Networking the desert plant microbiome, bacterial and fungal symbionts structure and assortativity in co-occurrence networks

Kenji Maurice, Liam Laurent-webb, Amélia Bourceret, Stéphane Boivin, Hassan Boukcim, Hassan Boukcim, Marc-André Selosse, Marc Ducousso

Environmental Microbiome

RESEARCH

Open Access

Networking the desert plant microbiome, bacterial and fungal symbionts structure and assortativity in co-occurrence networks

Kenji Maurice[1*], Liam Laurent-Webb[2], Amélia Bourceret[2], Stéphane Boivin[3], Hassan Boukcim[3,4], Marc-André Selosse[2,5,6] and Marc Ducousso[1]

Abstract

In nature, microbes do not thrive in seclusion but are involved in complex interactions within- and between-microbial kingdoms. Among these, symbiotic associations with mycorrhizal fungi and nitrogen-fixing bacteria are namely known to improve plant health, while providing resources to benefit other microbial members. Yet, it is not clear how these microbial symbionts interact with each other or how they impact the microbiota network architecture. We used an extensive co-occurrence network analysis, including rhizosphere and roots samples from six plant species in a natural desert in AlUla region (Kingdom of Saudi Arabia) and described how these symbionts were structured within the plant microbiota network. We found that the plant species was a significant driver of its microbiota composition and also of the specificity of its interactions in networks at the microbial taxa level. Despite this specificity, a motif was conserved across all networks, *i.e.,* mycorrhizal fungi highly covaried with other mycorrhizal fungi, especially in plant roots—this pattern is known as assortativity. This structural property might reflect their ecological niche preference or their ability to opportunistically colonize roots of plant species considered non symbiotic *e.g., H. salicornicum*, an Amaranthaceae. Furthermore, these results are consistent with previous findings regarding the architecture of the gut microbiome network, where a high level of assortativity at the level of bacterial and fungal orders was also identified, suggesting the existence of general rules of microbiome assembly. Otherwise, the bacterial symbionts Rhizobiales and Frankiales covaried with other bacterial and fungal members, and were highly structural to the intra- and inter-kingdom networks. Our extensive co-occurrence network analysis of plant microbiota and study of symbiont assortativity, provided further evidence on the importance of bacterial and fungal symbionts in structuring the global plant microbiota network.

Keywords Microbiome, Network, Symbiont, Desert, Arid, Co-occurrence, Assortativity

*Correspondence:
Kenji Maurice
kenji.maurice@gmail.com
Full list of author information is available at the end of the article

Introduction

Plants have a long history of interactions with microorganisms due to their intimate relationships with microbial communities such as bacteria and fungi since their emergence from aquatic ecosystems ~ 450 million years ago [1]. Soil microorganisms can be internalized or attached to the roots (endophytic or epiphytic communities), or located in their vicinity, the rhizosphere. These microorganisms are collectively referred to as microbiota [2], which play an important role in plant health [3]. Among their numerous associations, mutualistic symbioses, such as those involving nitrogen-fixing bacteria and mycorrhizal fungi, are the most widespread in the plant kingdom [4], and are also best known to have a seminal impact on plant survival and development [5]. These microbial symbionts differ in their life history strategies [6] and use the common symbiotic pathway to infect plants—initially programmed for the arbuscular mycorrhiza symbiosis—and thus share a long history of coevolution [7]. Mycorrhizae and nitrogen-fixing bacteria are mainly deterministically associated with their host, with the plant's phylogeny partly dictating the root colonization by these symbionts [4, 8]. The soil microbiota assembly is to a larger extent driven by deterministic (*e.g.,* host selection, environmental filtering) and neutral processes (*e.g.,* birth, death, and dispersion of microorganisms) [9, 10]. Niche preference—driven by selective pressures—is known to be influenced by environmental conditions. Factors such as land use, pH, salinity and nutrient content jointly shape the soil and plant microbiota composition [11–14]. Current research has also shed light on the importance of biotic interactions between plant hosts and their microbiota, and also within microbiota, as a driving force of microbiota assembly, and by extension of their host's fitness [15, 16]. This led to the holobiont concept, whereby selective forces are not limited to host-microbiome interactions but also encompass those within microbiota [17, 18]. For example, tripartite interactions commonly occur within microorganisms and plants, where mycorrhiza helper bacteria are known to interact with mycorrhiza, thus modulating plant mycorrhizal colonization success [19, 20]. Conversely, mycorrhizal fungi have also been found to promote nodulation in some cases, where *M. truncatula* nodulation could be improved by AMF symbiosis [21]. In another in vitro study, AMF were found to improve nodulation of *Lotus japonicus* by *Mesorhizobium loti* [22], confirming the importance of beneficial inter-kingdom interactions of symbionts. Plant symbiotic types have to a greater extent been shown to affect the differentially abundant plant root microbiota or symbiotype [23]. Using 17 greenhouse-grown plant species, including species symbiotically associated with AMF, rhizobia, AMF and rhizobia or non-symbiotic, Hartman et al. [23], showed that symbiotic taxa impacted the overall root microbiota assemblage. Relative abundance of Acidobacteria, Actinobacteria, Chlamydae and Verrucomicrobia were found to be impacted by the fungal symbionts' presence, while no fungi were found to be affected, hence supporting the possible inter-kingdom dependency of bacterial taxa. There are thus intricate relationships between what has been referred to as 'primary symbionts' *i.e.* which form symbiotic organs with the host plant (*e.g.*, AMF, rhizobia) and the extended microbiota, whereby their mutual influence could modulate the successful establishment of bacterial or mycorrhizal symbiosis with plants.

In natural environments, particularly those subject to strong abiotic constraints, presence of microbial symbionts can be critical to the establishment and survival of plants as they forage for water and nutrients [24–26]. Symbionts also improve the health status and resistance of plants to drought [27–29]. This is particularly true in desert ecosystems that are subject to high abiotic constraints such as low precipitation and poor soil nutrient content. Plants are thus spatially segregated in a discrete so-called fertility island pattern, while forming refuge niches that increase the microbial diversity and abundance [30, 31]. As a consequence of the harsh abiotic constraints, plant–microbe positive interactions may be strengthened in arid desert environments (*e.g.,* according to the stress gradient hypothesis for example [32]), through nutritional dependencies, cross-feeding, resource and niche competition, and metabolite exchange [17, 33]. Plants in desert environments thus constitute a unique model for studying microbial interactions, and for assessing the extent to which bacterial and fungal symbionts are central members in the plant microbiome. Deserts also constitute a major gap of knowledge for the understanding of the global terrestrial microbiome [34], an issue that should now be addressed considering the importance of desert ecosystems and their high vulnerability to climate change [35, 36]. Although these environments have long been considered to host scant biodiversity and provide few ecosystem services, it is now recognized that they contain up to 25% of global organic carbon [37] while hosting a high proportion of the global floristic diversity, with xeric shrubland plant diversity sometimes exceeding that of other biomes [38].

We are now aware that host plant species have an effect on their associated microbiomes [39], but research carried out in natural environments, particularly in hot deserts, still needs to be further investigated.We therefore conducted a study to assess whether the type of plant symbiosis modulates the co-occurrence of fungal and bacterial symbionts and of the extended microbiota, as well as their inter-kingdom interactions. We sought

to clarify the impact of symbionts in root and rhizosphere microbiota networks, by studying their conditional dependence using an extensive co-occurrence approach based on covariance relationships. Co-occurrence relationships do not necessarily support biotic interactions [40], but they provide a valuable tool for studying global covariance relationships within omics datasets [41], as covariance relationships might reflect ecological processes shaping the microbiome. This study was designed to determine whether the presence of microbial symbiont in plants affect the co-occurrence relationships of its microbiome. We more specifically focused on assessing whether fungal symbionts—namely AMF, ectendomycorrhizal fungi (EMF) and to a lesser extent dark septate endophytes—and bacterial symbionts Rhizobiales and Frankiales co-vary with the extended microbiome through both intra- and inter-kingdoms relationships and if their topology differs from other taxa. Herein, we studied six plant species with supposed contrasted symbiotic lifestyles and belonging to four families: the Fabaceae (*Retama raetam* Forssk. Webb & Berthel. and *Astragalus spinosus* Forssk. Muschl.), Amaranthaceae (*Haloxylon persicum* Bunge and *Haloxylon salicornicum* Moq. Bunge ex Boiss.), Poaceae (*Stipagrostis plumosa* L. Munro ex T. Anderson) and Cistaceae (*Helianthemum lippii* L. Dum. Cours.) in a highly constrained hot arid desert in Saudi Arabia. We sampled the roots and rhizosphere of each species during two seasons and profiled the bacterial and fungal microbiota using amplicon sequencing. We hypothesized that: (i) plant species have a major effect on the microbial community composition and they constrain covariance relationships between symbiont taxa, (ii) in networks, symbionts have a modular structure differing from that of other trophic modes considering their importance in plant life, and (iii) inter- and intra-kingdom covariance relationships of symbiotic taxa have distinct structures.

Material and methods

Sample collection

In the hot and arid desert conditions that prevail in the AlUla region (Medina province, Saudi Arabia Kingdom), six plant species were sampled in natural environments during the two studied seasons (summer in August 2021 and spring in March 2022). Samples were collected at five sites in the Sharaan Nature Reserve and in a natural environment close to AlUla city. Two Fabaceae species, *Astragalus spinosus* (Site 1, Site 2) and *Retama raetam* (Site 5), were chosen as nitrogen-fixing species associated with Rhizobiaceae [42]. *Stipagrostis plumosa* (Poaceae; Site 6) was chosen as an arbuscular mycorrhizal (AM) species [43]. *Helianthemum lippii* (Site 1, Site 2) was chosen as an ectendomycorrhizal species that is known to be associated with both *Glomus* spp. and Pezizaceae mycorrhizal fungi [44, 45]. Finally, two supposedly non-symbiotic (*i.e.*, that do not have a symbiotic relationship with Rhizobiaceae or mycorrhizal fungi) Amaranthaceae species were sampled, *Haloxylon persicum* (Site 3) and *Haloxylon salicornicum* (Site 4), (Fig. 1A) [43]. The features of the sites and their associated plant samples are outlined in Supplemental file 1 (Figs. S1–S7; Table S1).

Roots and rhizospheres of 25 individuals per plant species were sampled during two seasons (August 2021 and March 2022), thus resulting in 50 individual plants per species. For *S. plumosa*, an additional compartment was sampled i.e. the rhizosheath, which is the soil directly attached to the roots in a sheath-like shape [46] (Fig. S7J). It corresponds to a xerophytic trait that enables the plant to withstand desiccation and form a compartment distinct from the rhizosphere [47]. Samples were kept at 4 °C until laboratory analyses.

