Halophytes Increase Rhizosphere Microbial Diversity And Network Complexity In Inland Saline Ecosystem

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Research

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Abstract

Background: Salinization is an important global environmental problem influencing sustainable development of terrestrial ecosystems. Salt-tolerant halophytes are often used as a promising approach to remedy the saline soils. Yet, how halophytes affect rhizosphere microbial diversity, and microbes' association and functions in saline ecosystems remains unclear, restricting our ability to assess plant fitness to salt stress and to remediate saline ecosystems. Herein, we examined bacterial and fungal diversities, compositions, and co-occurrence networks in the rhizospheres of six halophytes and bulk soils in a semiarid inland saline ecosystem. We also established the relationship of microbial structure and network complexity to microbial functions.

Results: The microbial communities in rhizospheres were more diverse and complex than those the bulk soils. The connections of taxa in the rhizosphere microbial communities increased with fungi-fungi and bacteria-fungi connections and fungal diversity, but decreased with bacteria-bacteria connections and bacterial diversity. The proportion of the fungi-related central connections were larger in the rhizospheres (13-73%) than the bulk soils (3%). Additionally, fungi accounted for 27-63% of the keystone taxa identified in the microbial co-occurrence networks present in the rhizospheres, whereas the keystone taxa identified in the bulk soils were all bacteria/archaea. Moreover, microbial activity and residues were significantly higher in the halophyte rhizospheres than the bulk soils, and were significantly correlated with microbial composition and co-occurrence network complexity.

Conclusions: These results indicated that halophytes shaped rhizosphere microbiomes and increased microbial diversity and network complexity in inland saline ecosystem, while fungi enhanced rhizosphere microbiota associations. The increased microbial network complexity contributed to the higher microbial functions in rhizosphere soils.

Background

Salinization is one of the most important global environmental problems influencing sustainable development of ecosystems and agriculture. Globally, salinization affects approximately one billion hectares of land in more than 100 countries [1–2], with 36 million hectares in China [3]. World saline soils are predicted to increase with a rate of 1-1.5 million hectares each year, mainly due to sea level rise impacting coastal areas, temperature rise that inevitably leads to increasing evaporation and further salinization, and increased agricultural irrigation [4]. Salinization significantly affects resources availability, soil biodiversity, plant growth, and yield loss in agroecosystems, and such effects occur from site to landscape levels [5]. Additionally, saline soils are very fragile and associated with other forms of land degradation (e.g., erosion). Because saline soils are usually located in flat lands with deep soil layers [1], they can be used as an alternative arable land resource to meet rising requirement of land resources if adequately managed. Adequate use of saline soils has attracted increasing attentions from scientists, communities and policy-makers, and many approaches have been used to remediate saline soils [6].

Soil microbiota plays important roles in sustaining ecosystem functionality [7–10], remediating degraded land [11–14] and increasing the ability of plants to resist and/or tolerate stresses (drought, salt, etc.) [15–19] and diseases [20–22]. Soil microbial communities are incredibly diverse with complicated associations of microbes that support soil functionality. The associations among individual microbes can be assessed by examining the complexity of co-occurrence networks [23, 24]. Both the diversity and association of soil microbiota respond sensitively to environmental changes, and are tightly correlated with the services that the soil microbiota provide, including nutrient cycling, soil and ecosystem functions, restoration of degraded land, mitigation of environmental change, resistance to stress [23, 25–27]. Given that the response of soil microbial communities to environmental changes is closely associated with plant metabolism and root exudates [28–33], these associations occur mainly in the rhizosphere and depend on the plant fitness and health status [20, 34]. Although the diversity and composition of soil microbial community in saline soils were previously reported [35–39], it is still unclear how plant influence the microbial co-occurring network and microbes' associations in saline ecosystem, particularly for the rhizosphere soils.

Halophytes are naturally salt-tolerant plants and are often used as a promising approach to remedy the saline soils. Generally, the nutrients were higher while the salinity was lower in halophytes rhizosphere than bulk soils, providing an eliminated environmental for microbiota, leading to the shifts in microbial communities [40, 41]. Such changes in microbial diversity and community composition will definitely affect the interaction of microbiomes with nutrients cycling and plant growth in saline soils. Yet, how microbiomes associate in halophytes rhizosphere is limited addressed, hindering our ability to understand the interaction between microbiota and halophytes.

It has been demonstrated that microbial diversity was smaller and the associations among microbes were less complex in rhizosphere than bulk soils in non-saline condition [42, 43]. Such variations were mainly attributed to the host plant selection or root filtering [42, 44, 45] by secreting specific chemical compounds that offer a selective advantage to some specific microorganisms [46–48], or bioactive molecules that directly inhibit particular microbial taxa [46, 49], or plant compounds that act as signals to trigger microbes' changes [46, 49, 50]. Whereas, whether such response pattern exists in saline soils was not examined. On the other hand, while many studies have focused on soil-borne bacteria and archaea, soil fungi are also important for providing ecosystem services [15, 27, 51]. Some fungi (e.g., mycorrhizae) have the ability to help plants obtain resources under stressed environments and to tolerate salinity [15, 51]. Fungi also interact with bacteria to maintain the stability of soil microbial network and support ecosystem functionality [27]. However, it is still unclear how fungi interact with bacteria in saline soils. Such knowledge gaps highlight the need to investigate microbial associations in halophyte rhizosphere of saline soils.

Herein, we analyzed bacterial and fungal diversities, compositions, and co-occurrence networks in the rhizospheres of 6 halophytes (i.e., *Phragmites australis*, *Calamagrostis epigeios*, *Kalidium foliatum*, *Suaeda salsa*, *Nitraria sibirica* and *Tamarix chinensis*) and bulk soils in a semiarid inland saline ecosystem. We aimed to examine how halophytes alter rhizosphere microbial diversity and microbe's

association in saline soils. We measured the nutrient contents, soil pH, electric conductivity and salinity, and extracellular enzyme activities to assess nutrient availability and salt stress in rhizosphere. We measured extracellular enzyme activities because they play critical roles in biogeochemical cycles and nutrient availability and are closely related with microbial communities in saline soils [52], and also could be used to indicate microbial activity [53]. We also measured total amino sugars to indicate total microbial residues [54]. We quantified the diversity and composition of soil bacteria/archaea by using 16S rRNA gene amplicon sequencing and those of fungi by using ITS rDNA gene amplicon sequencing, respectively. We conducted indicator species analyses to identified bacterial and fungal taxa that significantly associated with a given habitats. We constructed co-occurrence networks to explore how halophytes influence the associations among microbes. We identified the central interactions and microbial keystone taxa, taxa that have an important influence on community structure and functioning [24, 25, 55, 56], to understand the associations among microbes. We also established the relationship of microbial composition and network complexity to microbial functions. We demonstrated that halophytes increased microbial diversities and co-occurring network complexity, and that fungi enhanced associations among rhizosphere microbiota in this inland saline ecosystem. Such facilitation in microbial community contributed to the higher microbial functions.

