) analysis was performed using the “mvpart” package to identify the soil factors that drive differences in plant physiological and biochemical traits.

The “vegan” package in R was employed to analyze the abundance and composition of microbial communities, while the “ggplot2” package was used to generate stacked bar plots at the phylum and genus levels. To assess differences in evaluation metrics, one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test was applied to detect significant differences. Prior to these analyses, the Shapiro-Wilk test confirmed that the data conformed to a normal distribution, and the Bartlett test was used to verify the homogeneity of variances (Zhang et al. 2023). The Mantel test was employed to evaluate the correlations between plant leaf physiological and biochemical traits and both phyllosphere microbial community composition and α -diversity, with significance determined via randomization tests to calculate P-values (Dixon 2003). Spearman correlation analysis was conducted to compute Spearman’s rank correlation coefficients, assessing the monotonic relationships between phyllosphere microbial genera, microbial community network topology, and the physiological and biochemical traits of plant leaves (Pan et al. 2017). For the microbial co-occurrence network, the total abundance of ASVs was calculated, and ASVs were ranked by abundance to select the top 1000 most abundant ASVs. Using the “WGCNA” and “igraph” packages, a correlation network among ASVs was constructed. Spearman’s method was used for correlation analysis, and ASVs with a Spearman correlation coefficient less than 0.8 and a P-value less than 0.0001 were excluded to ensure significant and reliable relationships between ASVs. The igraph package’s cluster_fast_greedy algorithm was utilized for module partitioning to identify potential functional modules within the community (Newman 2006; Blondel et al. 2008). The “microeco” package was used to calculate within-module connectivity (Zi) and among-module connectivity (Pi) of microbial networks, determining key species within the microbial community (Deng et al. 2012). The β -nearest taxon index (β NTI) and Bray-Curtis-based Raup-Crick metric (RCbray) were calculated using the “iCAMP” package. β NTI values less than -2 indicate homogeneous selection, while values greater than 2 suggest heterogeneous selection (Zhou and Ning 2017). For β NTI values between -2 and 2, RCbray values less than -0.95 indicate homogeneous dispersal, values greater than 0.95 suggest dispersal limitation, and all other values reflect stochastic drift (Stegen et al. 2015). Random forest analysis, implemented with the “randomForest” package, was used to identify key drivers of community structure, followed by the construction of a structural equation model (SEM) using the “plspm” package to further explore these relationships.

Results

Soil Environment and Physiological-Biochemical Traits of Halophytes

Linear regression analyses revealed significant negative correlations between plant MDA content, antioxidant enzyme activities (POD, CAT, and SOD), and soil EC, soil water content (SWC), and Na^+ and Cl^- concentrations. (Fig. 1a-d) To identify the key factors influencing plant physiological and biochemical traits, we conducted multivariate regression analyses, employing cross-validation and pruning of regression tree nodes. The results indicated that at high salinity ($\text{EC} \geq 2565 \mu\text{S/cm}$), SWC was a secondary determinant of these traits, whereas at low salinity ($\text{EC} < 2565 \mu\text{S/cm}$), EC was the primary driver. (Fig. S1) Furthermore, SS exhibited significantly higher CAT and POD activities, as well as elevated

MDA, SP, TN, Pro contents, compared to NS and SE. In contrast, SE showed significantly lower CAT, POD, SOD, TN, total phosphorus (TP), total carbon (TC), and Pro contents compared to SS and NS. (Fig. 2)

Phyllosphere Bacterial and Fungal Communities of Halophytes

Having established that soil salinity and soil moisture are decisive factors influencing the physiological-biochemical traits of halophytes, we further investigated their indirect effects on microbial communities by analyzing differences in the composition, diversity, and functional metabolism of phyllosphere microbiota from the three halophytic species. Pearson and Mantel analyses were employed to correlate these microbial attributes with plant physiological-biochemical traits.

Regarding community composition, our results revealed distinct structural differences among the phyllosphere microbial communities of the three halophytes. At the phylum level, Proteobacteria were predominantly enhanced in SE plants, while Firmicutes and the fungal phylum Ascomycota were primarily enhanced in SS plants. The fungal phylum Basidiomycota was also dominant in SS plants (Fig. 3a,c; Fig. S2a,c). At the genus level, *Planococcus* and the fungal genus *Acremonium* were enhanced in SS plants, *Vibrio* was enhanced in SE plants, and fungal genus *Pleospora* was predominantly found in SS plants, *Kocuria* was enhanced in SS and NS plants (Fig. 3b,d; Fig. S1b,d). To further elucidate the relationships between phyllosphere bacterial and fungal communities and plant physiological-biochemical traits, we conducted Mantel and Pearson correlation analyses. The results indicated significant or highly significant correlations between bacteria and fungi community composition and nutrient elements (TN, total phosphorus [TP], total carbon [TC]), reactive oxygen species (ROS) system indicators (MDA, CAT, POD), and osmotic adjustment substances (Pro, SP). (Fig. 3e) Further correlation analysis of the top 30 most abundant bacterial genera with plant traits revealed that *Vibrio* exhibited significant negative correlations with TN, TC, CAT, and SOD, whereas *Planococcus* and *Kocuria* showed significant positive correlations with TN, TC, CAT, and SOD. Key fungal genera, *Acremonium* and *Pleospora*, displayed significant positive correlations with TN, MDA, POD, CAT, SP, and Pro. (Fig. 3g,h)

Mantel analysis of the alpha diversity of bacterial and fungal communities showed that TC, TP, SOD, and Pro were significantly or highly significantly correlated with microbial α -diversity indices. (Fig. 3f) Spearman analysis further confirmed these associations. Specifically, TC, TP, SOD, and Pro were significantly positively correlated with bacterial Shannon, Simpson, Pielou, and Goods_coverage indices, while in fungal communities, these traits were positively correlated with the Obs index and negatively correlated with the Pielou index. (Fig. S3) Moreover, we found that the Shannon, Simpson, and Goods_coverage indices of phyllosphere bacterial communities in SE plants were significantly lower than those in SS and NS plants. In contrast, the Obs and Chao indices of phyllosphere fungal communities in SS plants were significantly lower than those in SE and NS plants, while the Pielou index of fungal communities in SE plants was significantly higher than in SS and NS plants. (Fig. 4)

Functional Prediction of Phyllosphere Microbial Communities in Halophytes

Given that the differences in soil physicochemical properties and plant physiological and biochemical characteristics are more pronounced in SE plants at the center of the saline-alkaline lake and SS plants at the lake edge compared to NS plants, we performed a differential metabolic pathway analysis of the phyllosphere microbial communities under high water stress (SS) and low water stress (SE) using PICRUSt2 to investigate the potential functional variations in bacterial and fungal communities. The results revealed pronounced functional differentiation between bacterial and fungal communities: metabolic functions associated with antioxidant stress responses were predominantly enhanced in SS phyllosphere microbial community, whereas functions related to competitive defense mechanisms or efficient resource utilization were more abundant in SE phyllosphere microbial community.

(Fig. 5) Specifically, in the SS phyllosphere bacterial community, secondary metabolic pathways including isoflavonoid biosynthesis, flavonoid biosynthesis, arachidonic acid metabolism, sesquiterpenoid biosynthesis, photosynthesis - antenna proteins, steroid hormone biosynthesis, carotenoid biosynthesis, and lipoic acid metabolism were significantly enhanced (Table S1). Similarly, in the fungal community, pathways such as phospholipid remodeling, NAD/NADP-NADH/NADPH metabolism, sulfate reduction I (assimilatory), palmitate biosynthesis I (animals and fungi), and phospholipases were enhanced, all of which are linked to antioxidant stress responses (Table S2).

In contrast, in the SE phyllosphere bacterial community, pathways such as biosynthesis of ansamycins, *Vibrio cholerae* pathogenic cycle, and plant-pathogen interaction exhibited significant expression, primarily associated with immune defense mechanisms (Table S1). Additionally, the superpathway of purine nucleotide salvage and the superpathway of pyrimidine ribonucleosides salvage were markedly enhanced in the SE phyllosphere fungal community, indicating efficient utilization of pre-existing environmental nutrient resources by fungi (Table S2).

Co-occurrence Network Patterns and Community Assembly of Phyllosphere Microbiota in Halophytes

Using co-occurrence network analysis, we constructed microbial networks for the phyllosphere of the three halophytes and further examined the correlations between the topological indices of bacterial and fungal networks and plant physiological-biochemical traits. The results revealed that TP, TC, SOD, and POD exhibited significant positive correlations with the degree, graph density, and clustering coefficient of bacterial networks (Fig. 6c). This suggests that as TP, TC, SOD, and POD levels increase, the co-occurrence network structure of bacteria becomes more tightly interconnected. In contrast, TP, TC, and SOD showed a significant positive correlation with the modularity of the fungal network, indicating that under high water and salinity stress, the modular organization of the SS fungal network becomes more distinct. However, these parameters were significantly negatively correlated with graph density and degree, suggesting that despite increased modularity, the fungal network structure remains relatively loose, with lower interdependence among community members (Fig. 6d). Furthermore, we employed Zi-Pi analysis to identify key network nodes, pinpointing core microbial taxa and screening genera that play pivotal roles in the microbial communities. The results indicated that bacterial communities were predominantly distributed within the dominant phyla Proteobacteria and Firmicutes. Specifically, the key genera in SS were *Planococcus* and *Kocuria*, in NS they were *Planococcus* and *Planomicrobium*, and in

SE it was *Vibrio*. For fungal networks, key nodes primarily belonged to the phylum *Ascomycota*, with the core genera identified as *Alternaria* and *Acremonium* (Fig. S4).

To further elucidate the influence of plants on the assembly of phyllosphere bacterial and fungal communities, we employed null model analyses based on β NTI and RCbray to assess the contributions of stochastic and deterministic processes. The results showed that β NTI values for phyllosphere bacterial communities were consistently less than -2 , indicating that deterministic processes dominated bacterial community assembly. (Fig. 6e) In contrast, fungal community assembly varied: the β NTI value for NS plants was less than -2 , suggesting a dominance of deterministic processes, whereas β NTI values for SE and SS plants fell within $[-2]$, indicating that fungal community assembly was influenced by both deterministic and stochastic processes. (Fig. 6f) Combined with RCbray analysis, we found that homogeneous selection was the primary process governing the assembly of bacterial communities and the fungal community in NS plants. Ecological drift and dispersal limitation dominated fungal community assembly in SE plants, while homogeneous selection (50%) and ecological drift (50%) jointly drove fungal community assembly in SS plants. (Fig. S5a,b) To identify the intrinsic factors driving community assembly, we conducted Mantel tests to explore correlations between β NTI and environmental variables. The results demonstrated significant correlations between plant nutrient content (TN, TC, TP), antioxidant enzyme activity (SOD, POD, CAT), Pro, SUG content and the β NTI values of both bacterial and fungal communities (Table S3,4).