Soil parameter measurements

Three rhizosphere and bulk soil sub-samples were processed in triplicate using X-ray fluorescence spectroscopy (XRF). Triplicate soil samples were pressed at 20 t for 2 min with 1:3 v:v of SpectroBlend® (SCP Science) wich allow polymerization of the samples without influence on the XRF results [48]. Three measure on each triplicate, using the 'geoexploration' parameter with a total exposure time of 105 s of the XRF S1 Titan analyzer (Bruker) was used to measure the soil atomic composition of elements from magnesium to uranium, leading to nine measurements for each bulk and rhizosphere soil sample. The rhizosphere and rhizosheath compartment of *S. plumosa* were not measured as the soil material collected

(See figure on next page.)

Fig. 1 The microbiota of six plant species presenting different symbiotic modes was characterized **A** Pictures of the six plants species in their natural environment and their associated trophic modes. *Astragalus spinosus* (AS) and *Retama raetam* (RR) are the two Fabaceae species associated with nitrogen-fixing bacteria. *Haloxylon salicornicum* (HS) and *Haloxylon persicum* (HP) are the two non-symbiotic Amaranthaceae species. *Stipagrostis plumosa* (ST) is a Poaceae species associated with AMF. *Helianthemum lippii* (H) is an ectendomycorrhizal Cistaceae species. **B** nMDS (k = 3) of the fungal (ITS, 18S) and bacterial (16S) community composition according to the compartment (shape = bulk, rhizosphere, rhizosheath or root) and associated species (in colors). The associated R^2 and p-values computed using PERMANOVA are presented for each amplicon according to the compartment or species in roots and rhizospheres

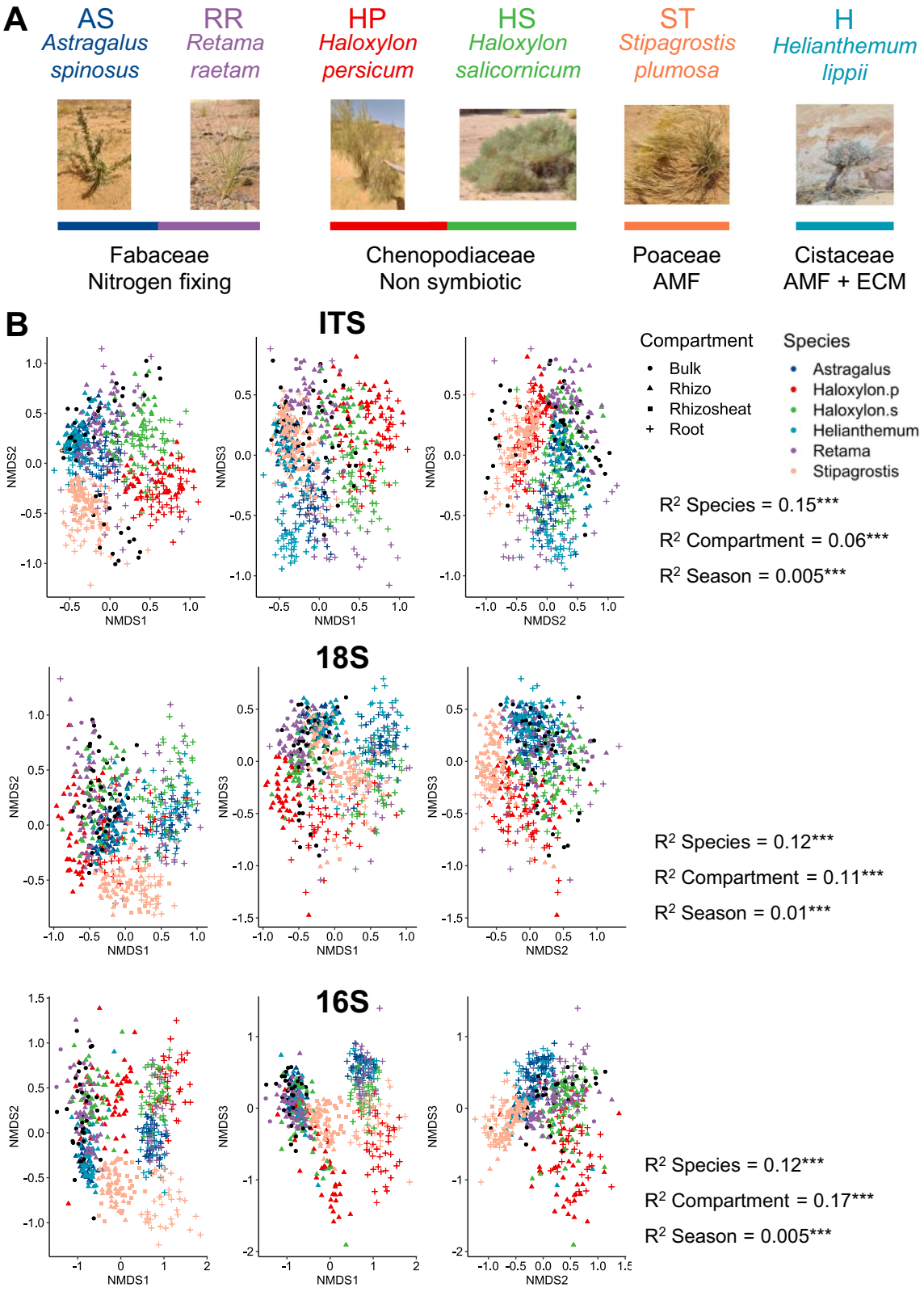

Fig. 1 (See legend on previous page.)

in the vicinity of their roots was insufficient for analysis. The soil pH was measured at a 1:5 v:v ratio of H_2O and KCl 1 M using a pH meter (pH Meter Knick 766, Knick International).

Library preparation and sequencing

Sample processing, sequencing methods and bioinformatic analyses were previously described in [48]. Briefly DNA extraction was performed with the FastDNA Spin Kit for Soil (MP Biomedicals™) and diluted to 0.3 ng.µL-1 before PCR amplification. 479F (5′ CAGCMGCYGCNGTAANAC 3′) and R888 (5′ CCGYCAATTCMTTTRAGT 3′) primers were used to amplify the V3-V4 regions of the 16S rRNA gene [49]. ITS86F (5′ GTGAATCATCGAATCTTTGAA 3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC 3′) primers were used to amplify the fungal ITS2 [50]. AMADf (5′ GGGAGGTAGTGACAATAAATAAC 3′) and AMADGr (5′ CCCAACTATCCCTATTAATCAT 3′) primers were used to amplify the 18S rRNA gene, thereby allowing enhanced resolution of AMF taxa [51]. Library sequencing was performed on a 2×250 NovaSeq 6000 system (Fasteris SA, Switzerland). 97% OTUs were constructed for taxonomic assignment of fungi (ITS and 18S), while ASVs were used for bacteria (16S). Additional information on the methods used for the sequencing library preparation and bioinformatic pipeline are available in Supplementary file 2, Supplemental methods 1.

Analysis of the microbial community composition

All statistical analyses were performed in R and details about each analysis workflow are presented in the Supplementary file 3 with their associated files and figures (Figs. S8, S9 and S10). We tested the effects of plant species on the microbial community composition by performing a permutational analysis of variance (PERMANOVA) on the Hellinger transformed root and rhizosphere OTU or ASV tables using the following formula: Hellinger transformed tables ~ Species*Season with 10,000 permutations. PERMANOVAs used to test 1°) the effect of species, compartment, and season on both the total dataset for each amplicon and 2°) the effect of season in each compartment of each species are available in Supplementary file 4 (Tables S2 and S3). Non-metric multidimensional scaling (nMDS) based on Bray–Curtis dissimilarity matrix of the Hellinger transformed data was performed to visualize differences in microbial community composition according to each compartment and species.

Analysis of the soil composition

Soil XRF data are compositional as they provide the relative abundance of each atomic element. A centered log ratio transformation was applied to remove the closure effect and each measure of each replicate was used for visualization using principal component analysis (n = 2364). Only major elements which varied across samples were retained as variables (Mg, Al, Si, P, K, Ca, Ti, Cr, Mn, Fe and Sr) for the PCA according to the 'species', 'sites' and 'soil type' conditions. ANOVA followed by TukeyHSD post-hoc tests were performed to test for pH differences between rhizosphere and bulk soils, the rhizospheres of each plant species and the differences between bulk soils of each site. Partial Mantel tests were carried out to identify the soil parameters that significantly affect the microbial composition (Supplemental methods 3).

Network construction and analysis

The sampling season had low impact on the community structure (Supplemental methods 2). Samples from both seasons were thus merged before network construction (Supplemental methods 4). Networks for each amplicon (ITS, 18S, 16S), compartment (roots and rhizospheres; with the addition of rhizosheaths for *S. plumosa*) and species (n = 6) combination were constructed using the SPIEC-EASI method [52], while inter-kingdom networks (ITS-16S, 18S-16S) we constructed using the extended SPIEC-EASI method [53]. Full details on the filters used for each amplicon and network construction are listed in Supplementary file 3 (Fig. S11). The networks were then visualized using Gephi [54] and their topological attributes were calculated using the igraph package in R [55]. As the networks were built with a different number of OTUs (or ASVs), the degree of nodes was normalized by dividing their degree by n-1, where n is the number of nodes in each network. Betweenness centrality was normalized against its maximum possible value in each graph using the igraph formula: $B^n = \frac{2B}{(n-1)(n-2)}$, where B^n is the normalized betweenness, B is the absolute betweenness and n is the number of nodes. Differences in node degree and betweenness centrality across compartments and species were assessed by ANOVA, followed by a post-hoc test based on the estimated marginal means [56]. We tested the extent to which the root microbiota centrality of nodes differed from that of the rhizosphere. To do so we specifically focused on the node degree and betweenness differences between rhizosphere and root compartments over plant species and amplicons.