Results

Soil properties and microbial diversities

The contents of organic carbon (SOC), total nitrogen (TN), nitrate nitrogen (NO_3^-), and soil moisture and the activities of extracellular enzymes were significantly higher, but electric conductivity (EC) and salinity were significantly lower in the rhizospheres of most halophytes tested than in bulk soils (Fig. S1), indicating that nutritional conditions were improved and the salinity stress were ameliorated in halophyte rhizospheres.

Halophyte rhizospheres had more diverse microbiota than did the bulk soils in this saline environment. Both bacterial and fungal alpha diversities were higher in the rhizospheres of the halophytes than in the bulk soils, except for *S. salsa* rhizosphere that had similar fungal Shannon index to that in the bulk soils (Fig. 1). Additionally, rhizosphere microbial diversity varied significantly by halophyte species. Among the six halophytes, *T. chinensis* had the highest bacterial diversity, while *C. epigeios* and *K. foliatum* had the highest fungal diversities. Moreover, the observed OTUs, Faith's phylogeny diversity, Chao1 and ACE of bacterial communities were negatively correlated with those of fungal communities across the six halophytes rhizospheres (Fig. S2).

The bacterial and fungal communities significantly differed among the rhizosphere and bulk soils and among the six halophytes (Table 1). Constrained analysis of principal coordinate showed that microbial communities clustered by habitat (rhizosphere vs. bulk soils) and halophyte species, which explained ~46% and 49% of the total variations for bacteria and fungi, respectively (Fig. 2). Many OTUs (1563 and 505 for bacteria and fungi, respectively) were shared among the seven habitats (six rhizosphere and one

bulk soils) and among the six halophytes rhizospheres (239 and 62 OTUs for bacteria and fungi, respectively), although less unique OTUs were identified in both bulk and rhizosphere soils (Fig. 2). This indicates that similar microbial species occur in both bulk and rhizosphere soils.

Table 1
The permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis distance among bulk soils and 6 halophyte rhizospheres.

comparison	Bacteria			Fungi		
	F	R ²	P	F	R ²	P
<i>Bulk soils - C. epigeios</i>	11.020	0.408	0.001	9.037	0.361	0.001
<i>Bulk soils - K. foliatum</i>	5.397	0.252	0.001	2.775	0.148	0.006
<i>Bulk soils - N. sibirica</i>	6.179	0.279	0.001	3.816	0.193	0.001
<i>Bulk soils - P. australis</i>	11.301	0.414	0.001	6.242	0.281	0.001
<i>Bulk soils - S. salsa</i>	9.005	0.360	0.001	11.983	0.428	0.001
<i>Bulk soils - T. chinensis</i>	7.920	0.331	0.002	3.200	0.167	0.001
<i>C. epigeios - K. foliatum</i>	13.011	0.448	0.001	22.035	0.579	0.002
<i>C. epigeios - N. sibirica</i>	6.377	0.285	0.001	10.044	0.386	0.001
<i>C. epigeios - P. australis</i>	7.287	0.313	0.001	11.402	0.416	0.001
<i>C. epigeios - S. salsa</i>	9.870	0.382	0.001	39.861	0.714	0.001
<i>C. epigeios - T. chinensis</i>	3.873	0.195	0.001	7.715	0.325	0.001
<i>K. foliatum - N. sibirica</i>	5.724	0.263	0.001	7.782	0.327	0.001
<i>K. foliatum - P. australis</i>	13.951	0.466	0.001	13.321	0.454	0.001
<i>K. foliatum - S. salsa</i>	9.093	0.362	0.001	36.998	0.698	0.001
<i>K. foliatum - T. chinensis</i>	7.920	0.331	0.001	6.628	0.293	0.001
<i>N. sibirica - P. australis</i>	7.794	0.328	0.001	6.697	0.295	0.001
<i>N. sibirica - S. salsa</i>	5.841	0.267	0.001	15.098	0.485	0.001
<i>N. sibirica - T. chinensis</i>	3.014	0.159	0.001	2.312	0.126	0.024
<i>P. australis - S. salsa</i>	9.376	0.369	0.001	20.752	0.565	0.001
<i>P. australis - T. chinensis</i>	4.281	0.211	0.001	4.300	0.212	0.001
<i>S. salsa - T. chinensis</i>	4.006	0.200	0.001	8.670	0.351	0.001

The dissimilarity distance of each rhizosphere microbial community was calculated by comparing with bulk soils (Fig. 3). The *S. salsa* and *K. foliatum* rhizospheres had lower dissimilarity distance than other halophytes. Among the six plants, the dissimilarity distances for bacteria (0.57–0.62) were smaller than that for fungi (0.69–0.85). Furthermore, the rhizospheres had significantly lower variation (distance from centroid) for both bacteria (0.23) and fungi (0.37) than the bulk soils (0.41 and 0.54), whereas rhizosphere bacteria had a smaller variation (0.19–0.27) than fungi (0.15–0.52) among the six halophytes (Fig. 3). The variations in both bacterial and fungal communities were mainly explained by the halophyte species. The explanation of host species was greater, but that of environments (nutrients and EC) was smaller for fungi (0.176 vs 0.017) than bacteria (0.108 vs. 0.030) (Fig. 3). Hence, halophytes had greater effects on fungal than bacterial community assemblies.

Rhizosphere microbial composition

The saline soil environment was comprised of 52 phyla for bacteria/archaea and 13 phyla for fungi. The bacterial/archaeal phyla were primarily composed of Proteobacteria (46%), Gemmatimonadetes (10%), Acidobacteria (7%) and Bacteroidetes (7%) (Fig. 2). The rhizosphere soils had significantly larger relative abundance of Proteobacteria, Acidobacteria, Myxococcota, Chloroflexi and Verrucomicrobia but smaller abundances of Bacteroidetes, Actinobacteria, Crenarchaeota, Halobacterota and Nanoarchaeota, respectively, than in the bulk soils. The fungal phyla were primarily composed of Ascomycota, which accounted for 79% of the sequences (Fig. 2). The rhizospheres had significantly larger relative abundance of Zoopagomycota, Rozellomycota, Chytridiomycota, Basidiomycota and Glomeromycota, but smaller abundances of Mortierellomycota and Apheliidiomycota, respectively, than the bulk soils.