Responses of Phyllosphere Bacterial and Fungal Communities to Physiological-Biochemical Traits of Halophytes

Random forest analysis was employed to identify the key factors influencing microbial community characteristics by constructing multiple decision trees and integrating them into a robust classifier for classification and regression. (Fig. S6) Based on the results of the random forest analysis, the most important factors were subsequently used to construct a SEM. The SEM results revealed that MDA content in halophytes exerted an indirect positive effect on both the composition and α -diversity of phyllosphere microbial communities. Specifically, at the initial stage, MDA directly modulated the ROS system of halophytes, and activation of the ROS system facilitated the accumulation of plant elements. This accumulation, in turn, positively influenced microbial community composition and α -diversity. Additionally, osmotic adjustment substances exhibited a negative effect on the α -diversity of phyllosphere fungal communities. (Fig. 7)

Discussion

Halophyte Responses to Soil Water Stress

In our study, soil water stress emerged as a key determinant of the physiological and biochemical traits of halophytes, significantly shaping the characteristics of phyllosphere microbial communities. Specifically, water stress-induced osmotic imbalance triggers excessive ROS production, leading to MDA accumulation, which in turn activates ROS signaling and detoxification pathways. Concurrently,

compensatory osmotic adjustment mechanisms are initiated, promoting the reallocation of nutrients such as C and N, which indirectly modulates bacterial and fungal community structures and drives differences in community composition. Linear regression analyses revealed that soil water stress significantly influences MDA content and the activities of antioxidant enzymes (POD, CAT, and SOD). Notably, under low salinity ($EC < 2565 \mu S/cm$) and water-limited conditions, SS exhibited elevated MDA and Pro contents, as well as increased POD, CAT, and SOD activities. These responses are attributed to low soil water content, which maintains a low soil matric potential, coupled with an imbalance between soil salinity and plant cell osmotic potential. This imbalance exacerbates the water potential gradient between plants and soil, further restricting water availability, leading to cellular dehydration, structural changes in leaves, and heightened ROS production. Consequently, plants activate antioxidant defense systems, resulting in enhanced antioxidant enzyme activities (Munns and Tester 2008; Miller et al. 2010; Flowers and Colmer 2015; Acosta-Motos et al. 2017). These findings also explain our multivariate regression results, which identified EC as the primary driver of physiological and biochemical traits under low salinity ($EC < 2565 \mu S/cm$). Surprisingly, soil salinity did not cause severe ion toxicity in halophytes, owing to their unique salt-tolerance mechanisms. Extensive research indicates that halophytes mitigate ion toxicity through ion compartmentalization, utilizing Na^+/H^+ antiporters (NHX) to sequester Na^+ in vacuoles, thus reducing cytosolic Na^+ concentrations (Apse et al. 1999; Blumwald 2000). Additionally, the succulent leaves and stems of halophytes store water and salts, diluting salt concentrations and minimizing ion toxicity (Kefu et al. 2003).

On the other hand, substantial evidence suggests that water stress inhibits photosynthesis, impairing the primary pathway for carbon acquisition (Zahra et al. 2022; Zhou et al. 2024). MDA, a marker of membrane damage, reflects oxidative injury caused by excessive ROS. Under normal conditions, elevated MDA content serves as a protective mechanism against water stress by regulating defense and developmental genes, providing cellular protection under oxidative stress (Morales and Munné-Bosch 2019). The significant positive correlation between MDA content and ROS-scavenging enzyme activities in our study confirms the proper functioning of ROS detoxification pathways. Under ROS signaling, antioxidant enzymes likely play dual roles in mitigating ROS damage and regulating ROS-mediated signal transduction. Notably, ROS, particularly H_2O_2 , act as signaling molecules by oxidizing cysteine residues in regulatory proteins, with SOD being a critical enzyme in H_2O_2 production. Loss of SOD activity can disrupt ROS signaling by reducing H_2O_2 levels, indirectly affecting protein and amino acid metabolism (Wang et al. 2018). We hypothesize that the activation of detoxification pathways may coincide with the induction of osmotic compensation mechanisms. Current studies indicate that under drought conditions, plants prioritize the transport of C and N to leaves to maintain Rubisco levels and photosynthetic efficiency, enhancing adaptation to environmental stress (Bota et al. 2004; Sala et al. 2010). The positive correlation between antioxidant enzyme activities and leaf nutrient (C, N, P) contents in our study further supports that water stress facilitates nutrient reallocation. (Fig. S9a,b) This nutrient accumulation may trigger compensatory amino acid and protein synthesis (Gagneul et al. 2007; Wang et al. 2009; Liu et al. 2011; Fan et al. 2011), as amino acids and proteins regulate osmotic imbalances caused by water stress. Certain amino acids also protect macromolecular subcellular structures,

mitigating oxidative damage from free radicals (Wang et al. 2016; Arbelet-Bonnin et al. 2020). Moreover, amino acids such as proline, tyrosine, alanine, amides, and non-protein amino acids accumulate under salt stress (Kumari et al. 2015; Behr et al. 2017). Additionally, nutrient accumulation may influence phyllosphere microbial communities by providing diverse secretory metabolites, recruiting beneficial microbes or suppressing pathogens, thereby shaping community diversity and functional differentiation.

Influence of Halophytes on Community Composition, α -Diversity, and Metabolic Functions of Phyllosphere Bacterial and Fungal Communities

Our study demonstrates that, under soil water stress, the nutrient content, antioxidant enzyme activity, MDA, and Pro levels in different halophytes regulate the structure of their phyllosphere bacterial and fungal communities to varying degrees. When facing environmental constraints, halophytes may emit distinct “distress signals” to the phyllosphere through diverse physiological-biochemical traits, secreting various amino acids, sugars, hormones, and phenolic compounds (Weibull et al., 1990; Nonomura et al., 2009; López-Guerrero et al., 2013; Chang et al., 2021). These secretions may facilitate the colonization of specific microbial strains or act as broad-spectrum inhibitors to restrict the proliferation of certain taxa, thereby influencing host preferences of key bacterial genera. Concurrently, bacterial genera with distinct ecological functions may become enhanced in specific phyllosphere environments under the selective pressure of halophytes, leading to functional differentiation in microbial metabolism. Notably, the differential distribution of key genera *Planococcus*, *Kocuria*, and *Vibrio* along soil salinity and moisture gradients provides strong evidence supporting our findings. We observed that as soil moisture and salinity increased, the abundance of *Vibrio* in the phyllosphere significantly rose, while bacterial α -diversity markedly declined. (Table S9c-e) This may be attributed to low water stress conditions, where halophytes exhibit reduced nutrient content and higher soil moisture creates a humid microenvironment conducive to pathogens, resulting in lower organic secretions or the release of antibacterial compounds. The antibacterial activity and broad-spectrum properties of compounds secreted by *Vibrio ruber* enable it to outcompete other taxa, reducing their niche space and resources, thus lowering overall diversity. Conversely, the distribution of *Planococcus* and *Kocuria* in the phyllosphere positively correlates with α -diversity. (Table S9c-e) Under high water stress, elevated MDA and nutrient levels in halophytes may enhance anabolic metabolism, increasing organic secretions and recruiting beneficial genera such as *Planococcus* and *Kocuria* to mitigate stress. (Satpute et al. 2010; Waghmode et al. 2020) Our functional prediction of phyllosphere microbiota further corroborates this observation: under low-stress conditions, functions associated with competitive defense mechanisms are significantly enhanced in the bacterial communities of the plant phyllosphere, whereas under high-stress conditions, metabolic functions linked to antioxidant stress responses predominate. For instance, photosynthesis - antenna proteins, carotenoids, lipoic acid, isoflavonoids, and flavonoids have been demonstrated to directly scavenge or reduce the production of reactive oxygen species (ROS) (Pospíšil, 2016; Vetoshkina et al., 2023; Suzuki et al., 1991; Ou et al., 1995; Kumar and Pandey, 2013; Platzer et al., 2022). In contrast, the significant expression of pathways such as naphthalene degradation, other glycan degradation, geraniol degradation, nitrotoluene degradation, and caprolactam degradation in the phyllosphere bacterial community of SE was associated with the degradation of complex organic compounds, indicating that

these bacteria efficiently utilize otherwise inaccessible carbon, nitrogen, and phosphorus resources in the phyllosphere environment. The functional differentiation of phyllosphere bacterial communities between the two halophytes not only highlights their adaptive strategies to distinct host environments but also underscores the flexible mechanisms employed by halophytes in response to diverse environmental stresses.

In contrast to bacterial communities, we found that compositional differences in fungal communities predominantly stem from the phylum *Ascomycota* (Fig. S7), which dominates the key fungal taxa in the phyllosphere of all three halophytes. Consequently, we infer that the regulatory influence of halophytes on phyllosphere fungal communities primarily targets the genera and abundance variations within *Ascomycota*. This phylum is currently recognized as an eutrophic group in microbial ecological strategies, playing a pivotal role in litter decomposition (Zheng et al. 2021; Liu et al. 2023). This ecological trait provides a plausible explanation for the increase of certain key genera (e.g., *Acremonium* and *Pleospora*) in the phyllosphere of halophytes with elevated TN content. Concurrently, the increase of *Acremonium* and *Pleospora* in SS under high water stress is a primary driver of functional differentiation in fungal communities (Fig. S8). Phospholipid remodeling, phospholipases, and palmitate biosynthesis protect microbial cell membranes from ROS damage by repairing compromised lipids, adjusting membrane composition (increasing the proportion of saturated fatty acids), and preventing lipid peroxidation (Leamy et al. 2014; Wang and Tontono 2019; Astudillo et al. 2019). Assimilatory sulfate reduction aids microfungi in synthesizing antioxidant molecules (e.g., glutathione, GSH), enhancing their survival in oxidative stress environments such as sulfide-rich or high-oxygen conditions (Traynor et al. 2019). Genera within the phylum *Ascomycota* not only positively contribute to nitrogen accumulation in plants but also actively participate in the stress-tolerance selection process of phyllosphere fungal communities by halophytes. Furthermore, in the phyllosphere fungal community of SE, the superpathway of purine nucleotide salvage and the superpathway of pyrimidine ribonucleosides salvage are significantly enhanced, indicating that fungal taxa utilize pre-existing pyrimidine and purine compounds in the environment rather than relying on energy-intensive *de novo* synthesis. This adaptation is particularly crucial in nutrient-limited settings and provides reasonable support for the hypothesis that the phyllosphere of SE plants under low water stress experiences a scarcity of nutritional resources. In terms of community diversity, the lower evenness and higher richness of the SS phyllosphere fungal community further confirm the enhancing effect of key *Ascomycota* genera in SS. As an eutrophic group, *Ascomycota* may enhance the colonization of other fungal genera by supplying nutrients, thereby increasing species richness to some extent. This phenomenon suggests that halophytes can modulate nutrient cycling and ecological functions of phyllosphere fungal communities by regulating specific *Ascomycota* genera. Our study provides preliminary insights into how interactions between halophytes and phyllosphere fungi drive community differentiation under water stress. However, the specific exudates through which halophytes mediate information transfer and material exchange with fungal communities remain to be elucidated.