Fungi were assigned to their trophic mode using FunGuild database [57]. Only AMF and EMF fungi were identified as mycorrhizal in our dataset and were thus retained as primary fungal symbionts. We also included endophytes such as dark septate endophytes, and epiphytes (which only represent a small fraction of the total fungal symbionts) as fungal symbionts. All these fungi, added to the primary symbionts will be referred to as

fungal symbiotrophs. For bacteria, we retained Rhizobiales and Frankiales as primary symbionts as they were the only nodule-forming orders identified. In order to assess how each symbiotic node was integrated in the overall covariance networks, we used the assortativity coefficient $r = \frac{\sum_i e_{ii} - \sum_i a_i b_i}{1 - \sum_i a_i b_i}$, where $a_i = \sum_j e_{ij}$, $b_i = \sum_i e_{ij}$, and e_{ij} represent the fraction of edges connecting each type i and j node [58]. The assortativity coefficient is a measure of network homophily, *i.e.,* the preferential attachment of nodes with the same attribute. This coefficient is bounded between −1 and 1, where values close to −1 are indicative of a dissortative network (*i.e.*, nodes with opposite attributes tend to preferentially attach), while values close to 1 are indicative of an assortative network (*i.e.*, nodes with the same attributes tend to preferentially attach). We thus assigned nodes to either i or j based on the different levels of assortativity to be examined (*e.g.,* symbiotrophic nodes = i, non-symbiotrophic nodes = j). We further checked if symbiotrophic nodes had a structural role in the networks by using their among- and within-module connectivity to assign their topological roles (Supplemental methods 2). Whereas assortativity is based on the node attributes, modularity is a community detection method, whereby nodes are assigned into modules based on their topology, regardless of their attributes. We used the Meconetcomp package in R [59] to examine the effect of each plant species on covariance relationships at the OTU (or ASV) level, as well as the intersection of network nodes and edges between the networks of each species for each rhizosphere and root compartment. Each independent node and edge were identified, as well as the species combinations in which they are shared across networks. Venns and UpSet plots were used to visualize each independent or common node and edge and their differences were assessed using the Jaccard distance matrix [60].

Results

Plant species is a major driver of the microbial community composition

The fungal community compositions were mainly driven by the plant species when compared to the compartment or season both for ITS2 (R^2 Species = 0.15*** > R^2 Compartment = 0.06*** > R^2 Season = 0.005***; Fig. 1B) and 18S markers (R^2 Species = 0.12*** > R^2 Compartment = 0.11*** > R^2 Season = 0.01***; Fig. 1B). Bacteria compositions however were mainly explained by the compartment (R^2 Compartment = 0.17*** > R^2 Species = 0.12*** > R^2 Season = 0.005***; Fig. 1B). Season had little impact on both fungal and bacterial community composition (Figs. S13, S14 and S15; Tables S2 and S3). The bacterial communities were better explained by the studied parameters than fungi, and this pattern was observed in the different plant species with regard to the compositions in their roots and rhizospheres (Fig. 1B; Supplemental file 4, Fig. S16). The rhizosphere soil composition was mainly driven by the Si content at the HP (*Haloxylon persicum*) site, while elements such as Fe, Mn and K were the main drivers at the other sites (Fig. 2A). Soil atomic composition explained 33.2% of variance in PCA dimension 1 and 17.7% in dimension 2 (Fig. 2A). Only minor differences in bulk soil compositions and pH were observed between sites (Fig. 2B). Bulk and rhizosphere soils did not differ greatly in their chemical composition (Fig. 2C), while pH_{H2O} was significantly more alkaline in the rhizospheres (Fig. 2A) than in the bulk

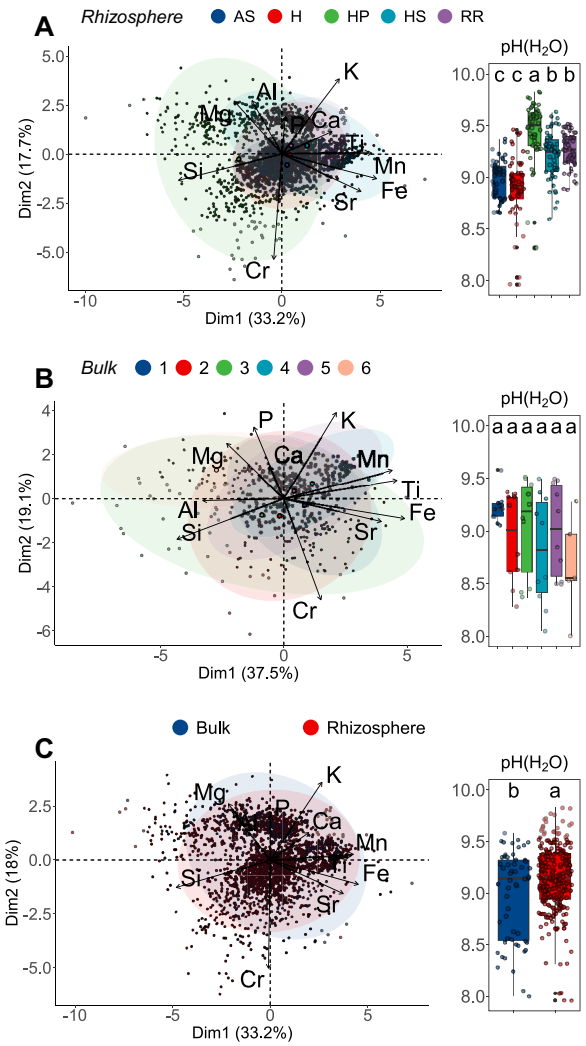

Fig. 2 Principal component analysis of soil composition. The rhizospheres of the different plant species (**A**), the bulk soils of the different sites (**B**) and the soil type (bulk or rhizosphere) (**C**) are presented. Differences of pH_{H2O} according to these conditions were tested using ANOVA followed by a Tukey HSD test. Different letters represent significant differences among groups

soils. Overall, the pH_{H2O} was highly alkaline, ranging from 8 to 9.8. Here again, the bulk soil pH did not differ between sites, while the pH_{H2O} of the *Haloxylon persicum* rhizosphere was the highest and the pH values observed for *A. spinosus* and *H. lippii* were the lowest. The site and plant species may be covariates in community composition analyses but the soil physico-chemical properties were similar between sites. Thus, microbiota differences were mainly due to the plant species (Fig. 1, Fig. S17). The main soil elements influencing the community composition were Mg, Fe, Ti and Al for ITS2, Mg and Fe for 18S and Mg, Ca, P, Al for 16S (Fig. S18). The pH_{H2O} was a strong driver of the microbial composition for both fungi and bacteria but varied more between compartments than between sites (Fig. 2; Fig. S18).

Plant species and compartment affect network topology

Fungal networks had a high proportion of positive edges in roots and rhizospheres, ranging from 90.2 to 98.1% for ITS2 and from 90.8 to 98.2% for 18S (Table 1). Despite the different number of nodes and edges across networks, topology measures such as modularity remained stable between ITS2 and 18S markers (*Student test*; p-value = 0.217). Bacterial networks had a higher proportion of negative edges in the roots and rhizospheres than fungal networks, ranging from 10.3 to 29.4% (Table 1). The modularity remained stable throughout all plant species' networks, but was higher in fungal networks than bacterial networks (Student test; p-value = 4.0×10^{-4}). The normalized node degree was higher in roots than in rhizospheres for bacterial and fungal networks (intra-kingdom and inter-kingdom networks), except for the 18S networks of the two Amaranthaceae species (*H. persicum* and *H. salicornicum*), while it did not change for the ITS2 networks of *A. spinosus* (As) (Fig. 3). The normalized betweenness centrality was also higher in roots for bacterial networks (16S, 16S-18S, 16S-ITS2). On the other hand, it was lower in roots for single domain fungal networks (ITS2, 18S) (Fig. 3). For *S. plumosa*, the rhizosphere and rhizosheath had closer values of betweenness and node degree than roots, while betweenness of ITS2 and 18S was higher in rhizosphere, but not different between roots and rhizosheath (Fig. S19A). Across species and compartments, differences in normalized degree and betweenness were conserved between bacterial intra-domain networks (16S) and inter-kingdom networks (16S-ITS, 16S-18S) to a greater extent than for fungi (ITS2 and 18S) (Fig. S19B).

Networks share a high number nodes but only a few edges

Overall, we identified a high proportion of nodes shared among plant species, while edges (*i.e.*, covariance between two nodes) were highly species-specific. The analysis of node sharing between networks showed that the highest proportion of sharing between plant species occurred in 18S networks, where 180 nodes (14.8% of nodes) in the rhizosphere and 97 nodes (13.1%) in the roots were common to all plant species (Venn diagrams, Fig. 4). For ITS, 9.7% of nodes in the rhizosphere and 9.8% of those in the roots were shared among all species. For 16S, only 4.3% of nodes in the rhizosphere and 6.6% of those in the roots were shared among all species.

Concerning differences between plant species, jaccard distances matrices revealed that *H. lippii* and *A. spinosus* had the more similar 18S node in the rhizosphere (UpSet plot: n = 80, 6.6% of total nodes; Jaccard distance = 0.22), while they were more dissimilar in the rhizosphere of Amaranthaceae species as compared to the other species. For the roots, *S. plumosa* and *H. persicum* had the most dissimilar 18S node sharing relative to the other species (Jaccard distance = 0.70). *A. spinosus* and *H. lippii* also had the most similar ITS nodes in the rhizosphere, a result in agreement with the 18S nodes (UpSet plot: n = 180, 10.9% of total nodes; Jaccard distance = 0.35), while *A. spinosus* had a high proportion of independent ITS nodes (n = 165, 10% of total nodes) In the roots, the highest proportion of shared or unique ITS nodes was found in *H.salicornicum* and *H.persicum,* which had the most unique nodes (n = 87, 85; 12.5, 12.2% respectively) compared to shared nodes among all species (n = 68, 9.8%).

Finally, for 16S, the proportion of nodes in the rhizosphere shared among all species was relatively low (n = 228, 4.3%) compared to the shared nodes of *A. spinosus* and *H. lippii* (n = 620, 12.9%) and to species-specific nodes. In the roots, *A. spinosus*, *H. salicornicum* and *H. persicum* had a higher proportion of independent nodes (n = 216, 16.3%; 191, 14.4%; 119, 9% respectively), while only 88 nodes (6.6%) were shared among all species. Otherwise, the higher proportion of independent or shared edges were unique to each species, as little to no edges were shared among all species in the rhizospheres and roots (0.1–2%; Figure S20). While most nodes were shared among plant species in the roots and rhizospheres, no edges were shared. This pattern was consistent in all single domain networks (ITS, 18S, 16S) but also in inter-kingdom networks (16S-ITS, 16S-18S).