The numbers of bacterial/archaeal indicator OTUs were larger in the bulk soils (216) than the rhizosphere soils (28–148). These OTUs were dominated by the members belonging to Halobacterota, Nanoarchaeota and Proteobacteria. Among the six halophytes, *C. epigeios*, *P. australis* and *K. foliatum* had more indicator OTUs than *T. chinensis*. Additionally, *C. epigeios* and *P. australis* rhizosphere soils shared many bacterial/archaeal indicator OTUs (46), mainly belonging to Proteobacteria and Gemmatimonadota (Fig. 4).

The numbers of fungal indicator OTUs were significantly larger in *C. epigeios* and *P. australis* rhizospheres (96 and 49) than the other plant rhizospheres and the bulk soils (17–36). The *K. foliatum* rhizosphere and the bulk soils shared many fungal indicator OTUs (93), most of which belonged to Ascomycota, while the rhizospheres of the six halophytes only shared a small number of fungal indicator OTUs among them (Fig. 4). Hence, indicator species composition varied by host plants in this saline ecosystem.

The top 50 most abundant OTUs from the rhizosphere and bulk soils were examined in more detail in order to better characterize the effects of halophytes on rhizosphere microbial composition. The relative abundances of the 50 most abundant OTUs together accounted for 29.1% and 60.0% of the bacterial and fungal sequences, respectively. Most of these OTUs (48 and 37 OTUs for bacteria and fungi) were also identified as indicator taxa and were enriched in the rhizosphere soils (Fig. 5). The abundant

bacterial/archaeal OTUs were mainly affiliated within Proteobacteria and Gemmatimonadota. The OTUs belonging to Halomonas and Candidatus_Nitrosop were identified as indicator OTUs in *K. foliatum* and *N. sibirica* rhizospheres. The relative abundance of OTUs related to the genera Limibaculum and Pelagibius were high in both rhizospheres and bulk soils, while those related to the genus Woeseia was significantly larger in the rhizospheres than the bulk soils. Most of the abundant fungal OTUs were related to the class Sordariomycetes and Leotiomyces (Fig. 5). Specifically, Trichoderma (Sordariomycetes) were enriched in the rhizospheres, particularly in those of *S. salsa*, *N. sibirica* and *K. foliatum*. The Trichoderma OTUs accounted for > 70% of the sequences identified in *S. salsa* rhizosphere, and for 2–52% in of the sequences in the other halophyte rhizospheres. In contrast, OTUs related to Blumeria was the major sequences enriched in the *C. epigeios* rhizosphere.

Microbial co-occurrence networks

When combined bacterial/archaeal and fungal OTUs altogether, the rhizospheres of the six halophytes had significantly larger number of nodes (559 to 725 nodes, highest in the *C. epigeios* rhizosphere) and connections between microbes (1037 to 1715 edges, highest in the *K. foliatum* rhizosphere) than the bulk soils (322 nodes and 645 edges) (Fig. 6; Table 2). All networks showed small, scale-free and non-random interaction patterns in the bulk and halophyte rhizosphere soils (Table S1). Although both co-occurrences (positive edge) and mutual exclusion (negative edge) were observed in the bulk and rhizosphere soils, the halophyte rhizospheres had higher proportion of mutual exclusion (3–16%) but lower proportion of co-occurrences (84–97%) in the microbial co-occurrence networks than those of the bulk soils (2% and 98%, respectively) (Table 2). In addition, the numbers of modules, negative cohesion and negative/positive cohesion were greater in the networks of the rhizospheres from the six halophytes (64 to 92, -0.25 to -0.31, and 0.71 to 0.91) than in those from the bulk soils (29, -0.24 and 0.65). Moreover, the values of modularity were also greater in four out of six halophyte rhizospheres (except *N. sibirica* and *K. foliatum*) (Table 2). Hence, halophytes increased complexity of microbial co-occurrence network in rhizosphere compared with bulk soils.

Table 2

The general properties of microbial co-occurring networks in bulk soils and halophyte rhizospheres.

Network properties	Bulk soils	<i>C. epigeios</i>	<i>K. foliatum</i>	<i>N. sibirica</i>	<i>P. australis</i>	<i>S. salsa</i>	<i>T. chinensis</i>
Total nodes	322	725	575	559	688	696	644
Bacterial nodes	290 (90%)	540 (74%)	345 (60%)	456 (82%)	537 (68%)	551 (79%)	557 (86%)
Fungal nodes	32 (10%)	185 (26%)	230 (40%)	103 (18%)	151 (22%)	145 (21%)	87 (14%)
Total edges	645	1332	1715	1177	1200	1037	1165
Positive edges	635 (98%)	1204 (90%)	1671 (97%)	1115 (95%)	1112 (93%)	876 (84%)	1073 (92%)
Negative edges	10 (2%)	128 (10%)	44 (3%)	62 (5%)	88 (7%)	161 (16%)	92 (8%)
Bacteria-Bacteria edges	531 (82%)	615 (46%)	328 (19%)	766 (65%)	557 (46%)	513 (49%)	695 (60%)
Fungi-Fungi edges	36 (6%)	260 (20%)	886 (52%)	124 (11%)	192 (16%)	202 (19%)	143 (12%)
Bacteria-Fungi edges	78 (12%)	457 (34%)	501 (29%)	287 (24%)	451 (38%)	322 (31%)	327 (28%)
Modularity	0.826	0.837	0.707	0.81	0.866	0.830	0.875
No. of Module	29	85	64	74	92	92	67
Negative cohesion	-0.31 ± 0.005	-0.25 ± 0.001	-0.25 ± 0.004	-0.28 ± 0.002	-0.28 ± 0.002	-0.30 ± 0.001	-0.27 ± 0.001
Negative/Positive cohesion	0.65 ± 0.010	0.86 ± 0.011	0.71 ± 0.018	0.75 ± 0.012	0.81 ± 0.004	0.91 ± 0.007	0.78 ± 0.004
The cohesion values are calculated as the sum of the significant abundance-weighted, with larger negative cohesion or Negative/Positive cohesion values indicating microbial networks tend to be more stable.							

Although the numbers of bacteria-bacteria connections were higher in some halophyte rhizosphere networks, the proportions of fungal nodes out of total nodes and those of fungi-fungi and bacteria-fungi connections out of total connections were larger in the halophyte rhizosphere networks than the bulk soil networks (Fig. 6, Table 2). Moreover, the total number of connections increased with the numbers of fungi-fungi and bacteria-fungi connections ($p < 0.05$ by correlation analysis) but not with bacteria-bacteria connections (Fig. S3). These results indicate that fungi might play more important roles than bacteria in maintaining rhizosphere microbial network complexity.