Influence of Halophytes on Phyllosphere Microbial Community Assembly

Within phyllosphere microbial communities, microorganisms do not exist in isolation; the metabolic activities and behaviors of each individual species influence neighboring taxa, while colonization and dispersal of all species are continuously shaped by the microenvironment provided by the host. Consequently, the assembly of phyllosphere microbial communities is often accompanied by the formation and expansion of microbial interaction networks. In this dynamic ecological process, environmental factors, inter-community relationships, and individual activities collectively play pivotal roles. Theoretically, the microbial community assembly is governed by three dominant mechanisms: host-mediated selection, biotic interactions, and dispersal limitation (Barner et al. 2018; D'Amen et al. 2018; Liao et al. 2022). In this study, the null model analysis revealed that the assembly of phyllosphere bacterial communities was entirely dominated by deterministic processes ($\beta\text{NTI} < -2$), with homogeneous selection as the core mechanism, consistent with previous findings (Liao et al. 2016; Xu et al. 2022). As plant nutrient content and antioxidant enzyme activity increase, bacterial co-occurrence networks exhibit enhanced connectivity and cohesiveness, potentially reflecting higher efficiency in intra-community information transfer. This also suggests that bacterial communities may be more responsive to host selection. Through the secretion of secondary metabolites (e.g., phenolics, terpenoids) or immune-regulating compounds (e.g., ROS) (Farré-Armengol et al. 2016; Pang et al. 2021), plants may significantly enhance the recruitment of bacterial taxa with specific functions (e.g., stress resistance, nutrient metabolism) (Li et al. 2022a). The community convergence driven by homogeneous selection and the efficient information transfer within bacterial co-occurrence networks indicate that hosts may employ a “distress” strategy to bolster their resilience against soil water stress.

In contrast to bacteria, while deterministic processes influence fungal community assembly, the proportion of stochasticity is significantly higher in fungal communities. This may partly stem from the more complex cellular structures of fungi, which confer greater stability under extreme conditions, thereby reducing the selective influence of halophytes, aligning with prior studies (De Vries et al. 2018). On the other hand, topological analysis of fungal networks revealed that under higher soil water stress, interactions and connectivity among fungal community members in the phyllosphere of halophytes were significantly reduced. The high modularity of these networks suggests that weak interactions among members within modules are confined to specific regions, limiting the propagation of environmental disturbances across the broader network. We hypothesize that the loose structure and weak interactions of fungal networks under strong environmental selective pressure may enhance the role of stochastic processes in community assembly. Conversely, under lower soil water stress, the networks exhibited high modularity, indicating relatively uniform interactions among community members. Moreover, elevated graph density and node degree pointed to a marked increase in direct microbial interactions, resulting in a more densely interconnected network. Environmental homogenization likely drives this highly interconnected, “integrated” network structure. Previous studies have suggested that in homogeneous habitats with weaker environmental selection pressure, ecological drift plays a more prominent role in shaping community structure, possibly because the absence of strong selective forces amplifies drift effects. In niche-poor, homogeneous habitats, stochastic processes such as drift may overshadow niche-based dynamics (Purves and Pacala 2005; Remmer et al. 2019; Milke et al. 2022).

Earlier research has also noted that fungal communities often exhibit pronounced stochasticity (Huang et al. 2022; Ye et al. 2023; Sheng et al. 2024). However, the theoretical underpinnings of this phenomenon warrant further exploration. By leveraging the perspective of co-occurrence network analysis, this study offers new insights into the underlying mechanisms of community assembly.

Mantel tests further demonstrated that the physiological responses of halophytes to water stress significantly shape the assembly of phyllosphere microbial communities. The significant correlations between plant nutrient content, antioxidant enzyme activity, Pro levels, and the β NTI values of bacterial and fungal communities suggest shared response strategies to environmental stress across these microbial groups. As a marker of membrane lipid peroxidation, MDA accumulation, alongside POD activity, reflects the extent of oxidative damage to plant cell membranes and the efficacy of antioxidant defenses (Morales and Munné-Bosch 2019). Meanwhile, Pro, as an osmoprotectant, indicates the host's physiological adaptation to water stress (Hasanuzzaman and Fujita 2022; Singh et al. 2022). Although the assembly patterns of bacterial and fungal communities differ, both form a coupled relationship with the host's stress-tolerant physiological traits through functional synergy. Bacteria enhance the dominance of core functional taxa via host-mediated selective filtering, whereas fungi reduce interspecies associations and simplify network topology. This dual mechanism enables SS to maintain the stability of community structure and function under high water stress to a certain extent. The synergistic interplay between the host-directed selection strategy of bacteria and the network simplification strategy of fungi suggests that microbial community assembly and co-occurrence networks are not isolated processes, but rather an integrated manifestation of host-microbe interaction networks adapting to saline environments.

Conclusion

Soil water stress is a critical determinant of the physiological-biochemical traits of different halophytes. Soil moisture and salinity exhibit a significant negative correlation with MDA, antioxidant enzyme activities in halophytes. Concurrently, the stress-tolerant physiological-biochemical characteristics of halophytes markedly drive the composition, diversity, and functional differentiation of phyllosphere microbial communities. Our findings demonstrate significant correlations between MDA, antioxidant enzymes, nutrient elements, and osmoregulatory substances in halophytes and the composition and diversity of their phyllosphere bacterial and fungal communities. Specifically, metabolic pathways associated with microbial antioxidant stress responses are predominantly enhanced in SS under high water stress, whereas functions related to microbial competition-defense mechanisms and oligotrophic traits are more abundant in SE under low water stress. This study reveals that water stress indirectly influences phyllosphere microbial communities: osmotic imbalance induced by water stress triggers excessive ROS production (leading to MDA accumulation), which activates ROS signaling and detoxification pathways. This signaling cascade concurrently affects the accumulation of nutrient elements such as C and N. The accumulation of these nutrients, potentially via synthetic metabolism and the secretion of diverse organic compounds, exerts functional selection on microbial communities. However, fungal communities exhibit lower dependence on the host compared to bacterial communities.

This study underscores that differential stress-tolerance strategies among halophytes under varying water stress conditions serve as a key driver in shaping the composition of their phyllosphere microbial communities.

Declarations

Funding

This work was supported by grants from the National Natural Science Foundation of China (42077054), Central guidance for local scientific and technological development funding projects (2023ZY0026), and the Natural Science Foundation of Inner Mongolia, China (2022LHMS03004, 2023LHMS03026).

Acknowledgements

We thank Jianjun Chen for help with setting up the experiment and collecting the plant and soil samples.

Author contributions

Xinyu Ge: Writing–review & editing, Writing–original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Wenbo Zhang:** Writing–review, investigation, Data curation. **Xiangjian Tu:** Investigation, Data curation. **Paul C. Struik:** Writing–review & editing. **Ke Jin:** Writing review & editing, Funding acquisition. **Rula Sa:** Investigation, Data curation. **Zhen Wang:** Supervision, Project administration, writing–review & editing, Funding acquisition.

Data availability

Data will be made available on request. All data generated or analyzed are included within the article and the supplementary information files.

Conflict of interests

The authors declare that they have no competing interests.

References

1. Abadi VAJM, Sepehri M, Rahmani HA et al (2020) Role of Dominant Phyllosphere Bacteria with Plant Growth–Promoting Characteristics on Growth and Nutrition of Maize (*Zea mays* L). *J Soil Sci Plant Nutr* 20:2348–2363. <https://doi.org/10.1007/s42729-020-00302-1>
2. Acosta-Motos J, Ortuño M, Bernal-Vicente A et al (2017) Plant responses to salt stress: Adaptive mechanisms. *Agronomy* 7:18. <https://doi.org/10.3390/agronomy7010018>
3. Al-Yasi H, Attia H, Alamer K et al (2020) Impact of drought on growth, photosynthesis, osmotic adjustment, and cell wall elasticity in damask rose. *Plant Physiol Biochem* 150:133–139. <https://doi.org/10.1016/j.plaphy.2020.02.038>

