

Differential recruitment of root bacterial community by inoculated inland spiny and spineless cactus in response to salinity stress

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ARTICLE INFO

Keywords:

Cactus-coastal inoculum
salinity
Spiny cactus
Spineless cactus
Bacterial microbiome
Root-endosphere
Microbial network
Opuntia

ABSTRACT

In the field of innovative challenges, it is essential to incorporate microorganisms into agricultural practices that promote and improve plant growth and health, particularly under conditions of salinity stress. This work elucidated the response of two *Opuntia ficus-indica* cultivars (spiny, *Gialla* and spineless, *Rossa*) inoculated inland with a coastal cactus rhizospheric soil (*Opuntia littoralis*) under NaCl treatment. The two cultivars reacted differently to salinity stress. The cladodes and roots of the *Rossa* cultivar were sensitive to salinity and accumulated both Na⁺ and Cl⁻. In contrast, the *Gialla* cultivar showed Na⁺ exclusion from the cladodes and root growth was unaffected by salinity. The diversity, richness, and correlation networks of root compartments bacterial communities were mainly determined while the cactus cultivar was subjected to salinity stress. Different subsets of key soil bacteria taxa were selected by the root systems of each cultivar after exposure to salinity. Our results highlight the importance of the rhizosphere of endemic coastal plants in improving plant resistance to salinity stress, particularly in the spiny cultivar compared to the spineless cultivar. The microbiome networks provide solid evidence that each cultivar adapts its bacterial community composition and interactions in response to salinity.

1. Introduction

Increased soil salinization is seen as a key factor contributing to desertification. It is closely linked to soil degradation processes, such as the abandonment of arable land and soil erosion (D'Odorico et al., 2013; Q. Zhao et al., 2020a,b). The reduction in plant growth in dry environments is due to the reduced availability of water, the upwelling of salts from shallow water tables, the reuse of degraded waters and the intrusion of salt water (Corwin, 2020). Improving the salinity tolerance of plants therefore deserves to be studied in depth, as it will not only improve our understanding of physiology fundamentals and evolution of plants but will also allow elaborating science-based management and

conservation strategies that will preserve plant cover and maintain agricultural productivity in arid areas in the face of the future effects of climate change.

It has recently been recognized that microbial symbionts have the potential to significantly reduce the effects of salt stress more effectively than conventional breeding techniques or genetic engineering approaches (Coleman-Derr and Tringe, 2014). The phenomenon of habitat-adapted symbiosis is one of the most important symbiont-based approach, in which the microbiome from a high-stress environment confers habitat-specific stress tolerance on non-host plants (Rodriguez et al., 2008). Several reports highlighted and emphasized the positive impact of this phenomenon on plants' tolerance to salinity. For example,

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The salinity tolerance of tomatoes has been demonstrated by endophytes isolated from dune grass plants of coastal saline soils (Rodríguez et al., 2008). In another study, a bacterial consortium collected from the rhizosphere of halophyte *Suaeda salsa* was inoculated to non-host plants to improve their growth and tolerance to salinity stress (Yuan et al., 2016). Finally, Rodríguez et al. (2008) and Yuan et al. (2019), reported that the rhizosphere microbiome favored the germination and growth of *Hibiscus hamabo* under salinity conditions. These pertinent results suggest the improved tolerance potential of plants would be mediated at the same time by individual microbes as well as by the microbial consortium as a whole (multi-microbe cooperation). This opens up promising avenues for exploiting microbial inoculants to increase plant productivity and salt tolerance in arid areas (Santos and Olivares, 2021; Trivedi et al., 2021).

Cacti are prominent plants in arid regions but are sensitive to NaCl, tolerating only up to 5–6 dS m⁻¹ in soil saturation extract (Le Houérou, 2002). Salinity can induce dormancy in cactus seeds (Podda et al., 2017), and their response to soil salinity varies by habitat (Goodman et al., 2012; Nobel, 1998). For instance, *Opuntia humifusa* from inland sites exhibited drastically inhibited growth when watered with 150 mM NaCl compared to those from marine sites (Silverman et al., 1988). This highlights how cacti-associated microorganisms can enhance tolerance to abiotic stresses. However, despite ongoing studies on the composition and diversity of these microbial communities (Flores-Nunez et al., 2020; Gargouri et al., 2021), the mechanisms underlying these interactions and their role in cactus adaptation to extreme salinity remain unclear. *Opuntia* plants provide multiple services, including food, fodder, bio-energy, and natural resource conservation (Cushman et al., 2015; Feugang et al., 2006; Inglese et al., 2017; Ranjan et al., 2016). Their utility depends on whether they are spiny or spineless (Felker et al., 2006). While spiny types, used for living fences and windbreaks, show higher cold tolerance, spineless types are favored for food and fodder due to ease of use. Yet, no study has explored the effects of salinity stress on both types or the role of microbiota in their salinity tolerance.

In our study we hypothesize that: i) Rhizosphere soils from coastal cacti (*Opuntia littoralis*) contain specific microbial communities which have evolved evolutionarily adaptive potentials to high salinity; ii) this tolerant rhizosphere microbiome may have conferred, via mutual beneficial interactions, tolerance abilities to the two inland cactus cultivars: *Opuntia ficus-indica* var. *inermis* cv. 'Rossa' (spineless) and *Opuntia ficus-indica* cv. 'Gialla' (spiny). These hypotheses were addressed by comparing the assembly and structure of bacterial communities associated with root endospheres of two inoculated cultivars under saline stress in greenhouses. We focused on quantifying various physiological and ion content parameters associated with salt adaptation. We then adopted a bottom-up approach (re-colonization and salt stress assay) to demonstrate changes in co-occurrence patterns and to identify potential bacterial hubs responsible for salt tolerance in each cactus cultivar.