We generated the taxonomic profile to identify the central interactions (edge betweenness centrality) [57]. Among the 30 central interactions, 29 were bacteria-bacteria co-occurrences in the bulk soils, mainly

between Proteobacteria, Gemmatimonadetes and Acidobacteria. For the rhizospheres, the number of central interactions originated from bacteria-fungi or fungi-fungi co-occurrences ranged from four in *T. chinensis* to 22 in *S. salsa* (Fig. S4). Most of these interactions were occurred between Ascomycota, Basidiomycota and Rozellomycota.

We identified five and 6–15 keystone taxa in the bulk and rhizosphere soils, respectively. The five keystone taxa identified in the bulk soils belonged to the bacterial phyla Acidobacteria, Actinobacteria, and Gemmatimonadetes, and archaeal class Nanosalinia (Nanosalinaceae family). For the rhizospheres, 27–63% of the keystone taxa were fungi, mainly those belonging to Ascomycota (mainly Hypocreales) (Table S2). These results suggest that fungi enhanced associations among rhizosphere microbiota in this inland saline ecosystem.

Linkages of soil microbial diversity and co-occurrence network complexity to microbial function

We calculated the average of the z-score of each extracellular enzyme activity to assess soil microbial activity [53, 58]. We measured total amino sugar to indicate microbial residue in soils [54]. The microbial activity and residues were significantly higher in rhizospheres soils than the bulk soils, with highest values in *S. salsa* (Fig. 7). The results from FAPROTAX and FUNGuild prediction showed that rhizospheres soils had significantly higher relative abundance of nutrients cycling functional groups and arbuscular mycorrhizal fungi than the bulk soils (Fig. S5). Moreover, both microbial activity and residues were significantly correlated with soil nutrients, microbial composition and co-occurring network complexity (Fig. S6). The results from structure equation model showed that microbial network complexity was more important than microbial composition in affecting microbial activity and residues. Soil microbial network complexity has significantly direct effects, while microbial composition has significant indirect effects through changing microbial network complexity (Fig. 7). Additionally, soil nutrients not only directly impact microbial activity and residues, but also indirectly exert their influences through changing microbial composition, while soil EC exerted its effects only through changing microbial composition (Fig. 7). Therefore, the increased microbial functions in the halophyte rhizospheres were mainly due to the increased co-occurrence network complexity.

Discussion

Halophyte increases microbial diversity in saline soils

The higher microbial diversity in the halophyte rhizospheres than the bulk soils was primarily ascribed to the higher available resources (nutrients) and less environmental stress. In this study, the contents of organic carbon, total nitrogen, nitrate nitrogen and soil moisture, and activities of various enzymes were significantly higher in rhizospheres than the bulk soils, whereas the EC and salinity were lower in four out of six rhizospheres than the bulk soils. The larger organic carbon, nutrient and moisture contents and lower salt stress in the rhizosphere might have promoted the microbial metabolisms and thereby

increasing the overall diversity. This explanation was supported by our observation that bacterial diversity increased with organic carbon, nutrient and moisture contents but decreased with EC and salinity (Fig. S7).

Our result was not in agree with previous understanding that rhizosphere usually has lower microbial diversity than bulk soils because of host plants select [42, 44, 45]. The host plant selection often decreases rhizosphere microbial diversity compared with bulk soils in managed ecosystem (e.g., agricultural ecosystem) or unstressed conditions because of root filtering [42, 44]. Such decreased microbial diversity in rhizosphere was observed in wheat [42] and wheat-maize/barley rotation systems [59]. However, Schmidt et al. [45] found that the bacterial diversity in maize rhizosphere was larger than bulk soils in long-term conventional agroecosystem, but the opposite was found in organically managed agroecosystem. Therefore, the effect of host selection on rhizosphere microbiome largely depends on plant type and environments [60, 61]. It has been suggested that, under salt or desert conditions, host plant selection increases rhizosphere microbial diversity because of the alleviated environmental stresses in rhizosphere [40, 41, 57]. For example, Marasco et al [57] reported higher fungal diversity (but similar bacterial diversity) in the desert speargrass rhizosphere than the bulk sand. Some recent studies also showed increased microbial diversity in the rhizosphere in coastal saline soils [40, 41].

Halophyte increases complexity of rhizosphere microbial co-occurrence network in saline soils

In this study, we observed that microbial co-occurrence networks were more complex in the halophyte rhizospheres than the bulk soils, probably because halophyte rhizosphere has greater potential for niche-sharing and interactions between microbes [62]. Similar to this study, more complex bacterial networks were identified in the rhizosphere than bulk soils from greenhouse microcosms [62] and field sand dune [63]. According to the Stress Gradient Theory, competitive (negative) interactions increase but facilitative (positive) interactions decrease with decreased stress [64, 65]. In this study, salt stress (the EC and salinity) were significantly lower (except for *S. salsa*), but the availability of resources (organic carbon and nutrients) were significantly higher in the rhizosphere than the bulk soils, while the competitive taxa, fast-growing species (e.g., Rhodobacteraceae, Nitrosomonadaceae, Cyclobacteriaceae, Desulfuromonadaceae, Nitrincolaceae, TRA3-20 and Blrii41 families, S0134 and AKAU4049 classes), was significantly higher, and replaced slow-growing, halophilic species (e.g., Balneolaceae, Haloferacaceae, Halomicrobiaceae and Salinisphaeraceae families and Woeseearchaeales order) (Fig. S8). Additionally, EC and salinity were negatively correlated with competitive taxa, but positively correlated with facilitative taxa (Fig. S8). The EC was also negatively correlated with the ratio of negative to positive connections and the ratio of negative to positive cohesions (Fig. S9). Hence, such decreased environmental stresses contributed to the increased complexity of microbial co-occurring networks in halophyte rhizospheres than the bulk soils.

The complexed microbial co-occurring network in rhizospheres was also due to the increased interaction of fungi with bacteria. The host plant usually selected some fungi in harsh environment because of the

high ability of these fungi to tolerate salt and desiccation [66]. Generally, coexisting fungal and bacterial communities are more stable than fungi- or bacteria-dominated communities [67], particularly under stressed conditions [25, 41, 68]. In this study, the proportion of bacteria-fungi and bacteria-bacteria interactions out of total interactions was larger and smaller, respectively, in the microbial co-occurrence networks of the halophyte rhizospheres, and the negative/positive cohesions increased with the proportion of bacteria-fungi connections. Therefore, increases in the functional and taxonomic diversities increase the complexity of halophyte rhizosphere microbial communities. While we quantified the co-occurrence network of microbial communities by using statistical tools, empirical studies should be conducted to further support our findings.