Symbiotrophic *fungi* are the most assortative

We then assessed the extent to which fungal symbiotrophic taxa were integrated in the covariance relationships with other taxa, using a so-called mixed patterns or assortativity metric which measures whether or not nodes with an attribute (*e.g.*, symbiotrophics taxa) tend to interact more with nodes of the same attribute. Compared to saprotrophs, pathogens or other fungal trophic

Table 1 Topological features for each intra-kingdom (ITS, 18S, 16S) and inter-kingdom (16S + ITS, 16S + 18S) co-occurrence networks according to the compartments of each plant species

	Root						Rhizosheath	Rhizosphere					
	AS	HS	HP	H	RR	ST	ST	AS	HS	HP	H	RR	ST
	ITS												
Node	294	354	283	214	205	219	530	1034	598	348	800	661	586
Edge	93	293	103	103	70	128	316	1155	512	272	682	561	583
Average degree	0.7	1.7	0.7	1.0	0.7	1.2	1.2	2.2	1.7	1.6	1.7	1.7	2.0
Average path length	2.2	11.9	3.1	3.1	1.7	5.1	5.4	9.0	10.7	11.3	11.7	13.1	9.4
Modularity	0.97	0.94	0.95	0.95	0.96	0.93	0.97	0.89	0.92	0.94	0.93	0.94	0.9
Positive edges (%)	97.9	96.3	98.1	98.1	97.1	97.7	94.0	93.1	95.1	95.6	90.2	95.5	94.4
Negative edges (%)	2.2	3.8	1.9	1.9	2.9	2.3	6.0	6.8	4.9	4.4	9.8	4.5	5.7
	18S												
Node	489	466	371	319	362	197	528	894	508	508	776	737	711
Edge	506	341	210	253	155	85	262	988	455	297	837	587	915
Average degree	2.1	1.5	1.1	1.6	0.9	0.9	1.0	2.2	1.8	1.2	2.2	1.6	2.6
Average path length	12.4	11.7	4.2	10.1	2.3	2.7	8.3	9.6	14.6	9.4	9.5	13.0	7.6
Modularity	0.91	0.93	0.95	0.92	0.97	0.93	0.96	0.89	0.93	0.93	0.89	0.94	0.84
Positive edges (%)	94.9	98.2	97.6	98.4	96.1	95.3	94.7	90.8	94.7	96.3	91.4	92.2	94.9
Negative edges (%)	5.1	1.8	2.4	1.6	3.9	4.7	5.3	9.2	5.3	3.7	8.6	7.8	5.1
	16S												
Node	747	677	374	386	462	251	1103	2921	1513	791	2654	2145	1769
Edge	1313	1026	397	252	462	277	1420	4676	3057	1443	4674	4815	4841
Average degree	3.5	3.0	2.1	1.3	2.0	2.2	2.6	3.2	4.0	3.6	3.5	4.5	5.5
Average path length	5.8	6.4	8.3	6.3	9.2	8.6	8.1	6.9	5.6	5.7	6.5	5.4	4.8
Modularity	0.77	0.8	0.87	0.94	0.88	0.89	0.82	0.74	0.89	0.74	0.7	0.62	0.6
Positive edges (%)	77.0	81.6	89.7	70.6	79.7	88.1	81.2	77.5	77.2	86.0	77.0	78.3	80.3
Negative edges (%)	23.0	18.4	10.3	29.4	20.4	11.9	18.8	22.5	22.8	14.0	23.0	21.6	19.7
	16S + ITS												
Node	1058	1031	657	600	667	470	1633	3955	2111	1139	3454	2806	2355
Edge	1414	1426	847	309	352	479	1694	5526	2931	1556	4854	5376	5120
Average degree	2.7	2.8	2.6	1.0	1.1	2.0	2.1	2.8	2.8	2.7	2.8	3.8	4.3
Average path length	7.9	7.6	7.1	8.6	5.9	9.3	10.8	8.3	7.8	7.7	7.9	6.1	5.7
Modularity	0.84	0.84	0.84	0.97	0.97	0.9	0.90	0.81	0.81	0.83	0.79	0.7	0.69
Positive edges (%)	77.7	82.7	85.1	76.1	82.4	87.5	82.4	79.2	80.7	85.2	78.8	79.3	80.7
Negative edges (%)	22.4	17.3	14.9	24.0	17.6	12.5	17.6	20.9	19.3	14.8	21.2	20.7	19.3
	16S + 18S												
Node	1236	1156	745	705	824	448	1631	3815	2021	1207	3430	2882	2480
Edge	1736	1527	705	573	541	413	1375	5840	3079	1352	5155	3380	4923
Average degree	2.8	2.6	1.9	1.6	1.3	1.8	1.7	3.1	3.0	2.2	3.0	2.3	4.0
Average path length	7.7	7.8	10.5	13.4	15.0	10.3	14.5	7.6	7.3	9.4	7.1	9.3	6.1
Modularity	0.84	0.84	0.9	0.93	0.96	0.91	0.933	0.78	0.78	0.88	0.78	0.86	0.71
Positive edges (%)	79.3	81.9	83.6	77.0	82.1	87.7	84.65	78.1	80.6	84.7	78.2	80.4	81.9
Negative edges (%)	20.7	18.1	16.5	23.0	19.9	12.4	15.3	21.9	19.4	15.3	21.8	19.6	18.1

modes, fungal symbiotrophs were highly assortative among the plant species where they occurred (r = 0.32–0.83; Fig. 5A; Table S4; see Figs. S21, S22, S23 and S24 for a more detailed picture). They formed highly assortative modules that preferentially interacted with other symbiotrophs, as compared to other fungi belonging to other trophic modes. This pattern was consistent among species and markers (ITS2 and 18S) but also in inter-kingdom networks (16S-ITS, 16S-18S; Fig. 5A). Highly assortative modules of *Glomus* spp. were identified in

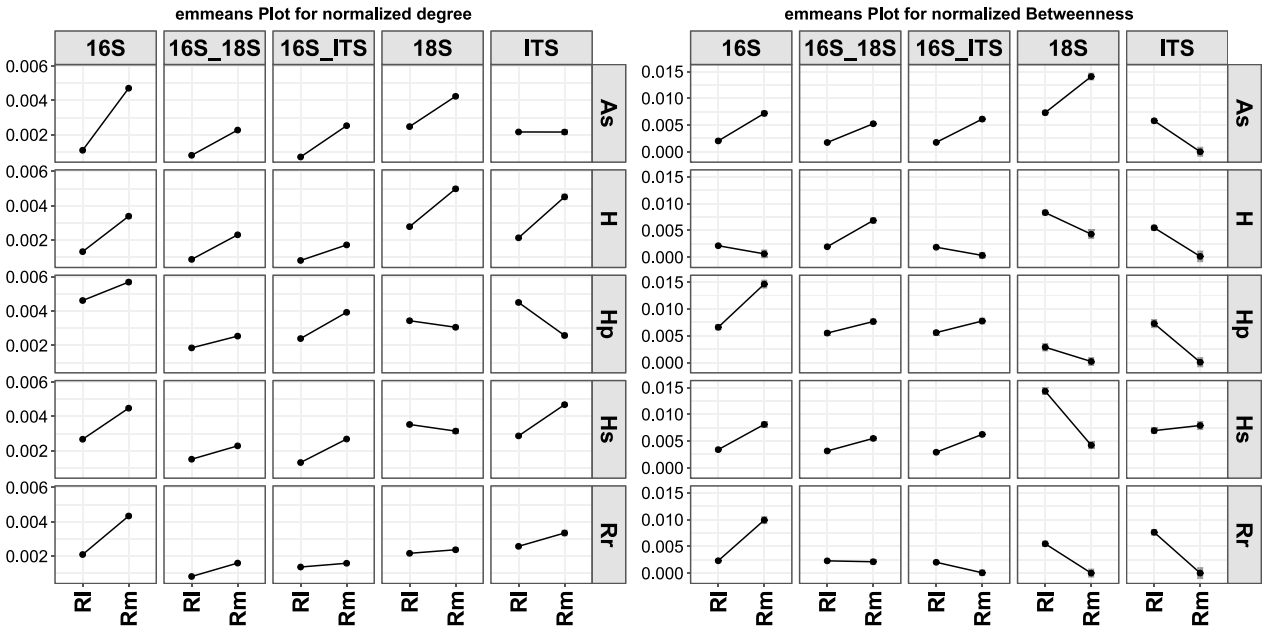

Fig. 3 Interaction plots of the estimated marginal means based on the different ANOVA models for the normalized node degree and normalized betweenness centrality. Increases or decreases in the normalized node degree and normalized betweenness across compartments (RI = rhizosphere; Rm = roots) are presented for each intra-kingdom networks (16S, 18S, ITS2), inter-kingdom networks (16S-18S, 16S-ITS2) and species (As, H, Hp, Hs, Rr). Confidence intervals are included in the plots

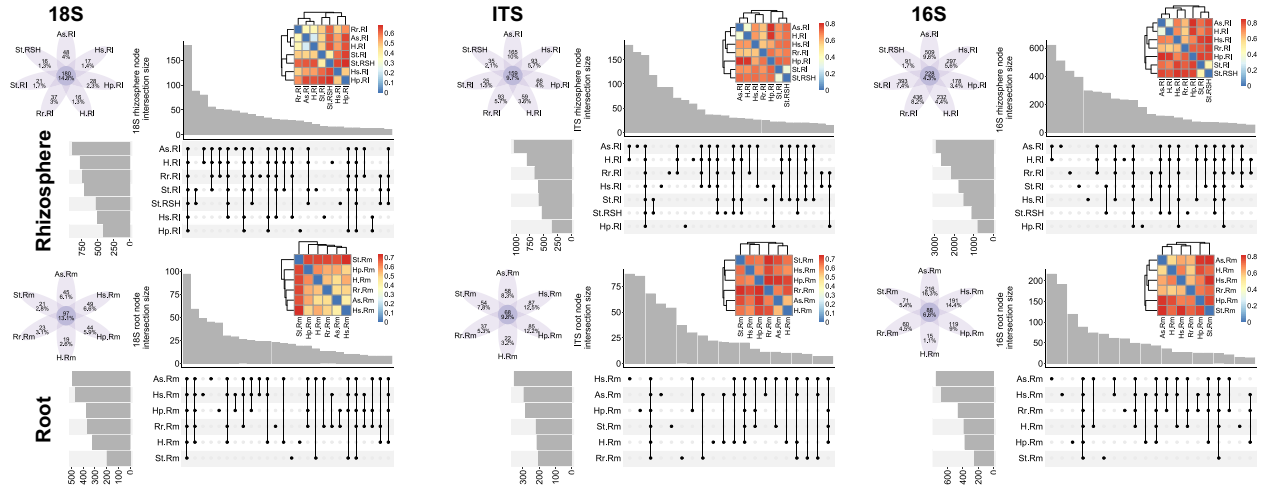

Fig. 4 Shared and independent nodes across each intra-kingdom network (18S, ITS2, 16S) according to the rhizosphere and root compartment. The Venns diagram represents the absolute number and the proportion of unique nodes for each network comparison for all species. The heatmap corresponds to the Jaccard distances of the node differences for all species networks. The UpSet plot represents the number of each node independent or shared for each species networks, for each single and combined features