4. Apse MP, Aharon GS, Snedden WA, Blumwald E (1999) Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in *arabidopsis*. *Science* 285:1256–1258.
<https://doi.org/10.1126/science.285.5431.1256>
5. Arbelet-Bonnin D, Blasselle C, Rose Palm E et al (2020) Metabolism regulation during salt exposure in the halophyte *Salicornia maritima*. *Environ Exp Bot* 177:104075.
<https://doi.org/10.1016/j.envexpbot.2020.104075>
6. Astudillo AM, Balboa MA, Balsinde J (2019) Selectivity of phospholipid hydrolysis by phospholipase A2 enzymes in activated cells leading to polyunsaturated fatty acid mobilization. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell. Biology Lipids* 1864:772–783.
<https://doi.org/10.1016/j.bbalip.2018.07.002>
7. Barner AK, Coblenz KE, Hacker SD, Menge BA (2018) Fundamental contradictions among observational and experimental estimates of non-trophic species interactions. *Ecology* 99:557–566.
<https://doi.org/10.1002/ecy.2133>
8. Bashir I, War AF, Rafiq I et al (2022) Phyllosphere microbiome: Diversity and functions. *Microbiol Res* 254:126888. <https://doi.org/10.1016/j.micres.2021.126888>
9. Behera TK, Krishna R, Ansari WA et al (2022) Approaches involved in the vegetable crops salt stress tolerance improvement: Present status and way ahead. *Front Plant Sci* 12:787292.
<https://doi.org/10.3389/fpls.2021.787292>
10. Behr JH, Bouchereau A, Berardocco S et al (2017) Metabolic and physiological adjustment of *Suaeda maritima* to combined salinity and hypoxia. <https://doi.org/10.1093/aob/mcw282>. *Ann Bot* mcw282
11. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J Royal Stat Soc Ser B: Stat Methodol* 57:289–300.
<https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>
12. Blondel VD, Guillaume J-L, Lambiotte R, Lefebvre E (2008) Fast unfolding of communities in large networks. *J Stat Mech* 2008:P10008. <https://doi.org/10.1088/1742-5468/2008/10/P10008>
13. Blumwald E (2000) Sodium transport and salt tolerance in plants. *Curr Opin Cell Biol* 12:431–434.
[https://doi.org/10.1016/S0955-0674\(00\)00112-5](https://doi.org/10.1016/S0955-0674(00)00112-5)
14. Bodenhausen N, Horton MW, Bergelson J (2013) Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. *PLoS ONE* 8:e56329.
<https://doi.org/10.1371/journal.pone.0056329>
15. Bota J, Medrano H, Flexas J (2004) Is photosynthesis limited by decreased rubisco activity and RuBP content under progressive water stress? *New Phytol* 162:671–681.
<https://doi.org/10.1111/j.1469-8137.2004.01056.x>
16. Bradford MMA rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding
17. Bunster L, Fokkema NJ, Schippers B (1989) Effect of Surface-Active *Pseudomonas* spp. on Leaf Wettability. *Appl Environ Microbiol* 55:1340–1345. <https://doi.org/10.1128/aem.55.6.1340->

18. Carillo P, Gibon YPROTOCOL Extraction and determination of proline
19. Chang JD, Vaughan EE, Liu CG et al (2021) Metabolic profiling reveals nutrient preferences during carbon utilization in bacillus species. Sci Rep 11:23917. <https://doi.org/10.1038/s41598-021-03420-7>
20. D'Amen M, Mod HK, Gotelli NJ, Guisan A (2018) Disentangling biotic interactions, environmental filters, and dispersal limitation as drivers of species co-occurrence. Ecography 41:1233–1244. <https://doi.org/10.1111/ecog.03148>
21. De Vries FT, Griffiths RI, Bailey M et al (2018) Soil bacterial networks are less stable under drought than fungal networks. Nat Commun 9:3033. <https://doi.org/10.1038/s41467-018-05516-7>
22. Deng Y, Jiang Y-H, Yang Y et al (2012) Molecular ecological network analyses. BMC Bioinformatics 13:113. <https://doi.org/10.1186/1471-2105-13-113>
23. Dixon P (2003) VEGAN, a package of R functions for community ecology. J Veg Sci 14:927–930. <https://doi.org/10.1111/j.1654-1103.2003.tb02228.x>
24. Fan P, Feng J, Jiang P et al (2011) Coordination of carbon fixation and nitrogen metabolism in *salicornia europaea* under salinity: Comparative proteomic analysis on chloroplast proteins. Proteomics 11:4346–4367. <https://doi.org/10.1002/pmic.201100054>
25. Farré-Armengol G, Filella I, Llusia J, Peñuelas J (2016) Bidirectional interaction between phyllospheric microbiotas and plant volatile emissions. Trends Plant Sci 21:854–860. <https://doi.org/10.1016/j.tplants.2016.06.005>
26. Flowers TJ, Colmer TD (2015) Plant salt tolerance: Adaptations in halophytes. Ann Botany 115:327–331. <https://doi.org/10.1093/aob/mcu267>
27. Gagneul D, Ainouche A, Duhazé C et al (2007) A reassessment of the function of the so-called compatible solutes in the halophytic plumbaginaceae *limonium latifolium*. Plant Physiol 144:1598–1611. <https://doi.org/10.1104/pp.107.099820>
28. Guo C, Yang A, Zhang W-H (2024) Host Identity Determines the Bacterial and Fungal Community and Network Structures in the Phyllosphere of Plant Species in a Temperate Steppe. Phytobiomes J 8:143–154. <https://doi.org/10.1094/PBIOMES-05-23-0038-R>
29. Hasanuzzaman M, Fujita M (2022) Plant responses and tolerance to salt stress: Physiological and molecular interventions. IJMS 23:4810. <https://doi.org/10.3390/ijms23094810>
30. Heath RL, Packer L (2022) Reprint of: Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 726:109248. <https://doi.org/10.1016/j.abb.2022.109248>
31. Hirano SS, Upper CD (2000) Bacteria in the Leaf Ecosystem with Emphasis on Pseudomonas syringae—a Pathogen, Ice Nucleus, and Epiphyte. Microbiol Mol Biol Rev 64:624–653. <https://doi.org/10.1128/MMBR.64.3.624-653.2000>

32. Huang L, Bai J, Wang J et al (2022) Different stochastic processes regulate bacterial and fungal community assembly in estuarine wetland soils. *Soil Biol Biochem* 167:108586. <https://doi.org/10.1016/j.soilbio.2022.108586>
33. Kefu Z, Hai F, San Z, Jie S (2003) Study on the salt and drought tolerance of *suaeda salsa* and *kalanchoe claigremontiana* under iso-osmotic salt and water stress. *Plant Sci* 165:837–844. [https://doi.org/10.1016/S0168-9452\(03\)00282-6](https://doi.org/10.1016/S0168-9452(03)00282-6)
34. Kefu Z, Hai F, Ungar IA (2002) Survey of halophyte species in China. *Plant Sci* 163:491–498. [https://doi.org/10.1016/S0168-9452\(02\)00160-7](https://doi.org/10.1016/S0168-9452(02)00160-7)
35. Kembel SW, O'Connor TK, Arnold HK et al (2014) Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proc Natl Acad Sci USA* 111:13715–13720. <https://doi.org/10.1073/pnas.1216057111>
36. Kong X, Pan J, Zhang M et al (2011) *ZmMKK4*, a novel group C mitogen-activated protein kinase kinase in maize (*zea mays*), confers salt and cold tolerance in transgenic *arabidopsis*. *Plant Cell Environ* 34:1291–1303. <https://doi.org/10.1111/j.1365-3040.2011.02329.x>
37. Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: An overview. *Sci World J* 2013:162750. <https://doi.org/10.1155/2013/162750>
38. Kumari A, Das P, Parida AK, Agarwal PK (2015) Proteomics, metabolomics, and ionomics perspectives of salinity tolerance in halophytes. *Front Plant Sci* 6. <https://doi.org/10.3389/fpls.2015.00537>
39. Leamy AK, Egnatchik RA, Shiota M et al (2014) Enhanced synthesis of saturated phospholipids is associated with ER stress and lipotoxicity in palmitate treated hepatic cells. *J Lipid Res* 55:1478–1488. <https://doi.org/10.1194/jlr.M050237>
40. Legay N, Baxendale C, Grigulis K et al (2014) Contribution of above- and below-ground plant traits to the structure and function of grassland soil microbial communities. *Ann Botany* 114:1011–1021. <https://doi.org/10.1093/aob/mcu169>
41. Li J, Jin M-K, Neilson R et al (2023) Plant identity shapes phyllosphere microbiome structure and abundance of genes involved in nutrient cycling. *Sci Total Environ* 865:161245. <https://doi.org/10.1016/j.scitotenv.2022.161245>
42. Li P-D, Zhu Z-R, Zhang Y et al (2022a) The phyllosphere microbiome shifts toward combating melanose pathogen. *Microbiome* 10:56. <https://doi.org/10.1186/s40168-022-01234-x>
43. Li Y, Gao Y, Zhang W et al (2019) Homogeneous selection dominates the microbial community assembly in the sediment of the three gorges reservoir. *Sci Total Environ* 690:50–60. <https://doi.org/10.1016/j.scitotenv.2019.07.014>
44. Li Y, Pan J, Zhang R et al (2022b) Environmental factors, bacterial interactions and plant traits jointly regulate epiphytic bacterial community composition of two alpine grassland species. *Sci Total Environ* 836:155665. <https://doi.org/10.1016/j.scitotenv.2022.155665>
45. Liao J, Bearup D, Strona G (2022) A patch-dynamic metacommunity perspective on the persistence of mutualistic and antagonistic bipartite networks. *Ecology* 103:e3686.

<https://doi.org/10.1002/ecy.3686>

46. Liao J, Cao X, Zhao L et al (2016) The importance of neutral and niche processes for bacterial community assembly differs between habitat generalists and specialists. *FEMS Microbiol Ecol* 92:fiw174. <https://doi.org/10.1093/femsec/fiw174>
47. Liu M, Wei Y, Lian L et al (2023) Macrofungi promote SOC decomposition and weaken sequestration by modulating soil microbial function in temperate steppe. *Sci Total Environ* 899:165556. <https://doi.org/10.1016/j.scitotenv.2023.165556>
48. Liu X, Yang C, Zhang L et al (2011) Metabolic profiling of cadmium-induced effects in one pioneer intertidal halophyte *Suaeda salsa* by NMR-based metabolomics. *Ecotoxicology* 20:1422–1431. <https://doi.org/10.1007/s10646-011-0699-9>
49. López-Guerrero MG, Ormeño-Orrillo E, Rosenblueth M et al (2013) Buffet hypothesis for microbial nutrition at the rhizosphere. *Front Plant Sci* 4. <https://doi.org/10.3389/fpls.2013.00188>
50. MacArthur RH, Wilson EO The theory of island biogeography
51. McGeorge WT (1954) Diagnosis and improvement of saline and alkaline soils: By staff of U. S. Salinity laboratory, agriculture handbook 60 U. S. Dept. Agric., supt. Documents, U. S. Government printing office washington 25, D. C., 1954, 160 pages, \$2.00. *Soil Sci Soc Amer J* 18:348–348. <https://doi.org/10.2136/sssaj1954.03615995001800030032x>
52. Milke F, Wagner-Dobler I, Wienhausen G, Simon M (2022) Selection, drift and community interactions shape microbial biogeographic patterns in the pacific ocean. *ISME J* 16:2653–2665. <https://doi.org/10.1038/s41396-022-01318-4>
53. Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R (2010) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ* 33:453–467. <https://doi.org/10.1111/j.1365-3040.2009.02041.x>
54. Morales M, Munné-Bosch S (2019) Malondialdehyde: Facts and artifacts. *Plant Physiol* 180:1246–1250. <https://doi.org/10.1104/pp.19.00405>
55. Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59:651–681. <https://doi.org/10.1146/annurev.arplant.59.032607.092911>
56. Nelson DW, Sommers LE (2018) Total carbon, organic carbon, and organic matter. In: Sparks DL, Page AL, Helmke PA et al (eds) *SSSA Book Series*. Soil Science Society of America, American Society of Agronomy, Madison, WI, USA, pp 961–1010
57. Newman MEJ (2006) Modularity and community structure in networks. *Proc Natl Acad Sci USA* 103:8577–8582. <https://doi.org/10.1073/pnas.0601602103>
58. Nonomura T, Xu L, Wada M et al (2009) Trichome exudates of *Lycopersicon pennellii* form a chemical barrier to suppress leaf-surface germination of *oidium neolycopersici* conidia. *Plant Sci* 176:31–37. <https://doi.org/10.1016/j.plantsci.2008.09.002>
59. Ou P, Tritschler HJ, Wolff SP (1995) Thiocctic (lipoic) acid: A therapeutic metal-chelating antioxidant? *Biochem Pharmacol* 50:123–126. [https://doi.org/10.1016/0006-2952\(95\)00116-H](https://doi.org/10.1016/0006-2952(95)00116-H)