Fungi enhanced associations among microbiota in halophytes rhizosphere

Our results demonstrated that fungi enhanced associations among rhizosphere microbiota in this inland saline ecosystem. The keystone taxa identified in the bulk soils were all bacteria and archaea, while fungi composed 27–63% of the keystone taxa in halophyte rhizospheres. We identified Ascomycota as the keystone taxa in the rhizospheres, and most central interactions were originated from the co-occurrence with them, indicating that Ascomycota played a key role in the interaction with other taxa in the rhizosphere. This is probably because Ascomycota has a strong adaptability to stresses and ability to utilize resources and thus niche preferences [69]. Among Ascomycota, five *Trichoderma* were identified as keystone taxa in four (*C. epigeios*, *K. foliatum*, *N. sibirica* and *P. australis*) out of six rhizospheres. In addition, 15 out of 50 top most abundant OTUs identified in the rhizosphere soils were related to *Trichoderma*, and 14 of which were identified as indicator OTUs. *Trichoderma* often inhibit the growth of plant pathogens and root-knot nematodes [70–72], and thus to promote plant growth. Additionally, we also identified Basidiomycota as the keystone taxa in the rhizosphere communities. Most members of Basidiomycota tend to live in relatively harsh environments, and their relative abundance in soils is related to their ability to decompose lignocellulose, and to the availability of resources [73, 74]. The increased nutrients in the rhizosphere might have resulted in the higher relative abundance of Basidiomycota.

We also observed that the total connection of rhizosphere microbial network was positively correlated with fungal diversity but negatively correlated with bacterial diversity (Fig. S10), further indicating that the complexity of halophyte rhizosphere microbial network was enhanced by fungi in this saline ecosystem. These results were consistent with recent evidences that fungi are more important than bacteria for distinguishing among root-zone microbiomes of halophytes [41]. However, whether fungi are more important than bacteria for sustaining rhizosphere microbial network complexity under managed conditions or unstressed conditions merits further examination.

Conclusions And Implications For Sustainable Development Of Saline Ecosystem

Given the continuous rising of saline soils as a result of intensified climate change and human activities, and the increasing dependence of human on saline ecosystems, it is timely and of the highest importance to comprehensively analyze the interactions between halophytes and soil microbiota for the remediation of saline soils. Here we demonstrated that halophytes shaped rhizosphere microbiomes and increased microbial diversities in inland saline ecosystem due to decreased salt stress. Combined with previous understandings, our results highlighted that host plant selection might have divergent effect on rhizosphere microbial diversity, with decreased diversity under management or natural conditions but increased diversity under some stressed conditions (i.e., drought and salt). Therefore, the improvement of microbial communities and functionality might be an important mechanism for the remediation of saline soils with halophytes, and also for any other practices that has ability to decrease salt stress. We further showed halophytes have greater effects on fungal than bacterial communities and that fungi enhanced microbiota associations, which provides new insight that halophytes sustain rhizosphere microbial network complexity by enhancing association of fungi with bacteria. These findings emphasize the need to develop technologies to increase rhizosphere microbial diversity and include important fungal taxa (e.g., *Trichoderma*) to facilitate sustainable development of saline ecosystem.

Materials And Methods

Study site and soil sampling

This study was conducted in an inland semiarid saline ecosystem around the largest salt lake, Huamachi lake, in Shaanxi, China. The Huamachi lake is located in Dingbian of Shaanxi province (37°68'N, 107°53'E). The study site has a temperate continental semi-arid monsoon climate, with a mean annual temperature of 7.9°C, mean annual precipitation of 312 mm, and mean evaporation demand of 2523 mm. Precipitation primarily occurs from July to September. The soil in the dry lakebed and the surrounding region is a saline soil, with a texture of sandy loam, an EC of 6.72–12.08 mS cm⁻¹ and a salinity of 2.79–5.79%. The covering vegetation is dominated by *Phragmites australis*, *Calamagrostis epigeios*, *Kalidium foliatum*, *Suaeda salsa*, *Nitraria sibirica* and *Tamarix chinensis*, with a canopy cover of > 60%.

In early July 2020, we established nine adjacent plots (10 m × 10 m) for the sampling of rhizosphere and bulk soils in the surrounding region of Huamachi lake. In each plot, we randomly selected six plants for each of the six halophytes (*P. australis*, *C. epigeios*, *K. foliatum*, *S. salsa*, *N. sibirica* and *T. chinensis*) for the field sampling of rhizosphere samples. The roots of each plant from 0–15 cm depth were carefully taken out, lightly shaken to remove loosely bound soils, then the soils that tightly attached to the roots (rhizosphere soils) were brushed off to compose the rhizosphere sample of each halophyte for the plot. We also collected 6 soil samples at 0–15 cm depth from bare land without any plant using a sterilized soil auger to combine as a composite bulk soil sample for the plot. Totally, we have 63 samples (six rhizosphere samples and one bulk soil sample for each of the nine plots). All the moisture rhizosphere and bulk soil samples were stored in ice box, transported to local laboratory and then divided into three

sub-samples: one air-dried for the measurement of soil physiochemical properties, one stored at -20°C for the measurement of extracellular enzyme activities, and one stored at -80°C for high-throughput sequencing.

Laboratory analysis for soil properties and extracellular enzyme activities

A small fraction of moisture soils was dried at 105°C to measure soil moisture content. Another fraction of moisture soil was used for the measurement of available nitrogen (NH_4^+ and NO_3^-). Air-dried soils were ground to pass through a 2-mm sieve for the measurement of available phosphorous (OP), soil pH, EC and salinity. A small fraction of < 2-mm samples were ground to pass through a 0.25-mm sieve for the measurement of SOC and TN. Soil metrics were measured using standard methods as described by Qiu et al. [75]. The SOC and TN were measured using the Walkley-Black and Kjeldahl method. Soil NH_4^+ and NO_3^- were measured using a continuous flow analyzer (AutoAnalyzer-AA3, Seal Analytical, Norderstedt, Germany) after extraction with 2 mol L^{-1} KCl. Soil OP was determined by the Olsen method. Soil pH and EC were measured in a soil: water (1:5) extract with a pH meter (Mettler Toledo, Germany). Soil salinity was measured by extracting soil samples with deionized water in a soil to solution ratio of 1:5 and drying the extracts to constant weight.