18S networks, and were persistent in the 16S-18S inter-kingdom networks. The ubiquity of these modules was further supported by the complementarity of the two ITS2 and 18S markers, which yielded different diversity profiles in the identification of fungal primary symbionts within Glomeromycotina (Fig. 5B) with similar assortativity levels (Table S4). While 18S had a higher proportion of assignation at the genus level (particularly *Glomus* spp.), ITS2 identified a high proportion of *Dominikia* spp. which were not revealed by 18S (Fig. 5B). The *r* assortativity coefficient was significantly higher for symbiotrophs compared to that of the other trophic modes, especially in the roots of the different plant species (Fig. 6). While the assortativity of fungal symbiotrophs

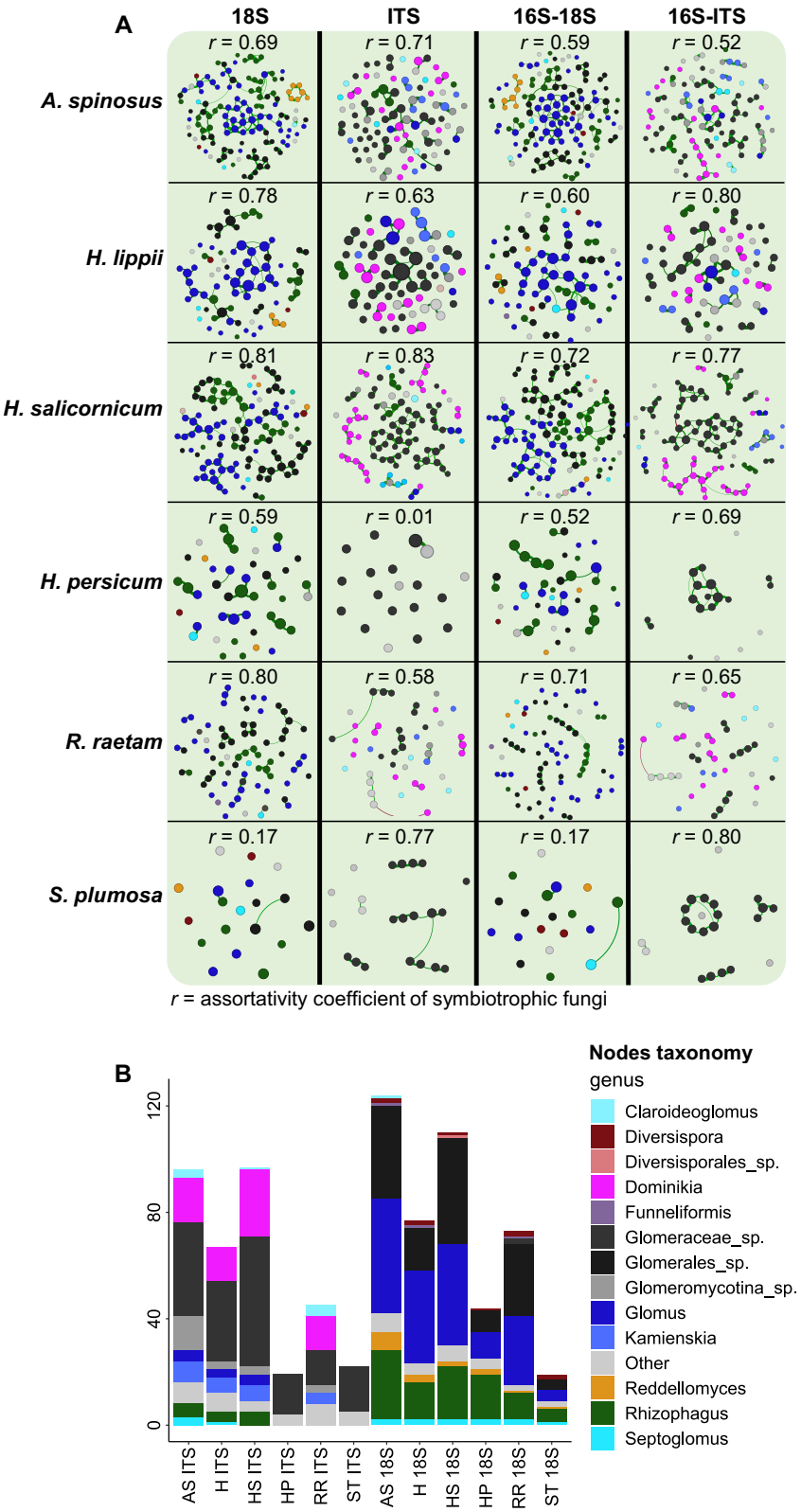

Fig. 5 Sub-networks of symbiotrophic fungi in the roots of all plant species. The assortativity coefficient of symbiotrophic fungi *r*, calculated on the whole network per plant species is also presented (**A**). Only symbiotrophic fungus nodes extracted from the total networks are presented for each intra-kingdom fungal network (ITS2, 18S) and inter-kingdom network (16S-ITS2, 16S-18S). Nodes are colored according to their genus and their abundance (number of nodes) is presented according to ITS2 or 18S (**B**)

was consistently higher in the roots compared to rhizosphere in both single- and inter-kingdom networks, they displayed a lower assortativity in the rhizospheric inter-kingdoms compared to single-kingdom networks (Fig. 6). In addition to the lower assortativity in inter-kingdom networks (fungi + bacteria) compared to single-kingdom networks, we identified a higher proportion of Glomeromycotina acting as module or networks hubs in the inter-kingdom networks as compared to intra-kingdom networks (Fig. S25). Bacterial symbionts (*i.e.* Rhizobiales and Frankiales) had a contrasted pattern compared to symbiotrophic fungi, as they covaried more with bacteria of different genus in the rhizospheres and roots. Their *r* assortativity coefficient at the order level was similar to that of other bacterial orders (Fig. S26), and lower compared to fungal symbiotrophs. Nonetheless, bacterial symbionts were acting as module and network hubs in the roots and rhizospheres, thereby highlighting their importance in structuring the bacterial networks, as well as in inter-kingdom interactions (Fig. S27).

Discussion

Despite the importance of symbiotic microbial taxa for plant health and their prevalence in the plant phylogeny, their effects on the extended plant microbiota has remained unfathomable and has yet to be characterized. Through massive sequencing and network analysis of the bacterial and fungal microbiome of various groups of symbiotic plants in a natural environment, we have characterized the overall covariance relationships of plant symbiotic taxa with the extended rhizosphere and root microbiome.

Plant species is a strong driver of its microbiota

In deserts, microorganism growth and activity are mainly limited by water availability, a phenomenon known as the pulse reserve paradigm [61]. In our study, the sampling season had little effect on the community composition (Fig. 1; Figs. S13–S17), mainly due to the absence of significant rainfalls between the two sampling periods (August 2021 and March 2022). This may explain why we observed little differences in microbial diversity and composition and soil composition between these two dry seasons. In addition, the soil composition was relatively homogeneous between sites (Fig. 2). Our sampling strategy therefore enabled us to specifically study the effect of plant species on the microbiome. Our extensive sampling effort over two seasons and the filters applied before network construction enabled us to characterize robust microbiome covariance relationships (Figs. S8–S11). More specifically, we were able to assess whether symbiotrophs impacted other taxa in microbial co-occurrence networks through intra- and inter-kingdom relationships, and if they had a different topology compared to that of non-symbiotic taxa.

Note that the selected plant species have diverse ecological niches, and covariation effects between the species and their niches linked to the prevailing environmental conditions cannot be ruled out. However, sites also had a significant effect on bulk soil microbial communities (Fig. S17; $R2 = 0.218^{***}$, 0.204^{***} and 0.269^{***} for ITS, 18S and 16S respectively). This covariation between sites and species can be explained by the restricted niches of certain species, such as H. persicum, which only thrives on mobile, very sandy soil. Replicating sites in future studies could help reduce this effect, in order to more accurately determine the proportion of variance attributable to the inter-site effect on microbial communities. Moreover, while the plant species phylogeny could be expected to partially drive its associated microbiome [62], particularly the mycorrhizal associations [63, 64], we identified one of the two Amaranthaceae species (*H. salicornicum*, a Amaranthaceae assumed to be non-mycorrhizal), being closely associated with AMF fungi (Table S5). AMF could thus withstand drought conditions by finding refuge in the roots of the only locally persistent plant species, as these fungi are able to interact with different partners [65], and modulate their interactions [66]. Otherwise, *H. persicum* (the other Amaranthaceae) was not associated with mycorrhizal fungi, though it is able to withstand extreme temperatures and low water availability [67]. *H. persicum* may thus rely less on fungal symbionts for growth and survival or AMF may have found another way to persist in the soil such as colonizing dead root tissue. The colonization of *H. salicornicum* in situ (Table S5) demonstrates the plasticity of AMF root colonization at the plant species level, even in plants that are considered to be non-mycorrhizal.

Then we confirmed that the rhizosphere microbiota was subject to significant edaphic constraints in desert environments. Elements such as Mg, P, Al, and Ca influenced microbial community assembly, and pH was a major driver of the microbial composition (Fig. S18), as previously described in various environments [11, 68].

(See figure on next page.)