60. Pan D, Nolan J, Williams KH et al (2017) Abundance and distribution of microbial cells and viruses in an alluvial aquifer. *Front Microbiol* 8:1199. <https://doi.org/10.3389/fmicb.2017.01199>
61. Pang Z, Chen J, Wang T et al (2021) Linking plant secondary metabolites and plant microbiomes: A review. *Front Plant Sci* 12:621276. <https://doi.org/10.3389/fpls.2021.621276>
62. Platzer M, Kiese S, Tybussek T et al (2022) Radical scavenging mechanisms of phenolic compounds: A quantitative structure-property relationship (QSPR) study. *Front Nutr* 9:882458. <https://doi.org/10.3389/fnut.2022.882458>
63. Pospíšil P (2016) Production of reactive oxygen species by photosystem II as a response to light and temperature stress. *Front Plant Sci* 7. <https://doi.org/10.3389/fpls.2016.01950>
64. Purves DW, Pacala SW (2005) Ecological drift in niche-structured communities: Neutral pattern does not imply neutral process. In: Burslem D, Pinard M, Hartley S (eds) *Biotic Interactions in the Tropics*, 1st edn. Cambridge University Press, pp 107–138
65. Remmer CR, Robichaud CD, Polowyk H, Rooney R (2019) The role of ecological drift in structuring periphytic diatom communities. *J Freshw Ecol* 34:363–377. <https://doi.org/10.1080/02705060.2019.1614104>
66. Richards D, Lavorel S (2023) Niche theory improves understanding of associations between ecosystem services. *One Earth* 6:811–823. <https://doi.org/10.1016/j.oneear.2023.05.025>
67. Rognes T, Flouri T, Nichols B et al (2016) VSEARCH: A versatile open source tool for metagenomics. *PeerJ* 4:e2584. <https://doi.org/10.7717/peerj.2584>
68. Roşca M, Mihalache G, Stoleru V (2023) Tomato responses to salinity stress: From morphological traits to genetic changes. *Front Plant Sci* 14:1118383. <https://doi.org/10.3389/fpls.2023.1118383>
69. Rosindell J, Hubbell SP, Etienne RS (2011) The unified neutral theory of biodiversity and biogeography at age ten. *Trends Ecol Evol* 26:340–348. <https://doi.org/10.1016/j.tree.2011.03.024>
70. Sala A, Piper F, Hoch G (2010) Physiological mechanisms of drought-induced tree mortality are far from being resolved. *New Phytol* 186:274–281. <https://doi.org/10.1111/j.1469-8137.2009.03167.x>
71. Satpute SK, Banat IM, Dhakephalkar PK et al (2010) Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. *Biotechnol Adv* 28:436–450. <https://doi.org/10.1016/j.biotechadv.2010.02.006>
72. Sheng M, Hu W, Liu C-Q et al (2024) Characteristics and assembly mechanisms of bacterial and fungal communities in soils from Chinese forests across different climatic zones. *CATENA* 245:108306. <https://doi.org/10.1016/j.catena.2024.108306>
73. Singh P, Choudhary KK, Chaudhary N et al (2022) Salt stress resilience in plants mediated through osmolyte accumulation and its crosstalk mechanism with phytohormones. *Front Plant Sci* 13:1006617. <https://doi.org/10.3389/fpls.2022.1006617>
74. Stegen JC, Lin X, Fredrickson JK, Konopka AE (2015) Estimating and mapping ecological processes influencing microbial community assembly. *Front Microbiol* 6. <https://doi.org/10.3389/fmicb.2015.00370>

75. Suzuki YJ, Tsuchiya M, Packer L (1991) Thiocctic acid and dihydrolipoic acid are novel antioxidants which interact with reactive oxygen species. *Free Radical Res Commun* 15:255–263.
<https://doi.org/10.3109/10715769109105221>
76. Traynor AM, Sheridan KJ, Jones GW et al (2019) Involvement of sulfur in the biosynthesis of essential metabolites in pathogenic fungi of animals, particularly aspergillus spp.: Molecular and therapeutic implications. *Front Microbiol* 10:2859. <https://doi.org/10.3389/fmicb.2019.02859>
77. Tyagi VK, Chauhan SK The effect of leaf exudates on the spore germination of phylloplane mycoflora of chilli (*capsicum annum* L.) cultivars
78. Vetoshkina D, Balashov N, Ivanov B et al (2023) Light harvesting regulation: A versatile network of key components operating under various stress conditions in higher plants. *Plant Physiol Biochem* 194:576–588. <https://doi.org/10.1016/j.plaphy.2022.12.002>
79. Vorholt JA (2012) Microbial life in the phyllosphere. *Nat Rev Microbiol* 10:828–840.
<https://doi.org/10.1038/nrmicro2910>
80. Waghmode S, Suryavanshi M, Sharma D, Satpute SK (2020) Planococcus species – an imminent resource to explore biosurfactant and bioactive metabolites for industrial applications. *Front Bioeng Biotechnol* 8:996. <https://doi.org/10.3389/fbioe.2020.00996>
81. Wang B, Tontonoz P (2019) Phospholipid remodeling in physiology and disease. *Annu Rev Physiol* 81:165–188. <https://doi.org/10.1146/annurev-physiol-020518-114444>
82. Wang L, Pan D, Lv X et al (2016) A multilevel investigation to discover why *kandelia candel* thrives in high salinity. *Plant Cell Environ* 39:2486–2497. <https://doi.org/10.1111/pce.12804>
83. Wang X, Fan P, Song H et al (2009) Comparative proteomic analysis of differentially expressed proteins in shoots of *salicornia europaea* under different salinity. *J Proteome Res* 8:3331–3345.
<https://doi.org/10.1021/pr801083a>
84. Wang Y, Branicky R, Noë A, Hekimi S (2018) Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *J Cell Biol* 217:1915–1928.
<https://doi.org/10.1083/jcb.201708007>
85. Weibull J, Ronquist F, Brishammar S (1990) Free amino acid composition of leaf exudates and phloem sap: A comparative study in oats and barley. *Plant Physiol* 92:222–226.
<https://doi.org/10.1104/pp.92.1.222>
86. Xu Q, Vandenkoornhuysen P, Li L et al (2022) Microbial generalists and specialists differently contribute to the community diversity in farmland soils. *J Adv Res* 40:17–27.
<https://doi.org/10.1016/j.jare.2021.12.003>
87. Yang Y, Guo Y (2018) Elucidating the molecular mechanisms mediating plant salt-stress responses. *New Phytol* 217:523–539. <https://doi.org/10.1111/nph.14920>
88. Ye F, Hong Y, Yi X et al (2023) Stochastic processes drive the soil fungal communities in a developing mid-channel bar. *Front Microbiol* 14:1104297.
<https://doi.org/10.3389/fmicb.2023.1104297>

89. Yuan F, Guo J, Shabala S, Wang B (2019) Reproductive physiology of halophytes: Current standing. *Front Plant Sci* 9:1954. <https://doi.org/10.3389/fpls.2018.01954>
90. Zahra N, Al Hinai MS, Hafeez MB et al (2022) Regulation of photosynthesis under salt stress and associated tolerance mechanisms. *Plant Physiol Biochem* 178:55–69. <https://doi.org/10.1016/j.plaphy.2022.03.003>
91. Zhang L, Xiao J, Li J et al (2012) The 2010 spring drought reduced primary productivity in southwestern China. *Environ Res Lett* 7:045706. <https://doi.org/10.1088/1748-9326/7/4/045706>
92. Zhang W, Li J, Struik PC et al (2023) Recovery through proper grazing exclusion promotes the carbon cycle and increases carbon sequestration in semiarid steppe. *Sci Total Environ* 892:164423. <https://doi.org/10.1016/j.scitotenv.2023.164423>
93. Zheng H, Yang T, Bao Y et al (2021) Network analysis and subsequent culturing reveal keystone taxa involved in microbial litter decomposition dynamics. *Soil Biol Biochem* 157:108230. <https://doi.org/10.1016/j.soilbio.2021.108230>
94. Zhou H, Shi H, Yang Y et al (2024) Insights into plant salt stress signaling and tolerance. *J Genet Genomics* 51:16–34. <https://doi.org/10.1016/j.jgg.2023.08.007>
95. Zhou J, Ning D (2017) Stochastic community assembly: Does it matter in microbial ecology? *Microbiol Mol Biol Rev* 81:e00002–17. <https://doi.org/10.1128/MMBR.00002-17>
96. Zieslin N, Ben-Zaken R (1991) Peroxidase, phenylalanine ammonia-lyase and lignification in peduncles of rose flowers. *Plant Physiol Biochem* 29:147–151