2. Materials and methods

2.1. Plant material and Experimental design

Two cactus cultivars were selected: *Opuntia ficus-indica* cv. 'Gialla', a spiny cultivar with dark yellow fleshed-fruits, and *Opuntia ficus-indica* var. *inermis* cv. 'Rossa' known to some farmers in Tunisia as 'Moore' a spineless cultivar with purple-fleshed fruits (Nefzaoui et al., 2010). In April 2021, two-year-old non-rooted cladodes were cut at the subterminal junction from vigorous *Opuntia* mother plants growing in the open field under a semi-arid climate in the El-Marja region, 50 km North-East of Tunis, Tunisia (35° 35' N; 8° 40' E) (Bouamama et al., 2011). Each cladode weighed approximately 500 g. From each cultivar, 10 fresh cladodes were air-dried, in the shade, for a fortnight to promote transplanting. They were then potted vertically, with the basal part 1/3 buried in 2 L pots filled with autoclaved sand (1 h at 100 °C repeated for 3 days), to ensure the absence of microbes, and clay stones in the bottom

of the pot to prevent waterlogging (Fig. 1). Prior to inoculation, all pots were maintained for 10 months and watered every 3 days with a half-concentration of Hoagland (Arnon and Hoagland, 1940) under similar greenhouse conditions (daytime temperature range: 25–30 °C; photoperiod of 8–10 h with an irradiance: 500–600 µE m⁻¹ s⁻¹). Before being exposed to the salinity treatments, all rooted cladodes were transferred to 10 L pots filled with a mixture of autoclaved substrate (2:1, sand: soil, v:v) inoculated with 500 g of rhizosphere soil taken from around the roots of *O. littoralis* located in a coastal area of the same region (36° 44' 04 N, 010° 28' 22 E). The microbial composition of the rhizosphere soil of *O. littoralis* was thoroughly analyzed to ensure that the inoculum contains beneficial microbes that could contribute to plant health and abiotic stress tolerance. To prevent the substrate drying out and encourage microbial colonization of the roots, all inoculated *Opuntia* cladodes were watered with tap water every 3 days and with a 0.1% of Hoagland solution at a frequency of once a week for 1.5 months (Fig. 1). Four treatments were defined as: Gialla control (GC), Rossa control (RC), Gialla stressed (GS), and Rossa stressed (RS). For the salinity treatments, five replicates of randomized cladodes from each group were watered with 150 mM NaCl (~9 g.L⁻¹), a concentration known to drastically affect the growth of *Opuntia* plants (Nerd et al., 1991), while the five control replicates from each group received only tap water with 0 mM NaCl. To prevent salinity build-up, plants were watered once a week with 50 ml of 0.1% Hoagland solution for 2 months (Fig. 1).

2.2. Physiological parameters assessment

To assess the salinity tolerance of the spiny and spineless *Opuntia* cultivar, we measured shoot and root growth and changes in shoot and adventitious root biomass over the course of the experiment. The roots and cladodes were separated, and the fresh weight was assessed. Root length cutting height from bottom basal to the top sub-terminal cladode were also assessed. The number of healthy cladodes (i.e., new developed sprouts) and damaged cladodes (i.e., cladodes with necrosis, excluding those with a yellowish color, flaccid phenotype or reduced succulence) of each plant was recorded. Based on fresh weight, water content was determined in three cross sections samples (2.5 cm in diameter each) per cladode from each plant.

2.3. Determination of Na⁺ and Cl⁻ contents

Na⁺ and Cl⁻ contents in cladodes and roots samples were determined according to the detailed protocol (Munns et al., 2010). Briefly, the cladodes and roots freed of substrate debris were oven dried at 60 °C for a week and ground to a fine powder using a cryogenic grinder MM400 (RETSCH). Forty mg of the dried samples were digested with 50 mL of 0.5 % nitric acid and mixtures filtered through Whatman filter papers. Na⁺ concentration was determined using a flame emission spectrophotometer (Jenway PFP7) and Cl⁻ concentration was determined by the Coulometric method using a Chloridometer (Buchler-Cotlove) with acetic acid buffer.

2.4. Metagenomic root DNA extraction and 16S rRNA analysis

To target the bacterial endosphere of the roots, another set of root samples were first washed for 2 min using a 1% sodium azide solution to remove the epiphytic micro-organisms and then washed by agitation for 30 min in sterilized water. Extraction of total DNA was carried out on 250 mg freeze-dried roots using "MO BIO PowerSoil DNA extraction kit" (MO BIO Laboratories) and the quantification used the "NanoDrop System 2000" (Thermo Scientific Co.). PCR amplicons amplification and generation of Illumina libraries were achieved according to Karray et al. (2020). Briefly, Sequencing of PCR amplicons of 16S rDNA was conducted using the Illumina MiSeq platform, specifically targeting the V3–V4 hypervariable regions of the 16S rRNA gene. We used the