Fig. 6 The *r* assortativity coefficient in the roots or rhizospheres of the different plant species for each intra-kingdom network (18S, ITS2) or inter-kingdom networks (16S-18S, 16S-ITS2). Different letters represent significant assortativity differences for the different trophic modes of the fungi tested using ANOVA followed by a Tukey HSD test

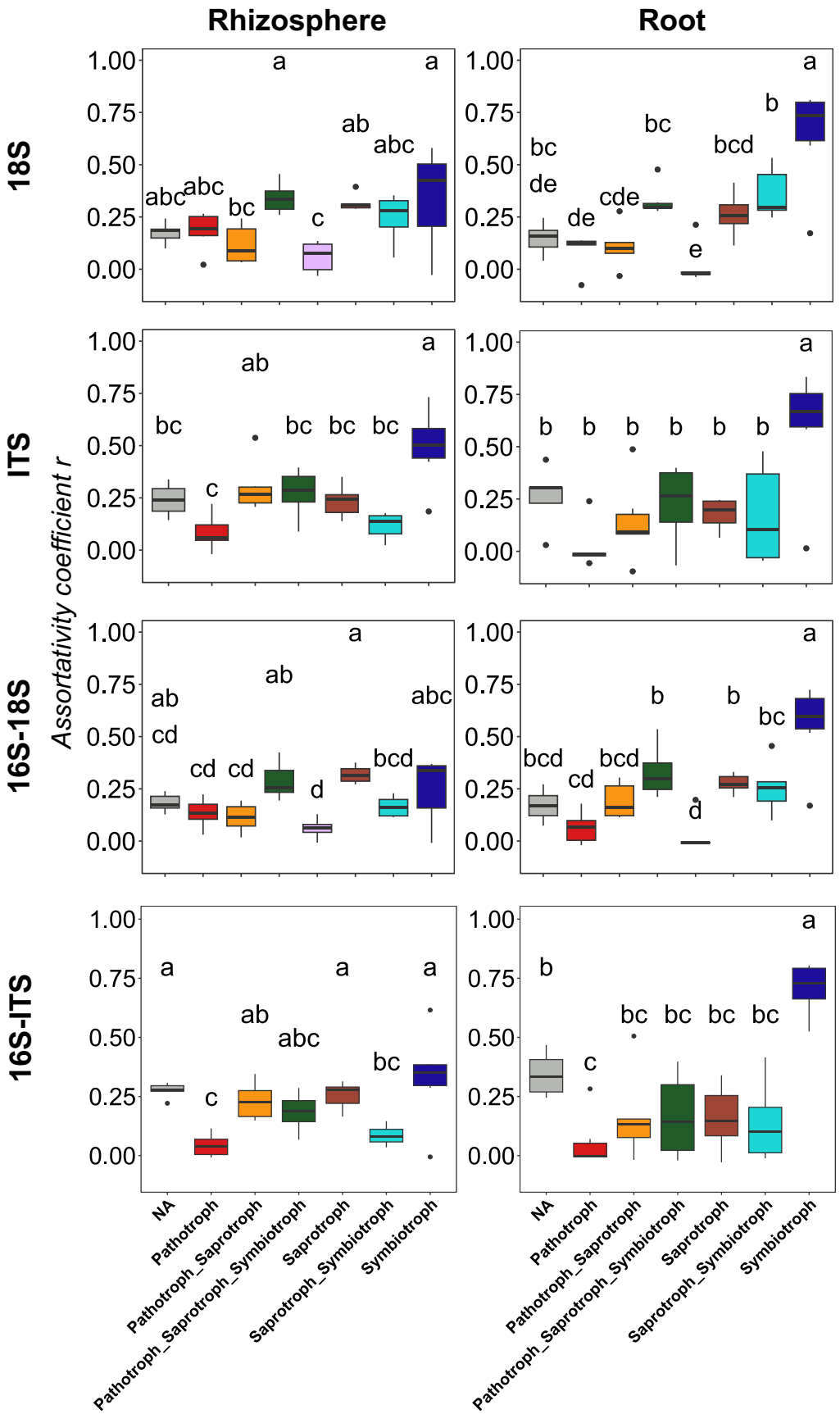

Fig. 6 (See legend on previous page.)

Above all, plant species had a pivotal effect on the rhizosphere and root microbiota in our study (Fig. 1), with respect to both bacteria and fungi, supporting our first hypothesis. In desert environments, where plants are heterogeneously distributed and form discrete patches (*i.e.* fertility islands), the plant species founding effect seems to have a greater impact on its microbiota than in temperate environments [69–71]. For example, it was found that date palm trees in a desertic agrosystem were consistently selecting Gamma- and Alpha-proteobacteria taxa in their roots, regardless of the surrounding soil microbiome [72]. This further suggests that plant host may have a greater effect on their associated microbiomes than the soil type in arid environments. However, in another study focusing on sympatric desert grasses, it was reported that the plant species was not a determining factor in the microbial assembly of the rhizosheath compartment [47], suggesting that stochastic processes may also be involved in microbiome assembly in desert plants. Nonetheless, intense selection related to the low concentration of nutrients and water, characteristic of the Arabian peninsula desert, may exacerbate the founding effect of plants on the microbial community composition, where microbial symbionts potentially play a crucial role as they are able to forage for soil water and nutrients or fix atmospheric nitrogen [25, 26, 29]. We also found that the normalized node degree and betweenness centrality of bacterial networks were higher in the roots than in rhizosphere (Table 1; Fig. 3), suggesting that there were stronger interactions in the endospheric community. This was also found to be the case in a comprehensive study of the network topology among plant habitats, where node centrality and taxonomic assortativity were higher in roots than rhizospheres [73].

Conserved assortativity of *fungi* across plant species despite the high edge specificity

Our co-occurrence network analyses further confirmed that plant species was a major factor in microbiota assembly. Networks had a high level of node sharing (Fig. 4), particularly for the 18S networks, suggesting a common core microbiota between plant species, whereas there was strong edge specificity at the OTU and ASV levels (Fig. S20). This suggests that interactions between microorganisms within the microbiota, more than microbiota community composition, are highly influenced by their host species. Despite these differences, a striking and ubiquitous pattern was revealed by our analysis: symbiotrophic fungi, mainly from the Glomeromycotina phylum, exhibited assortative interactions in different plant species, and this trend was particularly obvious in roots (Fig. 5; Table S4). While the network modularity was similar across species, it reflected the global network's modularity, thereby lacking the precision of different levels of modularity, such as local or assortative ones. Note that local mixing patterns often diverge from global ones such as network modularity, and more specifically assortative or dissortative mixing patterns, as they are not unimodally distributed universally [74]. Our assessment of the assortativity within specific symbiotrophic groups shed light on localized symbiotrophic fungus assortativity patterns in networks, supporting our second hypothesis that symbionts have a modular structure differing from that of other trophic modes. This demonstrates that, while interactions at OTU resolution may vary due to intra-genus or intra-trophic mode edge rearrangements, the organizational structure of the fungal microbiota may be higher throughout. This assortativity structuration of the root microbiota at the trophic mode level, which could be defined as a mesoscale level (*i.e.* intermediate between the OTU and the whole network) is in line with previous results on the gut microbiome [75, 76] or the brain connectome [77]. While assortativity seems to be a common feature of microbial networks [52], it was found to be a significant feature of the healthy gut microbiome [76]. Moreover, in the gut microbiome networks, segregated and autonomous assortative communities also exhibited core, peripheric, and dissortative communities, where densely connected assortative nodes benefit the whole network through metabolic functions. How these structural properties are shared in various host and environment, and the functional impact of a shared assortative module on peripheral network communities is a potential focus of research that still need to be addressed.

Assortativity as a key structural parameter of the plant microbiome network

The consistent assortative mixing pattern of symbiotrophic fungi across all species and primers could be due to various phenomena, especially in natural environments. First, it could be linked to the harsh abiotic constraints facing microbial communities during drought in desert environments. Highly assortative modules are more resistant to targeted degree-dependent attacks [58], whereby failure of a symbiotrophic node in the network would have less impact in a highly assortative network than a in dissortative one due to the high number of alternative paths between these nodes. These highly clustered patterns could thus reflect an ecological strategy of symbiotrophic fungi colonizing plant roots to withstand drought in line with desert microbiome dormancy strategies. For example, bacteria from Actinobacteria and Firmicutes species, commonly found in deserts, are able to form endospores which allow them to resist desiccation during drought [78, 79]. To which extent it may also be

the case for mycorrhizal fungi still need to be resolved. Strong co-occurrence between closely related OTUs could be the consequence of environmental filtering [80]. More specifically, these highly assortative clusters may be the result of niche partitioning, similar resource acquisition strategy or resource transfer [76], in line with mycorrhizal fungi ecology [81]. The conserved assortativity at the genus level for mycorrhizal fungi could be a strong niche partitioning effect, where conspecific AMF share similar niche requirement within the plant roots. However, Glomeromycotina polyploid genomes harbor multiple ITS2 and 18S copies, which could result in falsely positive co-occurrence links among phylogenetically close species [82]. Notwithstanding, the high assortativity of mycorrhizal fungi (delineated using OTUs to reduce this bias [83]), compared to fungi belonging to other phyla or trophic mode, was also found in other mycorrhizal genus such as *Dominikia*, and is therefore unlikely the result only of bias linked to primers choice.

While strong assortativity patterns were found to be stronger among symbiotrophic fungi than other trophic modes, this was not the case for bacterial symbionts such as Rhizobiales and Frankiales (Fig. S26). Their lower assortativity compared to symbiotrophic fungi indicated enhanced interactions with others bacterial and fungal taxa. However, their assortativity at the order level remained relatively high, therefore further supporting the involvement of signal of niche partitioning, resource acquisition and resource transfer [76]. As nitrogen is critical in nutrient-depleted ecosystems such as deserts, Rhizobiales and Frankiales functional involvement in nitrogen fixation seems to be of importance for the existence of other bacteria and fungi. However, this pattern was not specific to these bacterial taxa and, while they are known to improve the soil nutrient status and plant health [24], we could not differentiate them from other bacterial orders based on their assortativity. However, this high assortativity within different bacterial genera could be linked to their ability to horizontally transmit genes to each other [84, 85]. This ability enables them to adapt quickly to changing environmental conditions [86, 87], and could be facilitated between members of the same genus by this assortative structure. This could explain the concordance with other environments or hosts such as the intestinal microbiota, and could constitute a new direction for research.

Symbionts are structural in inter-kingdom networks

Bacteria and fungi are known to interact in soil and roots [20, 88, 89], but these interactions have yet to be comprehensively characterized. Given the importance of mycorrhizae for plant health, a high extent of covariances could be expected. Leung et al. [79], reported that mycorrhizal fungi co-occurred more frequently with bacteria compared to non-mycorrhizal fungi. Here we showed that fungal symbiotrophs remained assortative across the roots of several plant species in intra-kingdom but also in inter-kingdom networks. This minor interactions between mycorrhizal fungi and bacteria may be linked to non-overlapping niches during drought in desert. However, we also highlighted that symbiotrophs, notably fungal primary symbionts, were more structural in inter-kingdom relationships compared to the intra-kingdom's relationships, thereby suggesting that they had an enhanced structural role in fungal-bacterial interactions. This result supports our third hypothesis that the inter- and intra-kingdom covariance relationships of symbiotic taxa have distinct structures. For example, Glomeromycotina fungal nodes were highly structural in inter-kingdom networks and to a greater extent than in fungal networks alone (Fig. S25). This further supports previous findings on the ability of fungi to improve the stability and connectivity of bacterial networks as compared to intra-kingdom networks [48, 90]. This structural role may however have been due to indirect rather than direct interactions, such as interactions mediated through a third species, as suggested by the high assortativity. For instance, arbuscular fungi are known to alter litter decomposition by providing carbon to other decomposing microorganisms [91], potentially affecting their co-occurrence in the rhizosphere. Moreover, the structure of fungal-bacterial co-occurrence networks is driven by soil niches, while mycorrhizal fungi co-occurred more frequently with bacteria compared to non-mycorrhizal fungi [92]. Lastly, we confirmed the importance of considering inter-kingdom relationships when studying the microbiome. It is thus essential to assess the microbiome and its covariance relationships at different resolutions so as to gain greater insight into the microbiome assembly and interactions.