Figures

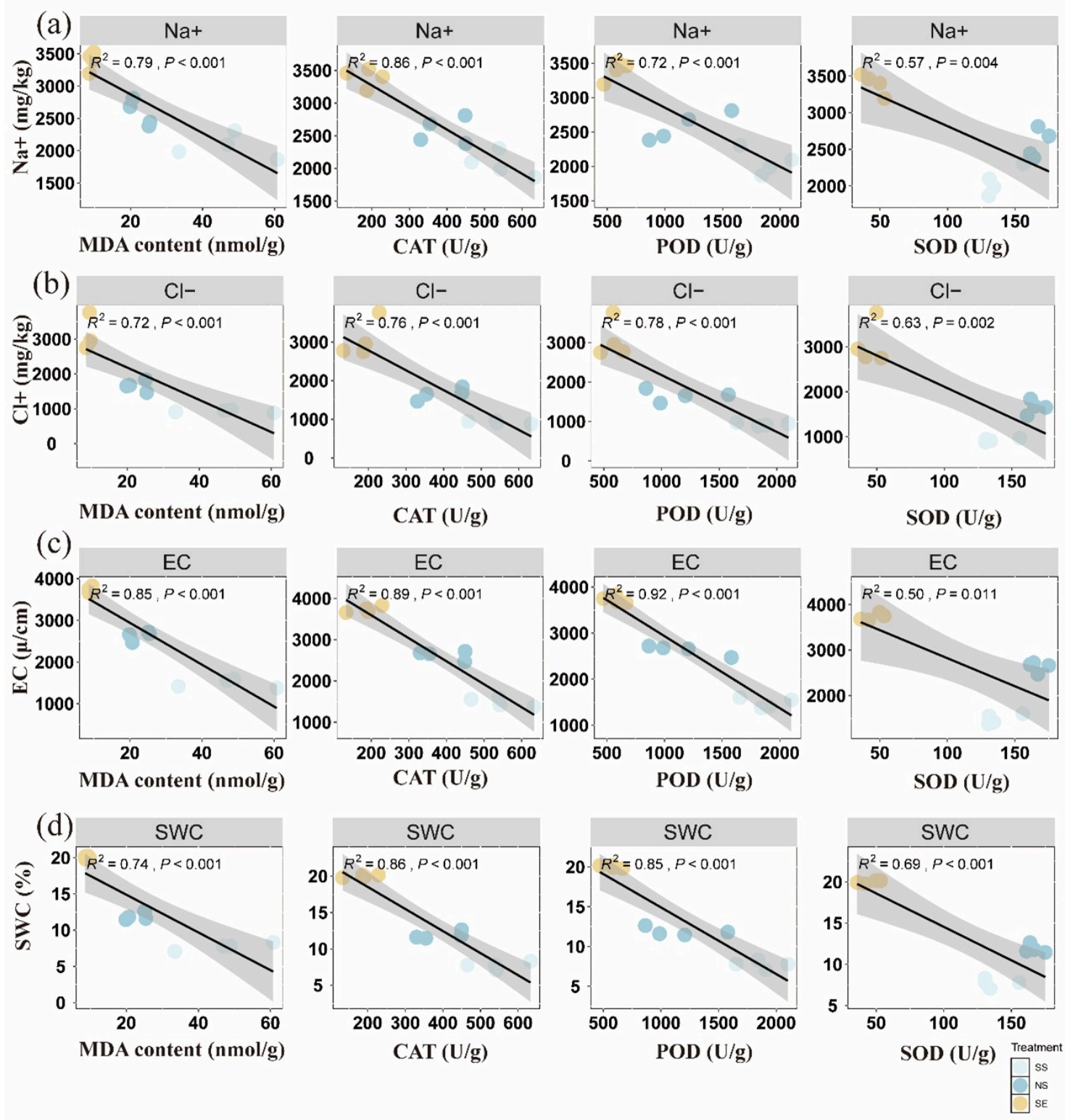


Figure 1

Correlation analysis of soil and plant physiological indicators ; Regression equations of plant MDA, CAT, POD, SOD with soil Na⁺, Cl⁻, EC, and SWC; The coefficient of determination (R^2) and statistical significance (P) are also shown. MDA plant malondialdehyde content, CAT plant catalase, POD plant peroxidase, SOD Plant superoxide dismutase, EC soil electrical conductivity, SWC soil water content.

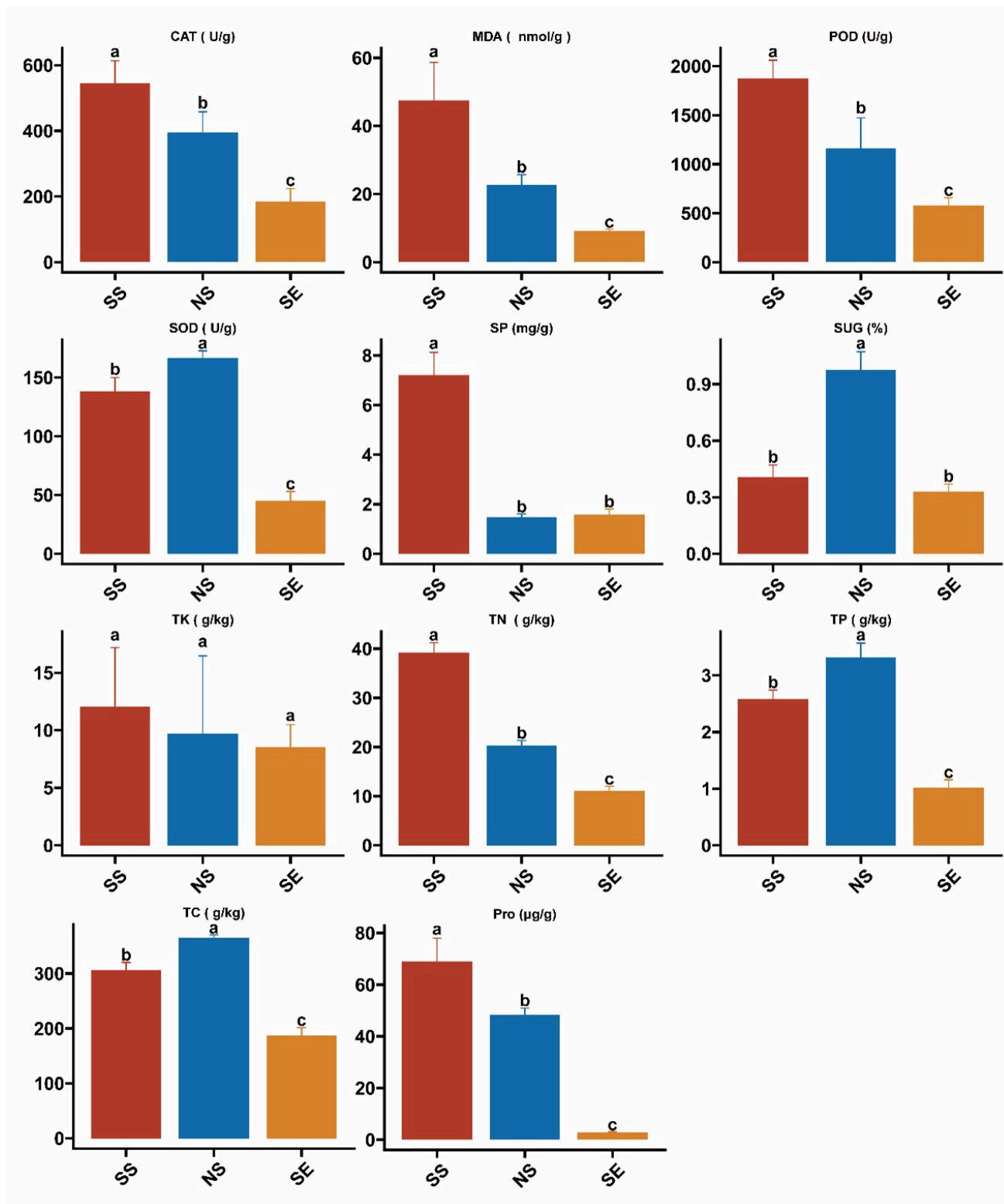


Figure 2

one-way analysis of variance (ANOVA) of physiological and biochemical parameters among three halophyte species; Results are reported as the mean $\pm$ sd (n = 4). Different letters indicate significant differences (P < 0.05), based on LSD tests. MDA plant malondialdehyde content, CAT plant catalase, POD plant peroxidase, SOD Plant superoxide dismutase, TN plant total Nitrogen, TC plant total carbon TP

plant total phosphorus TK plant total potassium SUG plant soluble sugar SP plant soluble protein Pro plant proline.