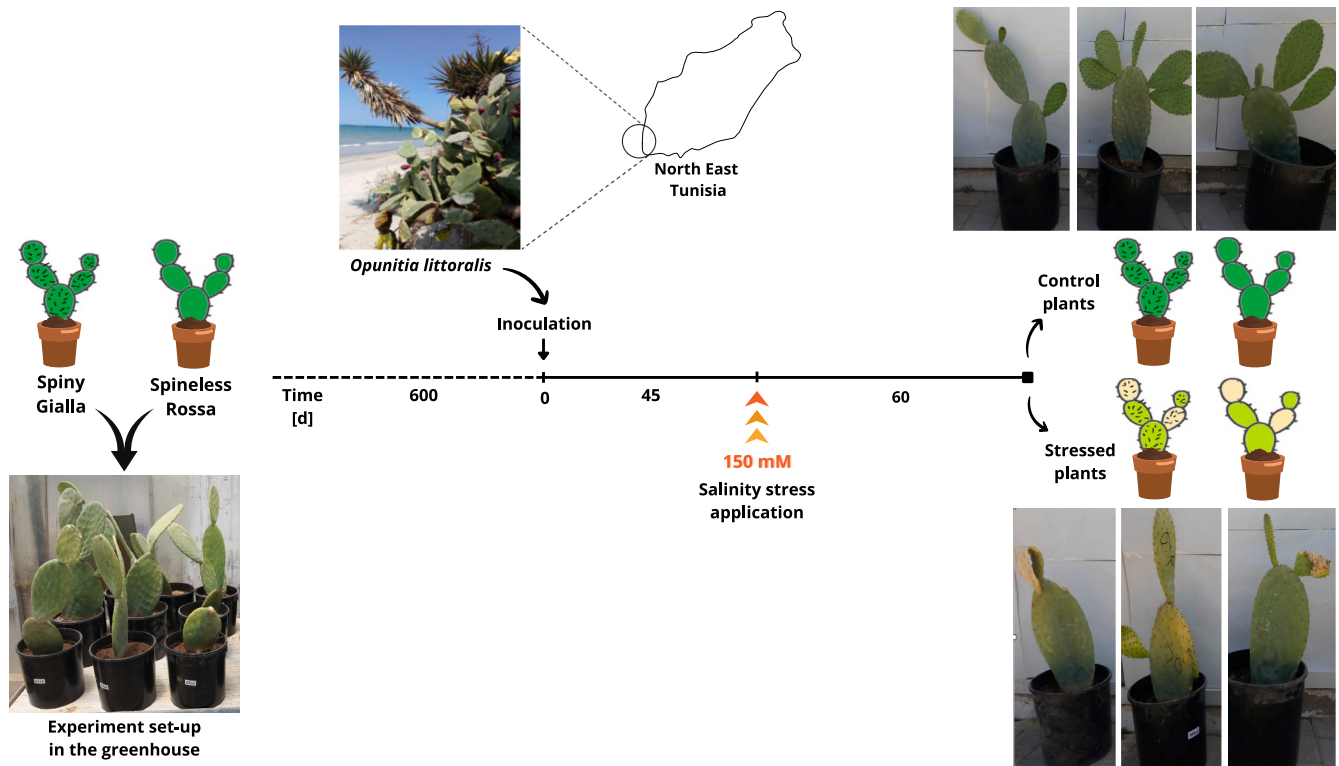


Fig. 1. Schematic overview of the inoculation trial with spiny (*Opuntia ficus-indica* cv. 'Gialla') and spineless (*Opuntia ficus-indica* var. *inermis* cv. 'Rossa') cacti plants. GC, Gialla control plants; GS Gialla stressed plants; RC Rossa control plants; RS Rossa stressed plants.

prokaryotic universal primer sets (Pro341F/Pro805R) for 2×300 bp paired-end sequencing (Illumina). The analysis of the raw data sequences was achieved with the QIIME software package 1.9.1 (Caporaso et al., 2010). Mitochondrial and chloroplast OTUs were subtracted from all sequence's samples. Finally, an OTU table was created and the raw 16S reads were registered in the NCBI Short Read Archive and received project no. PRJNA1112098.

2.5. Bacterial diversity, taxonomic distribution, phenotypical and functional analysis

QIIME (Version 1.9.0) was used to calculate the Simpson and Shannon Alpha diversity indices (Caporaso et al., 2010). A comparison of microbial communities on different samples was conducted using beta diversity. The matrices generated from Bray-Curtis dissimilarity distances were used to carry out a principal coordinate analysis (PCoA), and a permutational multivariate analyses of variance (PERMANOVA, 5000 permutations) as described by Ramette (2007). The OTUs commonly shared between different treatments were revealed by the Venn-diagram analysis involving the software available at <http://bioinformatics.psb.ugent.be>. The OTU table (Wilcoxon p-value: 0.05, LDA > 4) was subjected to a linear discriminant analysis effect size (LEfSe, <http://huttenhower.sph.harvard.edu/galaxy/>) to attempt identifying the discriminant bacterial clade (at family level) of both 'Gialla' and 'Rossa' cultivars in salt-stressed and control samples (Segata et al., 2011). The Similarity Percentages (SIMPER) analysis using PRIMER 6 was carried out to identify existing dissimilarities between cultivars and root treatments. The Unclassified OTUs, representing less than 3% of the relative abundance in the root system, were not considered in the analysis, as suggested by Clarke and Warwick (2001). BugBase was used to predict potential microbial phenotypes between the four treatments (Ward et al., 2017). Functional annotation of prokaryotic taxa (FAPROTAX) were used to predict the potential functional capacities of stressed bacterial communities of spiny and spineless cacti (Louca et al.,

2016).

2.6. Network Inference, Construction, and visualization

A co-occurrence network analysis was carried out on the root endophytic microbiome for each cultivar grown in the presence/absence of salinity, in order to explore significant relationships between OTUs (Weiss et al., 2016). The analysis used CoNet v. 1.1.1 beta based on the OTUs tab-delimited file with taxonomy annotation in Cytoscape 3.8.2 (Faust and Raes, 2016). In order to build the network, we filtered out those OTUs with a frequency < 0.05, then we performed Pearson and Spearman correlations between all pairs. We set the Pearson and Spearman correlation (r) threshold to 0.75 and combined it to Bray-Curtis (BC) and Kullback-Leibler (KLD) dissimilarity indices. To determine the statistical significance of co-occurrence and mutual exclusions, we computed the edge-specific permutation and bootstrap score distributions with 1000 iterations. The nodes in each network corresponding to different OTUs were classified according to their affiliation to phylum or class (97%) and were sized depending on the degree of connection. All of the P values were adjusted before to be subjected for multiple testing with Benjamini and Hochberg FDR controlling procedure (Benjamini et al., 2006). Networks of co-occurred microbes were visualized using Gephi (version 0.9.2) (Bastian et al., 2009). Topological analyses were carried out using "NetworkAnalyzer" (Doncheva et al., 2012) tool in the Cytoscape context to determine network topology parameters (i.e., network diameter, network heterogeneity, average number of neighbors, graph density, clustering coefficient, network centralization and shortest path). We consider as hubs those OTUs with the highest connection degree values, high closeness centrality and low betweenness centrality (Banerjee et al., 2018).

2.7. Statistical analysis

One-way analysis of variance (ANOVA) and mean comparison

procedures between treatments on 'Gialla' and 'Rossa' cultivars were carried out by the Tukey's HSD ($p < 0.05$). The relative abundance of each phylum, class, was summarized in histogram graphs using R software environment (v 163 0.3.2.5) (R Core Team, 2021). Statistical differences between the degrees of connection in the co-occurrence networks for control/stressed spiny and spineless cacti were analyzed with IBM SPSS Statistics software v.21 (IBM Corp., 2017).