The activities of extracellular enzymes involved in carbon, nitrogen and phosphorous cycles were measured using Microplate-scale fluorometric method [76]. The carbon-cycling related enzymes are β -1,4-glucosidase (BG), 1,4- β -D-cellobiohydrolase (CBH) and β -xylosidase (BX), the nitrogen-cycling related enzyme is β -1,4-N-acetyl-glucosaminidase (NAG), the phosphorous-cycling related enzyme is acid phosphatase (AP). Briefly, 3 g moisture soil sample was combined with 125 mL Tris buffer (50 mM Tris) in a crystal dish and homogenized to a uniform suspension using a blender for approximately 1 min. The pH value of the buffer was adjusted to be similar to that of soil sample with HCl or NaOH. The soil suspension (150 μL) and test enzyme substrate (50 μL) were added to the 96 microwell enzyme plate. We set up the blank control (150 μL soil suspension + 50 μL buffer), negative control (150 μL buffer + 50 μL enzyme substrate) and quench standard (150 μL buffer + 50 μL standard solution) for each sample. The microtiter plates were incubated at 25°C in the dark, AP for 0.5 h, BG for 2 h, CBH, BX and NAG for 4 h. The fluorescence signals for the enzymes were obtained at 365 nm excitation and 450 nm emission (BioTek, Synergy TM LX, USA). The average of the z-score of each extracellular enzyme activity was calculated to indicate soil microbial activity [53, 58].

The content of total amino sugar (TAS) was measured to indicate microbial residue in soils [54]. Briefly, the soil samples were hydrolyzed with 10 mL of 6 M HCl at 105°C for 8 h containing myo-inositol. The hydrolysate was filtered after adding myo-inositol, adjusted to pH 6.6–6.8 and centrifuged. The supernatant was freeze-dried and the amino sugars in residues were extracted with methanol. The recovered amino sugars were first cyanated with hydroxylamine hydrochloride and 4-dimethylaminopyridine, and then acetylated with acetic anhydride to form aldononitrile derivatives. The AS derivatives were analyzed via a Beifen 3420A gas chromatograph (Beijing Beifen- Ruili Analytical

Instrument. Co. Ltd., Beijing, China) equipped with a DB-5 column (50 m × 0.25 mm × 0.25 μm) and a flame ionization detector. The detailed temperature program was set by Zhang and Amelung [54]. Methylglucamine was added as the recovery standard before derivation. Each individual amino sugar was quantified, and then summed to calculate total amino sugars content.

DNA extraction, PCR and sequencing

Soil total genomic DNA of each sample was extracted using the MP FastDNA spin kit for soil according to the manufacturer instructions (MP Biomedicals, Solon, OH, USA). The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the targeting primer pairs 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The ITS1 rDNA gene was amplified by PCR using the targeting primer pairs ITS5-1737F (GGAAGTAAAAGTCGTAACAAGG) and ITS2-2043R (GCTGCGTTCTTCATCGATGC). The PCRs were carried out with 30 μL reactions mixture containing 6 μM primers, 10 ng template DNA, 15μL Phusion® High-Fidelity PCR Master Mix 2× (New England BioLabs) and 2μL H₂O. The samples were amplified by the following conditions: initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, extension at 72°C for 30 s and final extension at 72°C for 5 min. All samples were amplified in triplicate and pooled together in equal amounts, and then subjected to electrophoresis detection in a 2% (w/v) agarose gel. Then PCR products were purified with GeneJET Gel Extraction Kit (Thermo Scientific). The sequencing libraries with sample-specific index tags were constructed using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA). The library quality was assessed by the Qubit® 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Sequencing was performed on the Illumina NovaSeq platform to generate 250-bp paired-end reads at the Novogene Company, Beijing, China.

Bioinformatics analysis

Raw paired-end reads were assigned to samples based on the unique barcode and truncated by cutting off the barcode and primer sequence, and then merged using FLASH (V1.2.7) with default parameters [77]. Then, sequences with more than three consecutive low-quality base calls (Phred quality score smaller than 20) were truncated, and only reads with > 75% consecutive high-quality base calls and no ambiguous characters were retained for further analyses based on QIIME v1.9.1 [78]. Chimeric sequences were removed using a combination of de novo and reference-based chimera checking based on UCHIME algorithm [79]. Filtered sequences were clustered into operational taxonomic units (OTUs) using Uparse v7.0.1001 [80] based on 97% similarity threshold. For 16S sequencing, the Mothur algorithm [81] and SILVA_v138 database (<http://www.arb-silva.de/>) were used for taxonomy assignment. For the ITS sequencing, representative sequence annotations were carried out by BLAST algorithm in the *assign_taxonomy.py* script based on UNITE v8.2 database (<https://unite.ut.ee/>). Multiple sequence alignments were conducted using the MUSCLE v 3.8.31 [82]. We excluded chloroplast, mitochondria and rare OTUs (relative abundance < 0.005%) for bacteria and archaea, and filtered nonfungal and rare OTUs (relative abundance < 0.005%) for fungal dataset, respectively [83]. As a result, a total of 1,562,706 high-quality sequences (range 15,751 – 34,824; media 24,388 sequences per sample) and 3,638,843 high-quality sequences (range 36,914 – 67,776; media 58,007 sequences per sample) were remained.

Sequencing depths were rarefied to 15,751 and 36,914 sequences for bacteria/archaea and fungi, respectively. We also verified that the removal of rare OTUs did not affect our results based on the correlation analysis for alpha diversity (R^2 : 0.76–0.99, $p < 0.0001$; Fig. S11), Mantel tests (r : 0.9974–0.9995, $p < 0.0001$) and Procrustes analyses (M^2 : 0.0002–0.0027, $p < 0.0001$) in vegan package (Fig. S12) [84]. In addition, we constructed OTUs rarefaction curves to evaluate richness saturation using ggrare function in ranacapa package (Fig. S13) [85]. We used the Functional Annotation of Prokaryotic Taxa (FAPROTAX) [86], and FUNGuild [87] to extrapolate functional groups of bacteria and fungi in the soil, by using FAPROTAX_v1.2.1 and FUNGuild_v1.1 python script with default settings. We used only taxa that were rated as either “probable” or “highly probable” according to the confidence ranking for the guild assignment in order to avoid over-interpreting the fungal functional groups. The sequencing data were deposited into the NCBI (<https://www.ncbi.nlm.nih.gov/>) with bioprojects accession number PRJNA738372.