Conclusion

Here we obtained solid evidence indicating that the plant species is a strong factor in microbiome assembly in a desert environment under harsh abiotic constraints. More importantly, we found that symbionts, particularly mycorrhizal fungi, were consistently assortative across plant species despite the reorganization of their interaction at the OTU level in different plant species. This suggests that there is a strong niche partitioning under the high constraints that prevail during desert drought periods. Rhizobiales and Frankiales bacterial species were more structural of both, intra-kingdom and inter-kingdom network global architecture than symbiotrophic fungi, thus indicating their potential greater functionality in the microbiota of desertic

environments. By using covariance networks we were able to assess general assembly patterns in the plant microbiome, and the conserved assortative structure of mycorrhizal fungi. These patterns have already been identified in the gut microbiome and suggest that assortativity is a general assembly rule of microbial network across various hosts. While assortativity is a meaningful feature of microbial networks, these patterns still need to be studied under diverse environmental conditions to further assess how they are conserved, and how they impact microbial function and resilience across the global plant microbiome.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40793-024-00610-4.

Additional file1.

Additional file2.

Additional file3.

Additional file4.

Additional file5.

Acknowledgments
We are grateful to the INRAE MIGALE bioinformatics facility (MIGALE, INRAE, 2020. Migale bioinformatics Facility, doi: 10.15454/1.5572390655343293E12) for providing help and storage resources. Our warmest thanks to Benoît Perez-Lamarque for his help with NovaSeq sequencing demultiplexing. We also thank all members of the SoFunLand project who participated in the sample acquisition and biomolecular and soil analyses, particularly Odile Domergue, Coraline Damasio, Mathis Voisin, Romain Destabel and Sami Youssef.

Author contributions
MK Conceptualization, Methodology, Investigation, Data curation, Formal Analysis, Visualization, Writing—Original draft, Writing, Review & Editing. LL-W Resources, Writing, Review & Editing. BA Software, Data Curation, Writing, Review & Editing. SB Resources, Writing, Review & Editing. BH Supervision, Project administration, Funding acquisition, Writing, Review & Editing. SM-A Supervision, Project administration, Funding acquisition. DM Supervision, Project administration, Funding acquisition, Writing, Review & Editing.

Funding
This work was supported by the SoFunLand project, part of the Oasis program funded under the partnership between the RCU (Royal Commission for AlUla) and AFALULA (*Agence Française pour le development d'AlUla*).

Availability of data and materials
The bio-informatic pipeline can be found at: https://github.com/BPerezLamarque/Scripts/. The sequences supporting the conclusions of this article are available in the SRA GenBank repository (https://www.ncbi.nlm.nih.gov/genbank/) under project PRJNA1061359, Biosamples SAMN39266132 to SAMN39266820 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1061359?reviewer=toutf9o23hpsatatibti1gru1j).

Declarations

Ethics approval and consent to participate
All prevailing local, national and international regulations and conventions, and normal scientific ethical practices, have been respected.

Competing interest
The authors declare no competing interests.

Author details
[1]Cirad-UMR AGAP, Univ Montpellier, INRAE, 34398 Montpellier Cedex 5, France. [2]Institut de Systématique, Évolution, Biodiversité (UMR 7205 – CNRS, MNHN, UPMC, EPHE), Muséum National d'Histoire Naturelle, Sorbonne Universités, 57 Rue Cuvier, 75005 Paris, France. [3]Department of Research and Development, VALORHIZ, Montpellier, France. [4]ASARI, Mohammed VI Polytechnic University, Laayoune, Morocco. [5]Laboratory of Plant Protection and Biotechnology, Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, University of Gdansk, Abrahama 58, 80-307 Gdansk, Poland. [6]Institut Universitaire de France, Paris, France.

Received: 8 April 2024 Accepted: 28 August 2024
Published online: 02 September 2024

References

1. Kenrick P, Crane PR. The origin and early evolution of plants on land. Nature. 1997;389:33–9.
2. Berg G, Rybakova D, Fischer D, Cernava T, Vergès MCC, Charles T, et al. Microbiome definition re-visited: old concepts and new challenges. Microbiome. 2020;8:103.
3. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. Plant–microbiome interactions: from community assembly to plant health. Nat Rev Microbiol. 2020;18:607–21.
4. Martin FM, Uroz S, Barker DG. Ancestral alliances: plant mutualistic symbioses with fungi and bacteria. Science. 2017;356:eaad4501.
5. Van Der Heijden MGA, Bardgett RD, Van Straalen NM. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett. 2008;11:296–310.
6. Denison RF, Kiers ET. Life histories of symbiotic rhizobia and mycorrhizal fungi. Curr Biol. 2011;21:R775–85.
7. Oldroyd GED. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol. 2013;11:252–63.
8. Delaux P-M, Schornack S. Plant evolution driven by interactions with symbiotic and pathogenic microbes. Science. 2021;371:eaba6605.
9. Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, et al. Patterns and processes of microbial community assembly. Microbiol Mol Biol Rev. 2013;77:342–56.
10. Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH. Relative roles of niche and neutral processes in structuring a soil microbial community. ISME J. 2010;4:337–45.
11. Shen C, Xiong J, Zhang H, Feng Y, Lin X, Li X, et al. Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. Soil Biol Biochem. 2013;57:204–11.
12. Plassart P, Prévost-Bouré NC, Uroz S, Dequiedt S, Stone D, Creamer R, et al. Soil parameters, land use, and geographical distance drive soil bacterial communities along a European transect. Sci Rep. 2019;9:605.
13. Soudzilovskaia NA, Vaessen S, Van'T Zelfde M, Raes N. Global patterns of mycorrhizal distribution and their environmental drivers. In: Tedersoo L, editor. Biogeography of Mycorrhizal Symbiosis. Cham: Springer International Publishing; 2017. p. 223–35.
14. Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, et al. Global diversity and geography of soil fungi. Science. 2014;346:1256688.
15. Bonfante P, Anca I-A. Plants, mycorrhizal fungi, and bacteria: a network of interactions. Annu Rev Microbiol. 2009;63:363–83.
16. Basiru S, Ait Si Mhand K, Hijri M. Disentangling arbuscular mycorrhizal fungi and bacteria at the soil-root interface. Mycorrhiza. 2023;33:119–37.
17. Hassani MA, Durán P, Hacquard S. Microbial interactions within the plant holobiont. Microbiome. 2018;6:58.
18. Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. The importance of the microbiome of the plant holobiont. New Phytol. 2015;206:1196–206.
19. Frey-Klett P, Garbaye J, Tarkka M. The mycorrhiza helper bacteria revisited. New Phytol. 2007;176:22–36.
20. Boer WD, Folman LB, Summerbell RC, Boddy L. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev. 2005;29:795–811.