3. Results

3.1. Effect of salinity on length and fresh weight of shoots and roots of spineless and spiny cacti

Results showed there is a distinct effect of the salinity on the growth of the two inoculated cultivars (Fig. 2a). In spiny cultivar (Gialla), the shoot length of GS decreased around 2 times to the respect plants (GC), while the root length decreased about 0.7 of that control plants. In spineless cultivar (Rossa), the shoot length of RS decreased by 0.7, while the decrease of the RS root length was about 1.1 of that control plants (RC). The effect of salinity on the roots weight of GS was less pronounced than the length showing no significant changes, while the shoots decreased by half. However, shoots and roots weight of the spinless cultivars decreased by 0.6 as compared to their controls (Fig. 2b). The shoot water content % was distinctly decreased in RS and GS plants as compared to their respective controls (Fig. 2c).

It is worthy to mention that under control conditions (after inoculation and before salinity application), the spineless cultivar (RC) has a longer shoot length and the heavier roots than the spiny cultivar (GC).

To assess whether the salinity affects the sprouting of cladodes, we recorded the number of the newly developed sprouts (DS) and damaged cladodes (DC) at the end of experiment. The significant development of new sprouts was observed only in the cladodes of GC plants as compared to RC. Moreover, RS plants were presenting the highest DC number as compared to RC (Table 1).

3.2. Effect of salinity on Na^+ and Cl^- concentrations in shoots and roots of spineless and spiny cacti

Knowing that cladodes water content changes due to salinity (Fig. 2c), we used dry tissue ion contents to determine net ion accumulation in cuttings of spiny and spineless cacti. In general, roots displayed much higher Na^+ content than shoots (Fig. 3a). Our outcomes showed that salinity significantly increased Na^+ content in RS shoots around two times more than that in RC shoots. However, there was no significant increase of Na^+ in GS versus GC shoots (Fig. 3a). In the roots, GS cuttings accumulated the highest Na^+ amount (24.8 mg/gDW) as compared to GC (6.5 mg/gDW), RS (17.8 mg/gDW), and RC (8.8 mg/gDW). In contrary to Na content, Cl content was lower in roots than in cladodes (Fig. 3b). The stressed shoots from both cultivars significantly increased Cl^- amount as compared to their controls. Similarly, to Na accumulation, RS shoots have also the highest Cl^- amount. In the roots, Cl^- content was increased in the stressed cuttings RS and GS around four times than that in their controls (RC and GC) (Fig. 3b).

3.3. Richness, diversity and bacterial taxonomic profiles abundance between root endosphere of spineless and spiny cacti under salt stress

As a result of our high-throughput sequencing analysis (MiSeq Illumina), we obtained 670,260 high-quality reads with an average length of 442 bases. Good's coverage percentage of the root-endospheric samples of both control and stressed 'Rossa' and 'Gialla' cultivars was $>99\%$. To assess whether salinity affects diversity of prokaryotic communities of these two cultivars, we compared the root entophytic alpha- and beta-diversity of the controls and stressed cladodes. Average alpha diversity values were not significantly different between treatments ($p > 0.05$), whereas Shannon and Simpson indexes, which consider both

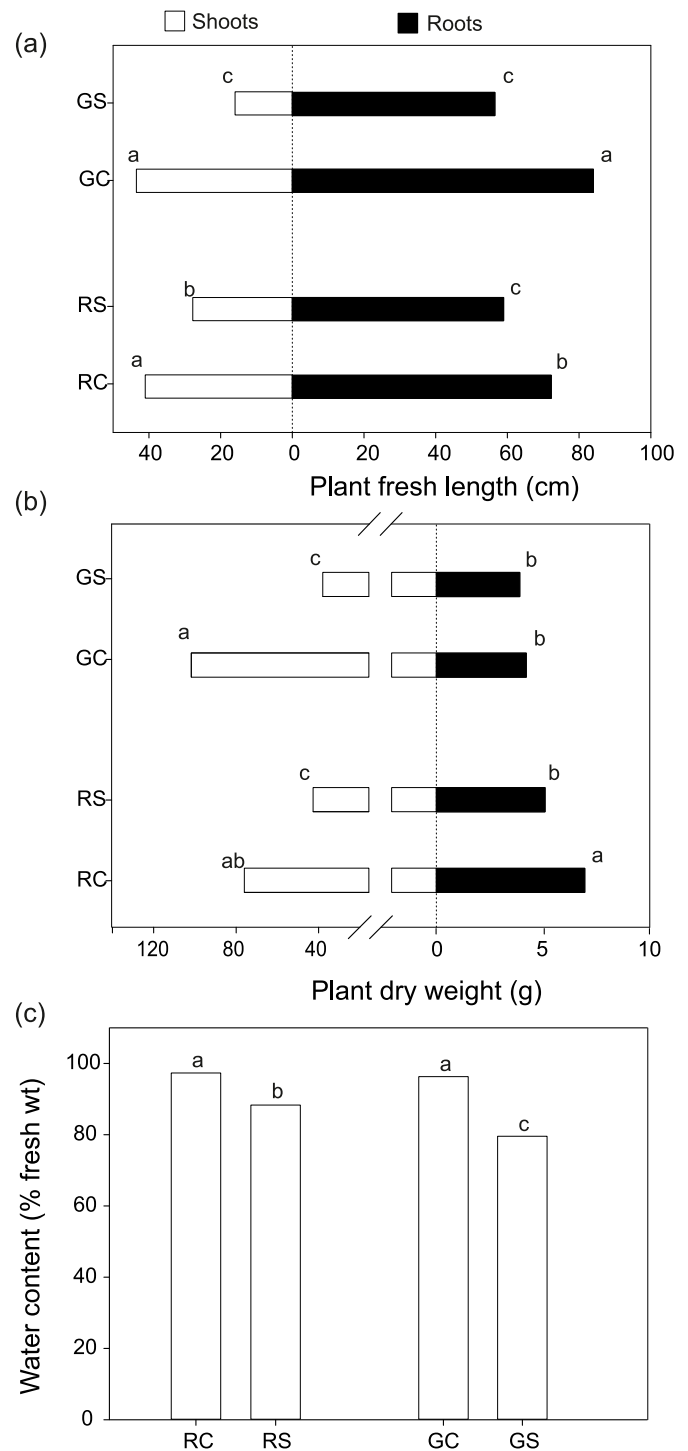


Fig. 2. Comparison of physiological parameters in shoots and roots of spiny and spineless *Opuntia*. (a) Average of plant fresh length in cm, (b) Average of plant dry weight in gram, (c) Water content in % fresh wt. White bars represent shoots samples and black bars represent roots samples of control plants (RC and GC) and stressed plants (RS and GS). Values are the means of five replicates. Graphs denoted with different letters are significantly different according to the Tukey test (HSD) at $p < 0.05$.

the richness and evenness of groups, indicated that the alpha-diversity decreased significantly in the stressed treatments as compared to the control treatment for both cultivars ($p < 0.05$). The GS cuttings presented the lowest richness and evenness values (Supplemental Fig. S1), suggesting that the diversity of bacterial community was highly related

Table 1
Means of newly developed sprouts (DS) and damaged cladodes (DC) per treatment during salt stress application. Means ± S.E. are presented.