Statistical analysis

All statistical analyses were conducted in the R environment (v3.6.3, <http://www.r-project.org/>). To assess the microbial alpha diversity, community richness (Observed OTUs, Chao1 and ACE) and diversity (Shannon and Faith’s phylogeny diversity) indices of bacteria and fungi were calculated based on the rarefied data. Differences in soil properties, extracellular enzyme activities, microbial alpha diversity and dominant phyla among halophyte rhizospheres and bulk soils (habitat) were determined by linear mixed effect models, including habitat as a fixed effect, and plot as a random effect using nlme package [88]. The assumptions of homoscedasticity and normality of residuals were inspected by Shapiro-Wilk test and residual *versus* fitted plots. Multiple comparisons were assessed by Tukey’s Honestly Significant Difference (Tukey’s HSD) in multcomp package [89] or Dunn’s test in dunn.test package [90] when the assumptions were not met. Venn diagrams were conducted by using the venn function in venn package [91] to show the numbers of shared and unique observed OTUs among halophyte rhizospheres and bulk soils.

Constrained analysis of principal coordinates (CAP) based on Bary-Curtis distance between each pair of samples were performed to test the host effects of halophytes on soil bacterial and fungal community structures. Statistical significance of the CAP was measured by the permutest function with 999 permutations in the vegan package [84]. To evaluate the significance of the host plant effects on soil bacterial and fungal community composition and heterogeneity, we performed permutational multivariate analysis of variance (PERMANOVA) and permutational analysis of multivariate dispersions (PERMDISP) on Bray-Curtis distance by using adonis and betadisper functions in the vegan package [84], respectively. The separations of mean values (distance to centroid) among habitats were evaluated by one-way ANOVA with Tukey’s HSD tests. All ordination analyses were performed using the phyloseq package [92]. We also calculated the pairwise dissimilarity distances between each halophyte rhizosphere and bulk soil bacterial or fungal community based on Bray-Curtis distance metric to explore the differences in bacterial or fungal community structures across halophyte rhizospheres and bulk soils.

Indicator species analyses were performed to identify bacterial and fungal taxa significantly associated with a given habitats on OTUs level using the `multipatt` function in the `indicspecies` package [93]. The analyses were permuted 999 times, and the significance of association between the taxa and the habitat was evaluated at a false discovery rate (FDR) corrected p value of < 0.05 [94]. Bipartite networks were implemented to visualize the identified indicator taxa using Gephi software (<https://gephi.org>). To better characterize the host effects of halophytes on rhizosphere microbial composition, we retrieved the dominant taxa (top 50 abundant for bacterial and fungal OTUs, respectively) and identified whether they belong to the indicator taxa. Phylogenetic distributions and relative abundance of the dominant taxa were constructed using `ggtree` function in `ggtree` package [95] and using `ggplot` function in `ggplot2` package [96].

The microbial co-occurrence networks were constructed based on 16S and ITS gene sequencing data to explore the bacterial-fungal co-occurrence relationships. Microbial co-occurrence networks were constructed using Molecular Ecological Network Analyses Pipeline (MENAP, <http://ieg4.rccc.ou.edu/MENA/>), using random matrix theory (RMT) to determine the correlation cut-off threshold automatically [97, 98]. The RMT theory was applied to define the transition point of nearest-neighbor spacing distribution of eigenvalues from Gaussian orthogonal ensemble (random) to Poisson (non-random) distribution, and this transition point was then used as the correlation cut-off for network construction [97, 98]. Briefly, only OTUs detected in at least eight replicates (out of nine replicates for each habitat) were used for each network construction. OTUs with missing values remained blank, and the pairwise correlation of the two pairs of OTUs were calculated based on relative abundance data and Pearson correlation coefficients. Then, the optimal threshold (0.92–0.94) recommended by RMT theory was used to filter the adjacency matrix (only correlations above the optimal threshold were used for defining the adjacency matrix) and construct co-occurrence networks.

Network topological indices were calculated in the MENAP interface, including node, edge, R^2 of power-law distribution, average path distance (geodesic distance), harmonic geodesic distance, modularity and numbers of module. The smaller average path distance and harmonic geodesic distance indicate that the co-occurring network is close [97]. In addition, we calculated the edge betweenness centrality by `edge.betweenness` function and counted numbers of bacterial node, fungal node, positive edge, negative edge, bacteria-bacteria edge, fungi-fungi edge, and bacteria-fungi edge in each network in `igraph` package [99]. To validate the nonrandom co-occurrence patterns, 100 random networks were constructed for each empirical network in an equal size (constraining numbers of node and edge), following the Maslov-Sneppen procedure [100]. The mean values and standard deviations of topology properties from 100 random network were calculated and compared with the corresponding empirical network. Topological roles of nodes in networks were determined by within-module connectivity (Z_i) and among-module connectivity (P_i) and classified into four categories: peripheral nodes ($Z_i < 2.5$, $P_i < 0.62$), connectors ($Z_i < 2.5$, $P_i > 0.62$), module hubs ($Z_i > 2.5$, $P_i < 0.62$), and network hubs ($Z_i > 2.5$, $P_i > 0.62$). Module hubs are highly connected to many nodes in their own modules, while network hubs act as both module hubs and

connectors. Network hubs, module hubs and connectors are regarded as keystone nodes [97, 98, 101]. All networks were visualized in R package igraph [99].

Cohesion, as a measure of the connectivity of microbial communities, was the sum of abundance-weighted and null model corrected index based on pairwise correlations across taxa [102, 103]. Cohesion can be used to identify associations among taxa caused by the interaction of positive and negative species and/or by the similarities and differences in the niches of microbial taxa [65, 102]. In order to evaluate the differences in cohesion values among different networks and to be consistent with the network analysis, we focused on those taxa that were present in the network [65, 104], and then calculating two cohesion values (positive and negative) for each sample:

$$Cohesion = \sum_{i=1}^n abundance_i \times connectedness_i$$

where n is the total number of taxa in a given community, and the construction process is mainly divided into two steps, including the calculation of the positive and negative connectedness matrix of all taxa in each sample and the final calculation of cohesion. For example, we first subtracted the pairwise associations matrix of all taxa within a given community (observed correlations) from the pairwise associations matrix of the null model iterations (expected correlations) to obtain pairwise correlations matrix corrected by null model of each taxa. Then, the positive and negative connectedness matrix of each sample was generated by averaging the positive and negative null model-corrected correlations separately. Finally, the positive and negative cohesion value of each sample was obtained by multiplying relative abundance table by the connectedness metrics according to the above formula [102]. The ranges of negative and positive cohesion were - 1 to 0 and 0 to 1, respectively. The more negative cohesion value indicates more complex microbial networks [65, 102]. Additionally, the absolute ratio of negative to positive cohesion were calculated to predict the co-occurrence network complexity, higher ratio indicates more complex microbial network [65, 102, 104, 105].