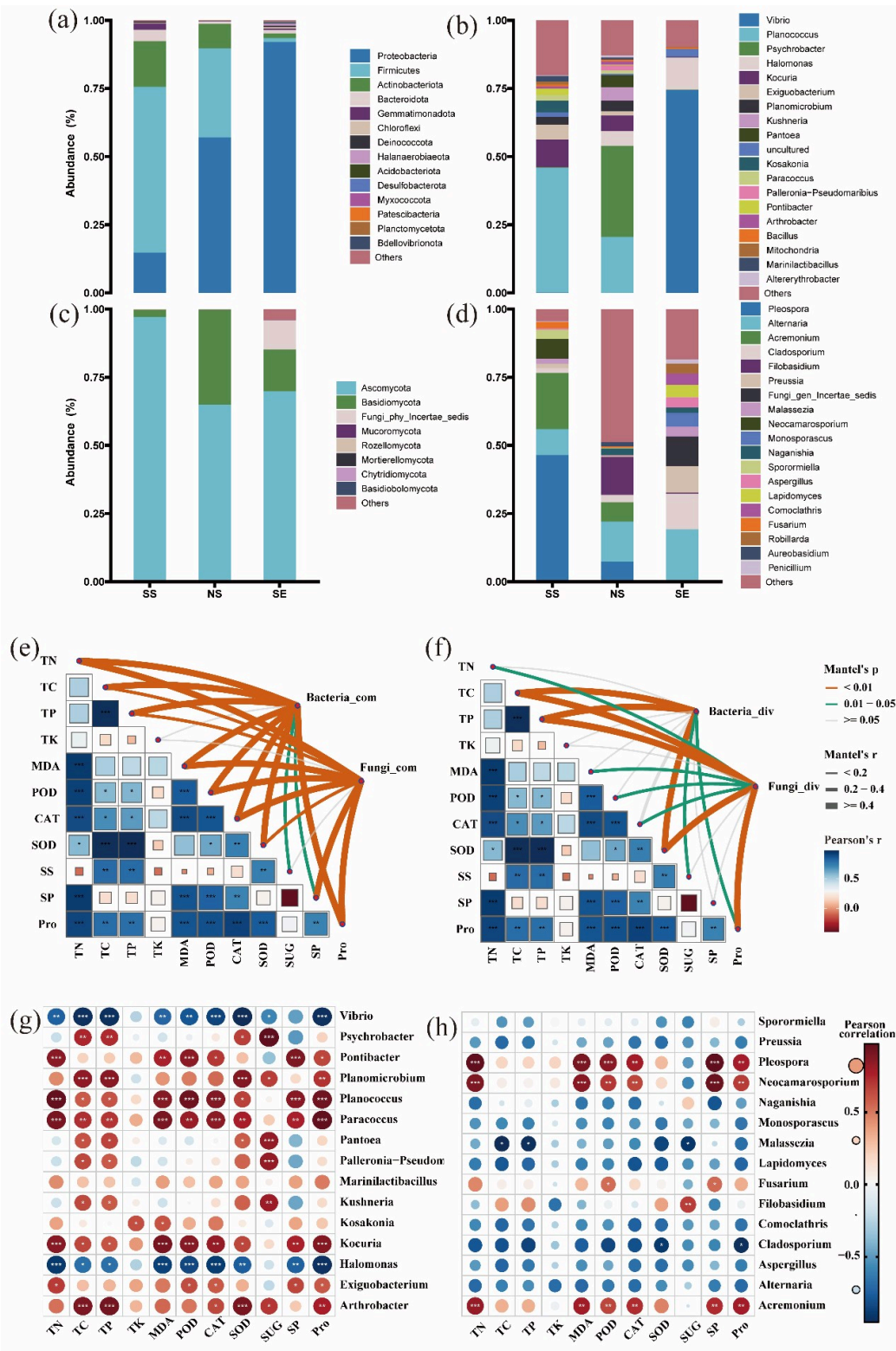


Figure 3

Relative abundance of phyllosphere bacteria (a) and (c) fungi (b) and (d) at the phylum ((a) and (b)) and genus ((c) and (d)) level across all plant species. Phyla with less than 0.1% of total reads were categorized as "others" and only the top 19 most abundant genera are displayed. (e) and (f) represent

Mantel analyses of plant physicochemical characteristics with the composition and α -diversity of phyllosphere bacterial and fungal communities. Bacteria_com: phyllosphere bacterial community composition; Bacteria_div: α -diversity of phyllosphere bacteria; Fungi_com: phyllosphere fungal community composition; Fungi_div: α -diversity of phyllosphere fungi. (g) and (h) represent Spearman correlation analyses between plant physicochemical characteristics and the abundance of the top 15 genera in phyllosphere bacterial (g) and fungal (h) communities. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ For meanings of acronyms, see the description in the fig 2.

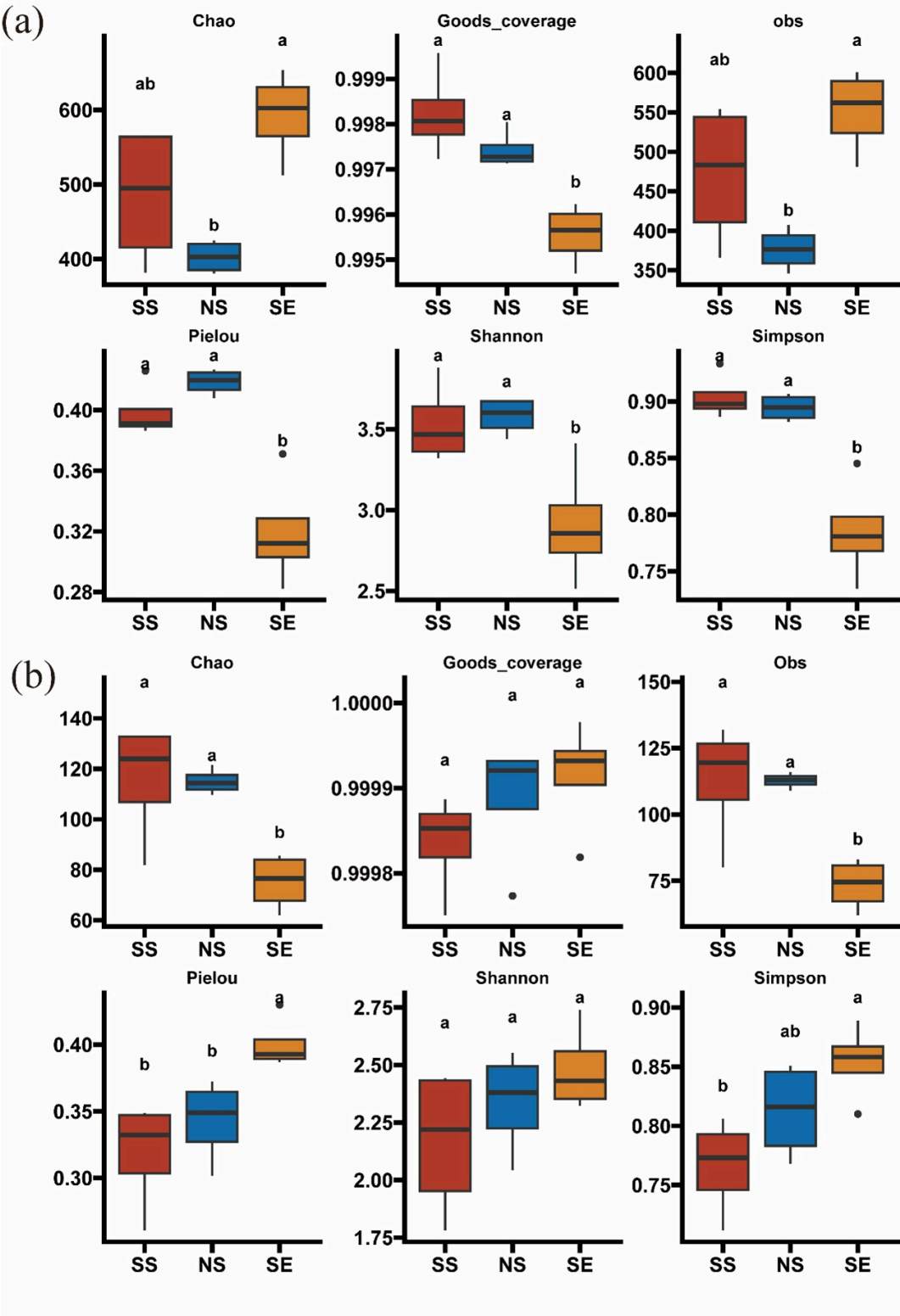


Figure 4

Comparative analysis of alpha diversity in phyllosphere bacteria (a) and fungi (b) among three distinct plant species. Results reported as the mean $\pm$ standard error ($n = 4$). For each parameter, a different letter indicates a significant difference at the 0.05 probability level ($P < 0.05$) based on Tukey's honest test. Alpha diversity indices include Observed_species, Chao, Shannon, Simpson, Pielou, and Good_coverage. SS, NS, and SE mean the phyllosphere bacterial or fungal communities of this halophyte species.

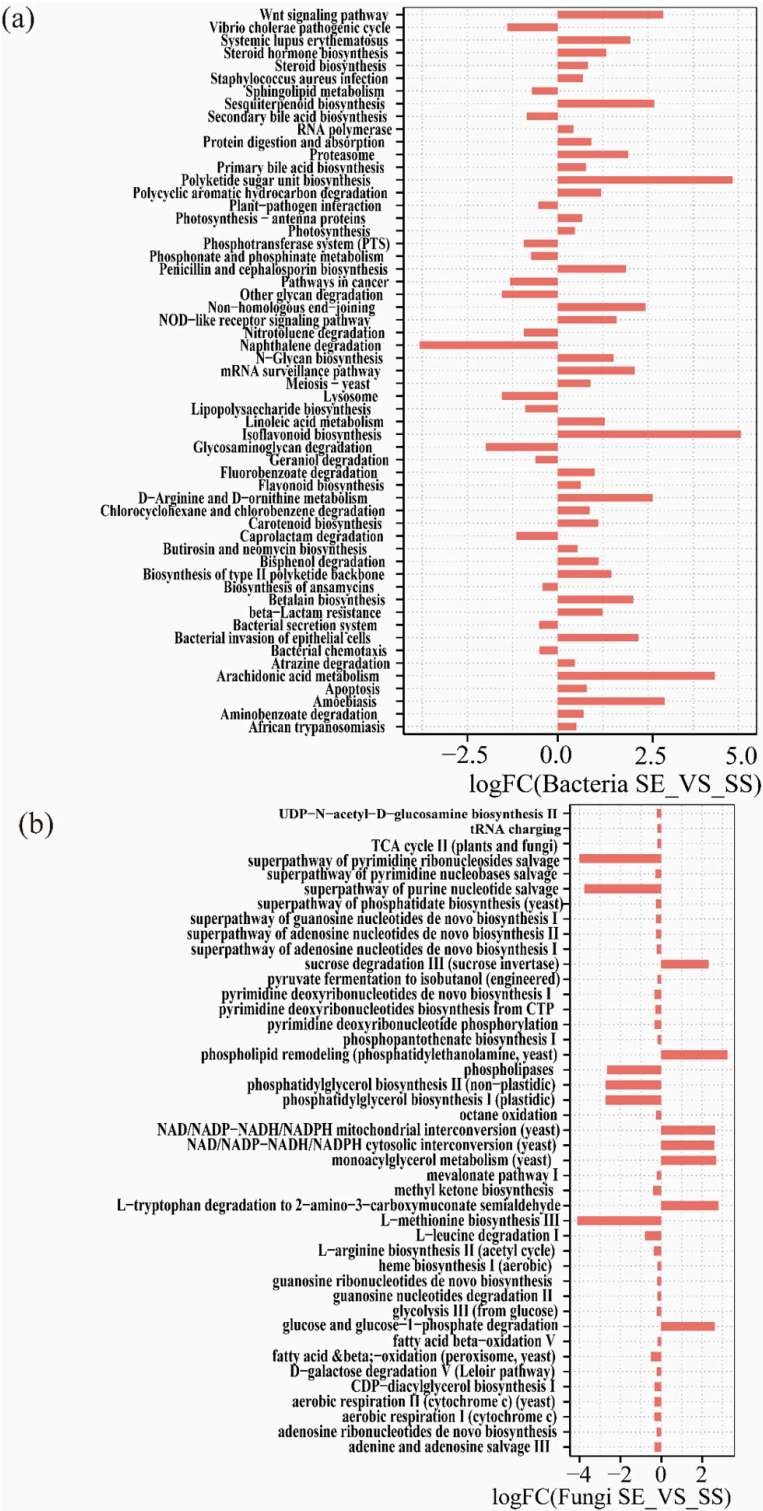


Figure 5

Differential analysis of secondary metabolic pathways in phyllosphere bacterial and fungal communities. (a) and (b) represent the metabolic pathway differential analysis of phyllosphere bacterial and fungal communities, respectively. In the figures, the horizontal axis logFC ($\log_2(\text{fold change})$) indicates differential expression, where positive values represent upregulation in the SS group relative to the SE control group, and negative values indicate downregulation. SS_vs_SE mean the differential analysis of predicted secondary metabolic pathways for phyllosphere bacterial or fungal communities between SS and SE halophyte plants.