Cultivar	Mean Developed Sprouts (DS)		Mean Damaged Cladodes (DC)	
	Control	Stressed	Control	Stressed
Rossa	0.00 ± 0.0 ^b	0.00 ± 0.0 ^b	0.00 ± 0.0 ^b	1.60 ± 0.2 ^a
Gialla	2.33 ± 0.2 ^a	1.66 ± 0.4 ^{ab}	0.00 ± 0.0 ^b	0.66 ± 0.4 ^{ab}

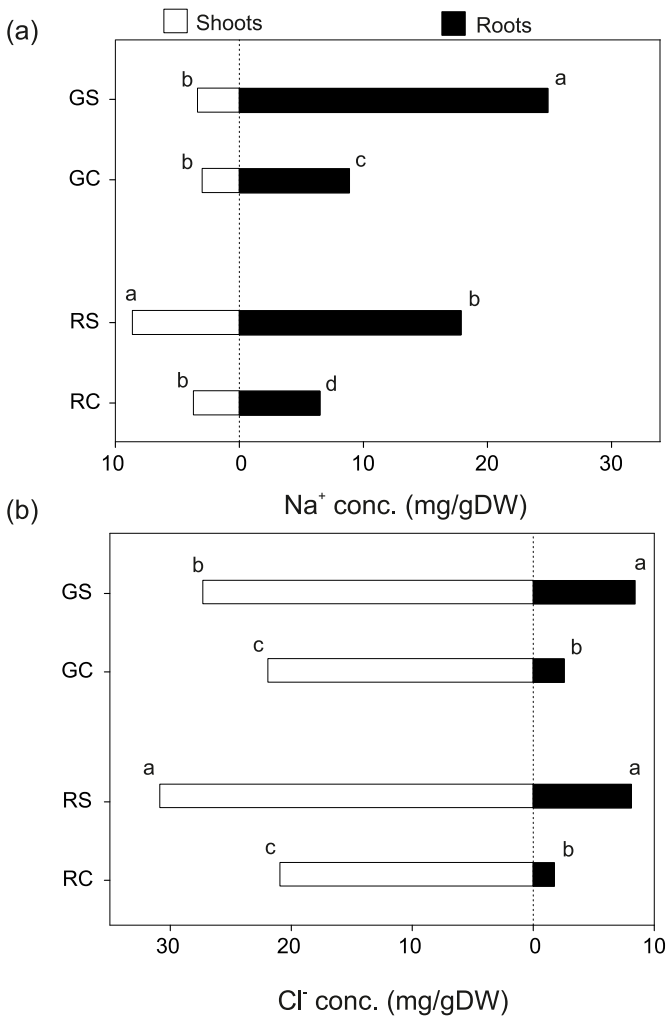


Fig. 3. Comparison of ion content in shoots and roots of spiny and spineless Opuntia. (a) Na⁺ content, (b) Cl⁻ content. White bars represent shoots samples and black bars represent roots samples of control plants (RC and GC) and stressed plants (RS and GS). Values are the means of five replicates. Graphs denoted with different letters are significantly different according to the Tukey test (HSD), $p < 0.05$.

to the cultivar (Rossa or Gialla) and the salinity application. The PCoA beta-diversity analysis showed also a significant dissimilarity between control and stressed treatments for each cultivar, which was more apparent and distinct than the clustering between control groups or stressed groups (Fig. 4a).

To identify the effect of salinity on the structure of prokaryotic communities in each cultivar, the OTUs were classified at phylum level, with the exception of *Proteobacteria*, which was divided by class. The OTUs were assigned to 22 bacterial phyla and 2 archaea phyla (Fig. 4b). Phylogenetic profiling showed significant differences in the root endosphere associated prokaryotic communities of Rossa and Gialla cacti following the application of salt stress. Indeed, the relative abundance of *Bacteroidetes* (5.9% RS - 9.7% RC), *Firmicutes* (1.3–3.7%),

Deltaproteobacteria (0.1–0.6%) and *Gammaproteobacteria* (12.8–19.8%) was enriched in RS vs. RC, while they were depleted in GS vs. GC (11.5–7.2%), (7.0–3.7%), (1.1–0.9%), and (19.8–13.8%), respectively. To the contrary, the relative abundance of *Chloroflexi* (1.9–0.5%) and TM7 (5.1–2.9%) was significantly decreased in RS and increased in GS (1.0–1.9%) and (1.1–2.4%). *Actinobacteria* was the only phylum significantly depleted (around 50%) in both RS and GS as compared to their controls. The relative abundance of *Betaproteobacteria* (2.7–0.8%) and *Verrucomicrobia* (3.7–2.6%) was decreased specifically in RS, while that of *Alphaproteobacteria* (20.1–41.9%) and *Cyanobacteria* (0.04–1.8%) were specifically enriched in GS. The comparative analysis of the prokaryotic community composition between controls samples (RC and GC) revealed that *Bacteroidetes*, *Firmicutes*, *Acidobacteria*, *Gemmatimonadetes*, *Deltaproteobacteria*, and *Gammaproteobacteria* were significantly more abundant in GC, while *Alphaproteobacteria*, *Chloroflexi* and TM7 were significantly larger in RC. It is worth to mention that the comparison between stressed samples (RS and GS) showed that *Gammaproteobacteria*, *Bacteroidetes*, *Firmicutes*, TM7, and *Gemmatimonadetes* were enriched in RS, whereas only the relative abundance of *Chloroflexi* was enriched in GS (Fig. 4b–Supplemental Table S1).