21. Wang X, Feng H, Wang Y, Wang M, Xie X, Chang H, et al. Mycorrhizal symbiosis modulates the rhizosphere microbiota to promote rhizobia–legume symbiosis. Mol Plant. 2021;14:503–16.
22. Tsikou D, Nikolaou CN, Tsiknia M, Papadopoulou KK, Ehaliotis C. Interplay between rhizobial nodulation and arbuscular mycorrhizal fungal colonization in *Lotus japonicus* roots. J Appl Microbiol. 2023;134:lxac010.
23. Hartman K, Schmid MW, Bodenhausen N, Bender SF, Valzano-Held AY, Schlaeppi K, et al. A symbiotic footprint in the plant root microbiome. Environ Microbiome. 2023;18:65.
24. Richardson AE, Barea J-M, McNeill AM, Prigent-Combaret C. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil. 2009;321:305–39.
25. Soussi A, Ferjani R, Marasco R, Guesmi A, Cherif H, Rolli E, et al. Plant-associated microbiomes in arid lands: diversity, ecology and biotechnological potential. Plant Soil. 2016;405:357–70.
26. Allen MF. Mycorrhizal fungi: highways for water and nutrients in arid soils. Vadose Zone J. 2007;6:291–7.
27. He A, Niu S, Yang D, Ren W, Zhao L, Sun Y, et al. Two PGPR strains from the rhizosphere of Haloxylon ammodendron promoted growth and enhanced drought tolerance of ryegrass. Plant Physiol Biochem. 2021;161:74–85.
28. Timmusk S, Abd El-Daim IA, Copolovici L, Tanilas T, Kännaste A, Behers L, et al. Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. PLoS One. 2014;9:e96086.
29. Naylor D, Coleman-Derr D. Drought stress and root-associated bacterial communities. Front Plant Sci. 2018;8:2223.
30. Bachar A, Soares MIM, Gillor O. The effect of resource islands on abundance and diversity of bacteria in arid soils. Microb Ecol. 2012;63:694–700.
31. Herman RP, Provencio KR, Herrera-Matos J, Torrez RJ. Resource islands predict the distribution of heterotrophic bacteria in chihuahuan desert soils. Appl Environ Microbiol. 1995;61:1816–21.
32. Bertness MD, Callaway R. Positive interactions in communities. Trends Ecol Evol. 1994;9:191–3.
33. Bakker MG, Schlatter DC, Otto-Hanson L, Kinkel LL. Diffuse symbioses: roles of plant–plant, plant–microbe and microbe–microbe interactions in structuring the soil microbiome. Mol Ecol. 2014;23:1571–83.
34. Guerra CA, Heintz-Buschart A, Sikorski J, Chatzinotas A, Guerrero-Ramírez N, Cesarz S, et al. Blind spots in global soil biodiversity and ecosystem function research. Nat Commun. 2020;11:3870.
35. Maestre FT, Salguero-Gómez R, Quero JL. It is getting hotter in here: determining and projecting the impacts of global environmental change on drylands. Phil Trans R Soc B. 2012;367:3062–75.
36. Moreno-Jiménez E, Plaza C, Saiz H, Manzano R, Flagmeier M, Maestre FT. Aridity and reduced soil micronutrient availability in global drylands. Nat Sustain. 2019;2:371–7.
37. Millennium Ecosystem Assessment Board. Ecosystems and Human Well-Being: Desertification Synthesis. 2005; Available from: http://hdl.handle.net/20.500.11822/8719
38. Kier G, Mutke J, Dinerstein E, Ricketts TH, Küper W, Kreft H, et al. Global patterns of plant diversity and floristic knowledge. J Biogeogr. 2005;32:1107–16.
39. Uroz S, Courty PE, Oger P. Plant symbionts are engineers of the plant-associated microbiome. Trends Plant Sci. 2019;24:905–16.
40. Peterson AT, Soberón J, Ramsey J, Osorio-Olvera L. Co-occurrence networks do not support identification of biotic interactions. Biodiv Inf. 2020;15:1–10.
41. Barberán A, Bates ST, Casamayor EO, Fierer N. Using network analysis to explore co-occurrence patterns in soil microbial communities. ISME J. 2012;6:343–51.
42. Doyle JJ, Luckow MA. The rest of the Iceberg. Legume diversity and evolution in a phylogenetic context. Plant Physiol. 2003;131:900–10.
43. Soudzilovskaia NA, Vaessen S, Barcelo M, He J, Rahimlou S, Abarenkov K, et al. FungalRoot: global online database of plant mycorrhizal associations. New Phytol. 2020;227:955–66.
44. Gutierrez A, Morte A, Honrubia M. Morphological characterization of the mycorrhiza formed by Helianthemum almeriense Pau with Terfezia claveryi Chatin and Picoa lefebvrei (Pat.) Maire. MYCORRHIZA. USA: Springer-Verlag; 2003. p. 299–307.
45. Torrecillas E, Del Mar Alguacil M, Roldán A, Díaz G, Montesinos-Navarro A, Torres MP. Modularity reveals the tendency of arbuscular mycorrhizal fungi to interact differently with generalist and specialist plant species in gypsum soils. Appl Environ Microbiol. 2014;80:5457–66.
46. Pang J, Ryan MH, Siddique KHM, Simpson RJ. Unwrapping the rhizosheath. Plant Soil. 2017;418:129–39.
47. Marasco R, Mosqueira MJ, Fusi M, Ramond J-B, Merlino G, Booth JM, et al. Rhizosheath microbial community assembly of sympatric desert speargrasses is independent of the plant host. Microbiome. 2018;6:215.
48. Maurice K, Bourceret A, Youssef S, Boivin S, Laurent-Webb L, Damasio C, et al. Anthropic disturbances impact the soil microbial network structure and stability to a greater extent than natural disturbances in an arid ecosystem. Sci Total Environ. 2024;907:167969.
49. Terrat S, Dequiedt S, Horrigue W, Lelievre M, Cruaud C, Saby NPA, et al. Improving soil bacterial taxa–area relationships assessment using DNA meta-barcoding. Heredity. 2015;114:468–75.
50. Op De Beeck M, Lievens B, Busschaert P, Declerck S, Vangronsveld J, Colpaert JV. Comparison and validation of some its primer pairs useful for fungal metabarcoding studies. PLoS One. 2014;9:e97629.
51. Berruti A, Desirò A, Visentin S, Zecca O, Bonfante P. ITS fungal barcoding primers versus 18S AMF-specific primers reveal similar AMF-based diversity patterns in roots and soils of three mountain vineyards. Environ Microbiol Rep. 2017;9:658–67.
52. Kurtz ZD, Müller CL, Miraldi ER, Littman DR, Blaser MJ, Bonneau RA. Sparse and compositionally robust inference of microbial ecological networks. PLoS Comput Biol. 2015;11:e1004226.
53. Tipton L, Müller CL, Kurtz ZD, Huang L, Kleerup E, Morris A, et al. Fungi stabilize connectivity in the lung and skin microbial ecosystems. Microbiome. 2018;6:12.
54. Bastian M, Heymann S, Jacomy M. Gephi: an open source software for exploring and manipulating networks. In: Proceedings of the international AAAI conference on web and social media; 2009. p. 361–2.
55. Csardi G, Nepusz T. The igraph software package for complex network research. InterJournal, complex systems. 2006;1695:1–9.
56. Searle SR, Speed FM, Milliken GA. Population marginal means in the linear model: an alternative to least squares means. Am Stat. 1980;34:216–21.
57. Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, et al. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 2016;20:241–8.
58. Newman MEJ. Mixing patterns in networks. Phys Rev E. 2003;67:5026126.
59. Liu C, Li C, Jiang Y, Zeng RJ, Yao M, Li X. A guide for comparing microbial co-occurrence networks. iMeta. 2023;2:e71.
60. Conway JR, Lex A, Gehlenborg N. UpSetR: an R package for the visualization of intersecting sets and their properties. Bioinformatics. 2017;33:2938–40.
61. Collins SL, Sinsabaugh RL, Crenshaw C, Green L, Porras-Alfaro A, Stursova M, et al. Pulse dynamics and microbial processes in aridland ecosystems: pulse dynamics in aridland soils. J Ecol. 2008;96:413–20.
62. Bouffaud M, Poirier M, Muller D, Moënne-Loccoz Y. Root microbiome relates to plant host evolution in maize and other Poaceae. Environ Microbiol. 2014;16:2804–14.
63. Brundrett MC, Tedersoo L. Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol. 2018;220:1108–15.
64. Tedersoo L, May TW, Smith ME. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza. 2010;20:217–63.
65. Kiers ET, Denison RF. Sanctions, cooperation, and the stability of plant-rhizosphere mutualisms. Annu Rev Ecol Evol Syst. 2008;39:215–36.
66. Olsson PA, Rahm J, Aliasgharzad N. Carbon dynamics in mycorrhizal symbioses is linked to carbon costs and phosphorus benefits. FEMS Microbiol Ecol. 2010;72:125–31.
67. Casati P, Andreo CS, Edwards GE. Characterization of NADP-malic enzyme from two species of Chenopodiaceae: Haloxylon persicum (C4) and Chenopodium album (C3). Phytochemistry. 1999;52:985–92.
68. Tripathi BM, Stegen JC, Kim M, Dong K, Adams JM, Lee YK. Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. ISME J. 2018;12:1072–83.
69. Sun Y, Zhang Y, Feng W, Qin S, Liu Z, Bai Y, et al. Effects of xeric shrubs on soil microbial communities in a desert in northern China. Plant Soil. 2017;414:281–94.
70. Berg N, Steinberger Y. Role of perennial plants in determining the activity of the microbial community in the Negev Desert ecosystem. Soil Biol Biochem. 2008;40:2686–95.

71. Ochoa-Hueso R, Eldridge DJ, Delgado-Baquerizo M, Soliveres S, Bowker MA, Gross N, et al. Soil fungal abundance and plant functional traits drive fertile island formation in global drylands. J Ecol. 2018;106:242–53.
72. Mosqueira MJ, Marasco R, Fusi M, Michoud G, Merlino G, Cherif A, et al. Consistent bacterial selection by date palm root system across heterogeneous desert oasis agroecosystems. Sci Rep. 2019;9:4033.
73. Fitzpatrick CR, Copeland J, Wang PW, Guttman DS, Kotanen PM, Johnson MTJ. Habitats within the plant root differ in bacterial network topology and taxonomic assortativity. MPMI. 2023;36:165–75.
74. Peel L, Delvenne J-C, Lambiotte R. Multiscale mixing patterns in networks. Proc Natl Acad Sci USA. 2018;115:4057–62.
75. Jackson MA, Bonder MJ, Kuncheva Z, Zierer J, Fu J, Kurilshikov A, et al. Detection of stable community structures within gut microbiota co-occurrence networks from different human populations. PeerJ. 2018;6:e4303.
76. Hall CV, Lord A, Betzel R, Zakrzewski M, Simms LA, Zalesky A, et al. Co-existence of Network Architectures Supporting the Human Gut Microbiome. iScience. 2019;22:380–91.
77. Fornito A, Zalesky A, Breakspear M. The connectomics of brain disorders. Nat Rev Neurosci. 2015;16:159–72.
78. Leung PM, Bay SK, Meier DV, Chiri E, Cowan DA, Gillor O, et al. Energetic basis of microbial growth and persistence in desert ecosystems. mSystems. 2020;5:e00495-19.
79. Lennon JT, Jones SE. Microbial seed banks: the ecological and evolutionary implications of dormancy. Nat Rev Microbiol. 2011;9:119–30.
80. Cadotte MW, Davies TJ. Phylogenies in ecology: a guide to concepts and methods. Princeton: Princeton University Press; 2016.
81. van der Heijden MGA, Martin FM, Selosse M-A, Sanders IR. Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytol. 2015;205:1406–23.
82. Egan CP, Rummel A, Kokkoris V, Klironomos J, Lekberg Y, Hart M. Using mock communities of arbuscular mycorrhizal fungi to evaluate fidelity associated with Illumina sequencing. Fungal Ecol. 2018;33:52–64.
83. Tedersoo L, Bahram M, Zinger L, Nilsson RH, Kennedy PG, Yang T, et al. Best practices in metabarcoding of fungi: from experimental design to results. Mol Ecol. 2022;31:2769–95.
84. Sun D. Pull in and push out: mechanisms of horizontal gene transfer in bacteria. Front Microbiol. 2018;9:2154.
85. Thomas CM, Nielsen KM. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat Rev Microbiol. 2005;3:711–21.
86. Aminov RI. Horizontal gene exchange in environmental microbiota. Front Microbio. 2011;2.
87. Arnold BJ, Huang I-T, Hanage WP. Horizontal gene transfer and adaptive evolution in bacteria. Nat Rev Microbiol. 2022;20:206–18.
88. Zhang L, Zhou J, George TS, Limpens E, Feng G. Arbuscular mycorrhizal fungi conducting the hyphosphere bacterial orchestra. Trends Plant Sci. 2022;27:402–11.
89. Warmink JA, Nazir R, Corten B, Van Elsas JD. Hitchhikers on the fungal highway: the helper effect for bacterial migration via fungal hyphae. Soil Biol Biochem. 2011;43:760–5.
90. Yang T, Tedersoo L, Liu X, Gao G, Dong K, Adams JM, et al. Fungi stabilize multi-kingdom community in a high elevation timberline ecosystem. iMeta. 2022;1:e49.
91. Herman DJ, Firestone MK, Nuccio E, Hodge A. Interactions between an arbuscular mycorrhizal fungus and a soil microbial community mediating litter decomposition. FEMS Microbiol Ecol. 2012;80:236–47.
92. Yuan MM, Kakouridis A, Starr E, Nguyen N, Shi S, Pett-Ridge J, et al. Fungal-bacterial cooccurrence patterns differ between arbuscular mycorrhizal fungi and nonmycorrhizal fungi across soil niches. mBio. 2021;12:e03509-20.

Publisher's Note