Variance partitioning analysis were conducted to evaluate the relative effects of types of host species, soil properties and electric conductivity on the variation in soil bacterial and fungal community composition, using the varpart function in the vegan package [84]. Correlation analysis of microbial diversity and soil properties was conducted by using Pearson correlation coefficient with FDR corrected p value < 0.05 [94]. Heatmaps of correlation coefficients were produced using the ggplot2 package [96].

We constructed a piecewise structural equation model to examine the causal pathways through which changes in soil nutrients and EC influence on microbial activity (multiple extracellular enzyme activity index) and total microbial residue (total amino sugar content), where both direct and indirect (via changing various aspects of community composition and network complexity) pathways were considered. Because SOC was positively correlated with soil nitrogen and phosphorous, we only included SOC in the model to indicate soil nutrients. The SOC was log-transformed and EC was sqrt-transformed. First, Akaike information criterion (AIC) was used to compare the linear mixed model with plot as random

effect and the general linear model, and the model with the smaller AIC was selected to construct piecewise SEM. We use Shipley's test of d-separation to test whether any paths are missing from the model and remove gradually the least significant paths from the models to improve model fit [106, 107]. We reported the standardized coefficient for each path from each component model. The final model was selected among the non-significant test, with higher Fishers-C (if $P > 0.05$, then no paths are missing and the model is a good fit) [108] and the lowest AIC. The SEMs were fitted by using the piecewiseSEM package [109].

Declarations

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Author contributions

L.Q. and X.W. conceived this project. X.W., W.K., H.Z., J.G., C.F., Y.R., J.W. and T.L. processed the soil samples and collected data. W.K. conducted the bioinformatics analyses. L.Q., W.K. and X.W. wrote the first draft of the manuscript and Q.Z., S.B., S.I., M.J.S. and M.S. contributed to subsequent revisions. All authors contributed to the final written product.

Availability of data and materials

The dataset supporting the conclusions of this article is available in the NCBI Sequence Read Archive repository under the accession number PRJNA738372.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

Figure 1

Alpha diversity of bacteria (A-E) and fungi (F-J) in bulk soils and halophyte rhizospheres. The curvilinear polygons of violin show the estimates of frequency densities. The box plots display the first (25%) and third (75%) quartiles, and median (bold line) value. The whiskers above/below box plot extend to the maximum/minimum values within the range of <1.5 quantile. The data points outside the range are plotted separately. Different lowercase letters indicate significant differences among halophyte rhizospheres and bulk soils.

Figure 2

Soil bacterial (A-C) and fungal (D-F) community compositions in bulk soils and halophyte rhizospheres. (A and D) Constrained analysis of principal coordinates (CAP) based on Bray-Curtis distance testing the bacterial and fungal community changes, respectively. The explained fractions of the total variation are 46% and 49% (with 95% confidence interval), respectively. (B and E) Venn diagram showing the numbers of shared and unique bacterial and fungal OTUs among bulk soils and halophyte rhizospheres, respectively. (C and F) The relative abundances (%) of major taxonomic groups at the bacterial and fungal phyla level, respectively. Phyla labeled with asterisk indicates significant differences in relative

abundance among halophyte rhizospheres and bulk soils. *, ** and *** indicate significance at $0.01 < P < 0.05$, $0.001 < P < 0.01$ and $P < 0.001$, respectively.

Figure 3

Variations in soil bacterial and fungal communities. (A and D) Dissimilarity distance showing the differences in bacterial and fungal community structure between bulk soils and halophyte rhizospheres. (B and E) Permutational analysis of multivariate dispersions (PERMDISP) compares the within-group dispersion among bacterial and fungal communities from bulk soils and halophyte rhizospheres using the average value of the individual observation distances to the group centroid based on Bray-Curtis distances, respectively. Greater distances to the centroid (y-axis) indicate more variation in microbial community composition. (C and F) The contributions of halophyte types, soil nutrients and soil electric conductivity (EC) to bacterial and fungal community compositions based on variance partitioning analysis, respectively.

Figure 4

Bipartite network of bacterial (A) and fungal (B) communities showing the indicator OTUs for each halophyte rhizosphere and bulk soils based on indicator species analysis. Circular nodes represent individual bacterial/fungal OTUs. Color indicates their phyla affiliation.

Figure 5

Phylogenetic distribution of the top 50 most abundant taxa (OTUs), and their relative abundance and indicator roles in the bacterial (A) and fungal (B) communities. The phylogenetic trees were colored by phyla level for bacteria and by class level for fungi. Taxa that could be affiliated to genus level are shown as genus, otherwise as OTU ID. The heatmaps in the middle showed the relative abundance of the taxa across halophyte rhizospheres and bulk soils. The bars on the right indicated whether the taxa were an indicator or non-indicator OTUs identified by indicator species analyses.

Figure 6

Soil microbial co-occurrence networks in bulk soils and halophyte rhizospheres. The networks are constructed based on random matrix theory using Molecular Ecological Network Analysis Pipeline. The circles and triangles represent bacterial/archaeal and fungal OTUs, respectively. The nodes are colored by module (A) and phyla (B) level, respectively. (C) The topological roles of nodes in the microbial networks were classified into four categories according to within-module connectivity (Z_i) and among-module connectivity (P_i), among which the network hubs, module hubs and connectors are identified as the keystone taxa. The circles and triangles represent bacterial/archaeal and fungal OTUs, respectively.

Figure 7

Soil microbial activity index (A) and microbial residue (B) in halophytes rhizospheres and bulk soils and their responses to soil environments, microbial compositions and co-occurring network complexity (C and D). Structural equation model was established to explore the significant effects of soil nutrient (SOC), electrical conductivity (EC), bacterial and fungal community compositions (first axis of nonmetric multidimensional scaling) and network complexity (network nodes, edges, degrees and negative/positive cohesion) on microbial activity (multiple extracellular enzymes activities index) (C) and microbial residue (total amino sugar content) (D). Arrows represent significant unidirectional relationships among variables. Blue and red arrows indicate positive and negative relationships, respectively. Path thickness were scaled by the magnitude of the standardized regression coefficients. The average of the z-score of each extracellular enzyme activity was calculated to indicate soil microbial activity. Significant tests were conducted by dunn's test. Columns labeled with asterisk indicates significant differences among halophyte rhizospheres and bulk soils (A and B).

Supplementary Files

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