3.4. Dominant taxa, specific and shared prokaryotic assemblages in the root endosphere of spiny and spineless cacti under salinity stress

The high-quality reads were clustered to a total of 1144 bacterial OTUs with a 97% identity threshold within the spineless and spiny samples, averaging 513 in RC, 484 in RS, 513 in GC, and 503 in GS. Despite the differences between the controls and the stressed cultivars, 98 OTUs were detected in all the treatments, being the core bacterial microbiome (Fig. 5a). Moreover, differences in OTUs were observed, suggesting that each cultivar under the two salinity conditions had its own specific bacterial population. Notably, the number of specific OTUs of both control cultivars RC (161) and GC (151) was higher than their respective stressed cultivars RS (133) and GS (135), with distinct bacterial community structures (>1%). OTUs related to *Actinobacteria*, *Alphaproteobacteria*, *Bacteroidetes*, *Gammaproteobacteria* and *Verrucomicrobia* were the dominant taxa among the common and the specific bacterial population in all treatments. Nevertheless, TM7 dominated specifically the non-common Rossa OTUs, while *Acidobacteria* and *Betaproteobacteria* were specific dominant taxa of the non-common Gialla OTUs.

The linear discriminant analysis effect size (LEfSe) revealed that salinity stress elicited unique sets of bacterial enrichment within both cultivars (Fig. 5b and c). RS showed a higher number of differentially abundant family clade than GS (15 and 5, respectively). We used a cut off of LDA score >4.0 for further analysis. In particular, stress-enriched bacteria associated with RS largely belonged to *Glycomycetaceae*, *Halomonadaceae*, *Oceanospirillaceae*, *Saccharospirillaceae*, *Pelagibacteraceae*, *Alcanivoracaceae*, and *Kiloniellaceae* (Fig. 5b). This latter was found to have the highest LDA score in both stressed population and it was the only family bloomed in stress-enriched bacteria associated with GS (Fig. 5c). *Chloroflexaceae* and *Chthoniobacteraceae* were the most differentially abundant families in RC, whereas *Thermomonosporaceae* and *Alcaligenaceae* were detected with high abundance in GC.

3.5. Phenotypic, functional prediction, and simpler analysis of prokaryotic communities in the root endosphere of spineless and spiny cacti under salinity stress

Using BugBase, we were able to predict biological interpretable phenotypes of the bacterial community. The bacterial phenotypes were classified according to their oxygen tolerance (aerobic, anaerobic, or facultatively anaerobic), gram stains (gram-positive and gram-negative bacteria), mobile element, biofilms formation, pathogenicity, and stress tolerance (Fig. 6a, Supplemental Table S2). BugBase revealed that all phenotypes were not significantly different between all treatments

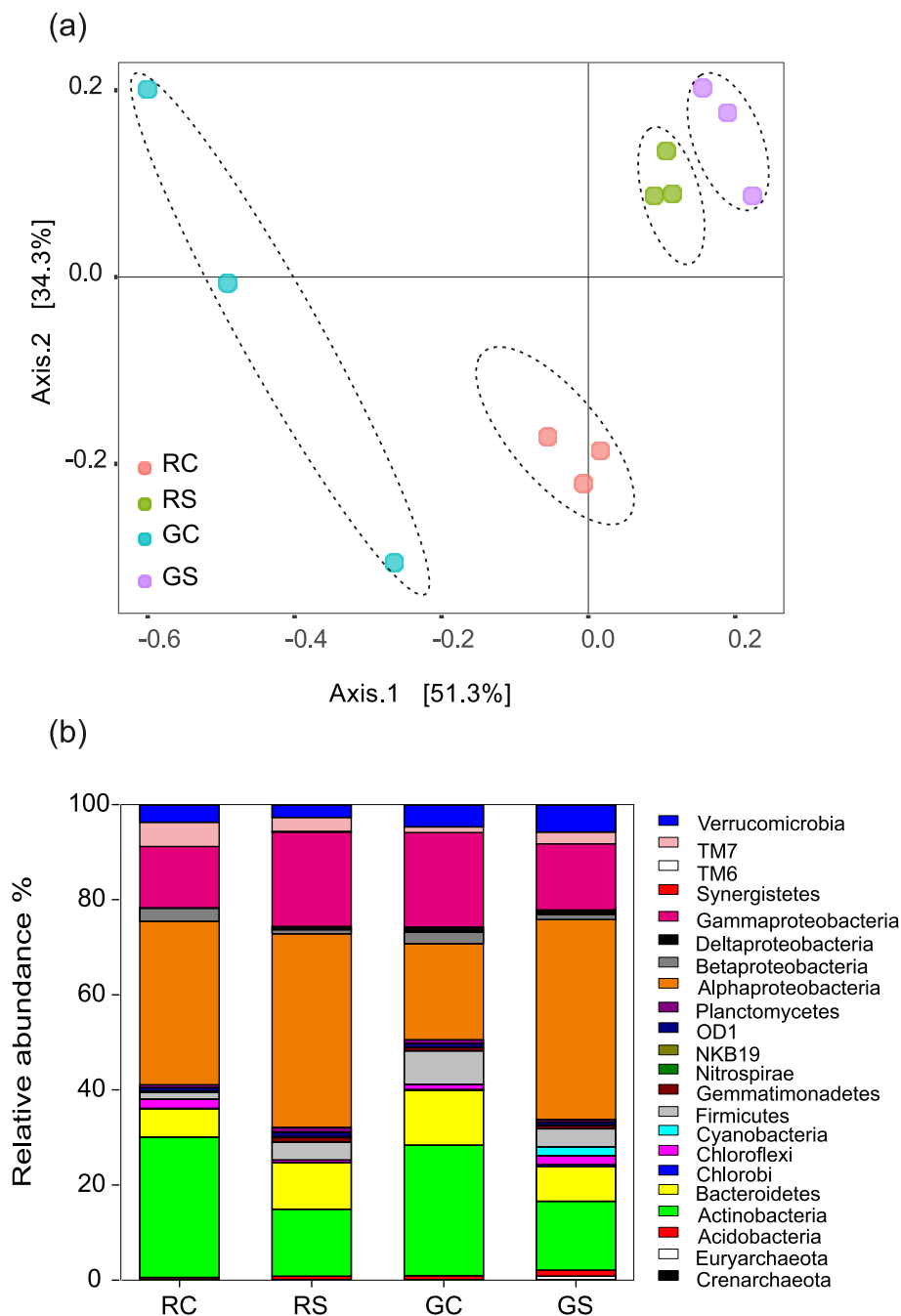


Fig. 4. Relative abundance of root endosphere prokaryotic communities of spiny and spineless *Optunia*. (a) Principal coordinates analysis (PCoA) plot for Bray–Curtis distances of the bacterial and archaeal communities associated with *Optunia* in endosphere of the two cultivars (stress value = 0.20714, PERMANOVA, $p = 0.0120$, R-Squared = 0.08595, F-value = 2.22). (b) Relative abundance based on frequencies of 16S rRNA gene sequences affiliated with different bacterial phyla, Proteobacterial classes and archaea of different endospheric root treatments of the two cultivars. RC: Rossa control samples, RS: Rossa stressed samples, GC: Gialla control samples and GS: Gialla stressed samples.