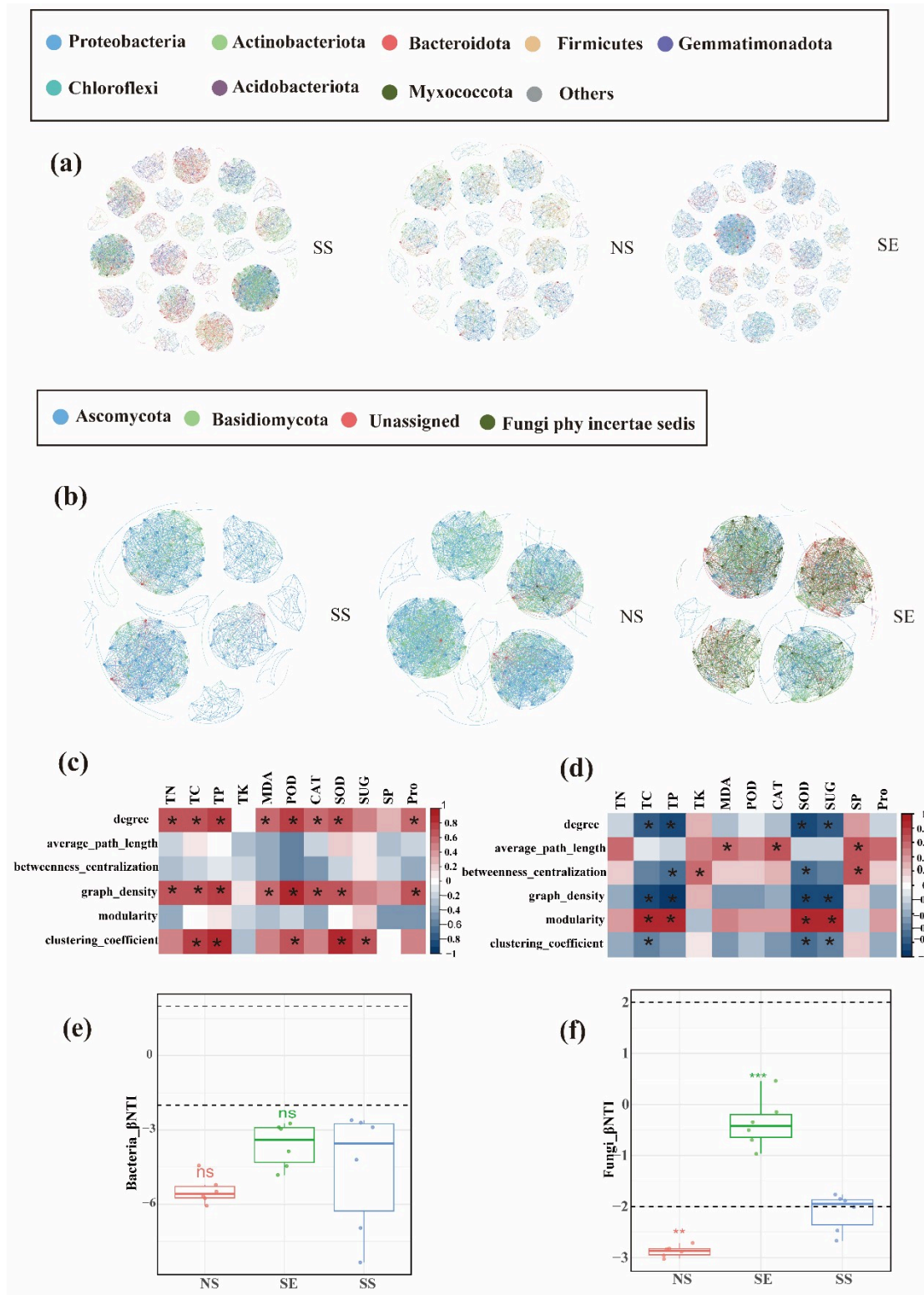


Figure 6

Co-occurrence networks of phyllosphere bacterial (a) and fungal (b) communities of three different halophytic plants, with the figures displaying the co-occurrence networks of phyllosphere bacteria and fungi for SS, NS, and SE plants. (c) and (d) represent Spearman correlation analyses between bacterial network topological indices and fungal network topological indices, respectively, and plant physiological and biochemical characteristics, with significance marked as $*p < 0.05$. The network topological indices

include degree, average_path_length, betweenness_centralization, graph_density, modularity, and clustering_coefficient. For meanings of acronyms, see the description in the materials and methods and result section. (e) β NTI differential analysis of phyllosphere bacterial communities across three different halophytic plants; (f) β NTI differential analysis of phyllosphere fungal communities across three different halophytic plants. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$. SS, NS, and SE mean the β NTI values (left panel) or ecological processes (right panel) of phyllosphere bacterial or fungal communities for this plant species.

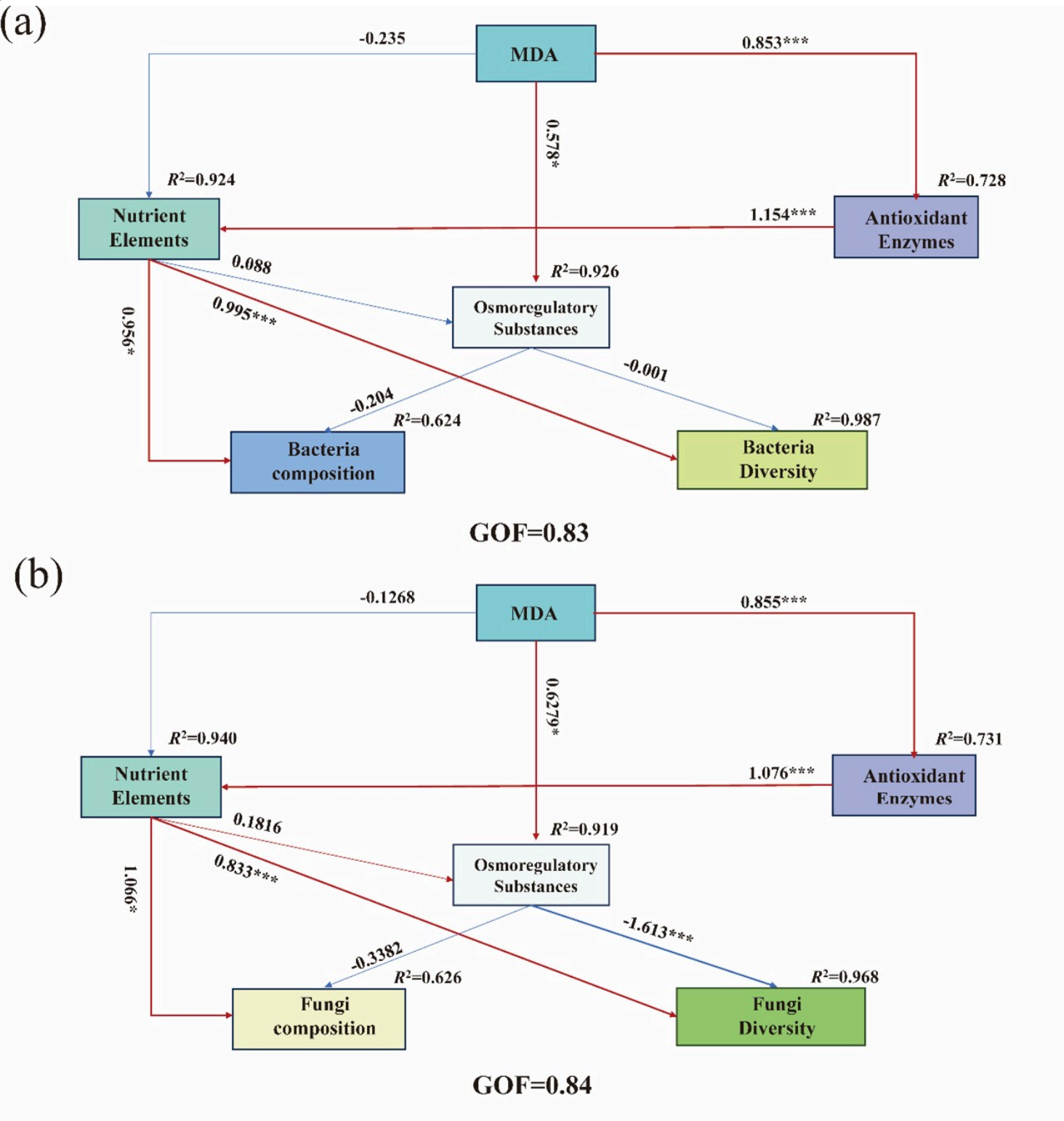


Figure 7

The PLS-PM analysis results reveal the direct and indirect effects of plant physiological and biochemical characteristics on the composition and diversity of phyllosphere bacterial (a) and fungal (b) communities. Large path coefficients are shown as thicker arrows, and red and blue colors represent positive and negative effects, respectively. The numbers adjacent to the arrows are the standardized path coefficients, R^2 represents the proportion of variance explained for each dependent variable in SEM. Nutrient Elements: TC, TN, TP; Antioxidant Enzymes: SOD, POD, CAT; Osmoregulatory Substances: Pro, SP. For meanings of acronyms, see the description in the result section. For meanings of acronyms, see the description in the fig 2.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementmaterials.docx](#)