except the phenotype associated with stress tolerance. Interestingly, a significantly higher abundance of stress tolerant bacteria in GS samples was observed in comparison with either GC or RS. Moreover, predicted functions based on FAPROTAX analysis revealed that 36.6 % (410 out of 1118) of the bacterial OTUs were at least assigned to one ecological type. (Fig. 6b). A total of 32 metabolic and ecological types were predicted, among which 11 out were significant ($p < 0.05$, Supplemental Table S3) with chemo-heterotrophy and aerobic chemo-heterotrophy as the dominant functions. For both cultivars, stressed samples had significantly fewer fermentation function profile as compared to their respective controls. Interestingly, salinity stress also affected

significantly several estimated functions especially associated with anoxygenic photoautotrophy_S oxidizing, anoxygenic photoautotrophy, photoautotrophy, nitrate denitrification, nitrous oxide denitrification, denitrification, nitrite respiration, observed in GS vs. GC. Altogether, the above results suggest that phenotypic and functional profiles revealed specifically a clear shift in the bacterial community of stressed Gialla cultivars. This was further confirmed by similarity percentages (SIMPER) analysis (Supplemental Table S4), which identified *Streptomyces*, *Bacillus* and *Actinoplanes* among the top three contributing genera to the dissimilarity between GC and GS treatments. Furthermore, *Microbulbifer*, *Devosia* and *Thalassospira* genera were decisive in the

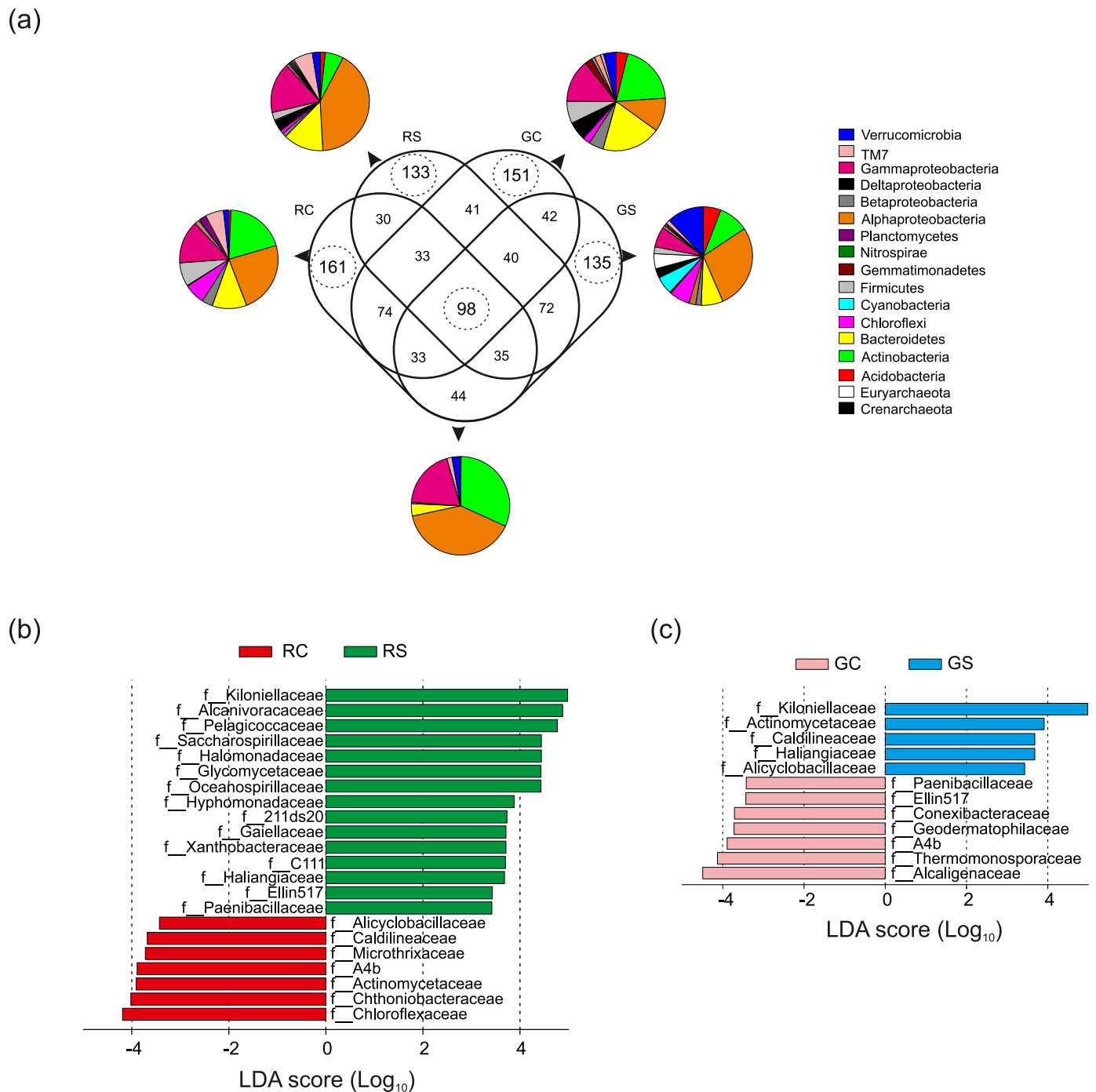


Fig. 5. Dominant taxa, specific and shared prokaryotic assemblages in the root endosphere of spineless and spiny cacti. (a) Venn diagram showing the distribution of the common and exclusive prokaryotic OTUs of control and stressed root cultivars of opuntia. Specific number of OTUs for Rossa and Gialla roots and their taxonomic affiliation were changed by the application of salinity treatment. Pie chart showing the relative abundance of the major bacterial phyla/Class for Rossa control (RC), Rossa stressed (RS), Gialla control (GC) and Gialla stressed (GS) plants. The characteristics of specific OTUs from the four treatments with a frequency higher than 1%. (b) Linear discriminant analysis (LDA) displaying differences between bacterial communities associated with control and stressed roots of Opuntia. The bar charts show the linear discriminant analysis scores determined by LefSe analysis, computed for features (at the operational taxonomic unit level), of differentially abundant taxa (at the family level) on root samples.

differentiation of the endophyte communities from GS and RS.

3.6. Identification of prokaryotic-network hubs that correlate with salinity stress in the root endosphere of spineless and spiny cacti

An analysis of the co-occurrence of the four generated bacterial networks in the root endosphere of control and stressed plants revealed different connectivity patterns influenced not only by the cactus cultivar

but also by the NaCl treatment (Fig. 7). We also counted a number significantly higher of mutual exclusion than co-presence interactions in all of the networks (Table 2). The subnetwork topologies analyses showed also significantly lower clustering coefficient, network density, and network centralization and longer characteristic path length, in the stressed subnetworks of both cultivars (RS and GS) than in the controls (RC and GC, Fig. 7). Furthermore, more nodes and edges were significantly identified in networks of spiny plants (Gialla) than in spineless

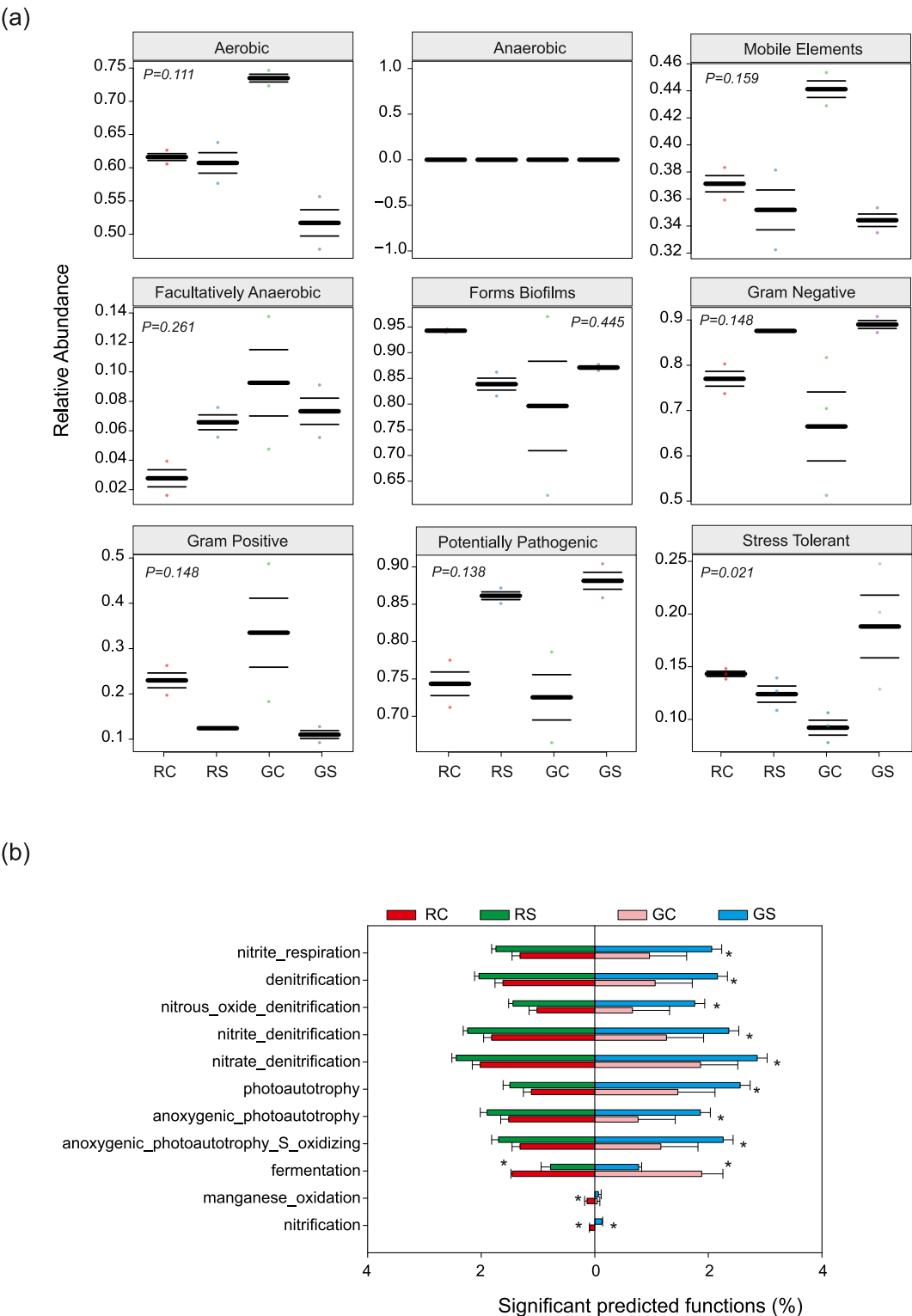


Fig. 6. Phenotypic and functional prediction analysis. (a) BugBase analyses, based on the NGS dataset. Outcome is grouped into four treatments (RC, RS, GC, and GS) on the x-axis. Relative abundance is given on the y-axis. BugBase identified phenotypes associated with aerobic, anaerobic, facultatively anaerobic, potentially pathogenic, stress tolerance, mobile element, biofilms formation, gram negative bacteria and gram positive bacteria. (b) Barplot of predicted functions based on FAPROTAX database. Stars indicate $P < 0.05$ according to the Tukey test (HSD). Error bars denote the SD of ($n = 3$) samples of each treatment.

plants (Rossa).

The taxonomy of the connected nodes was significantly different for the spineless and spiny root endosphere as compared to their respective controls after NaCl treatment ($p < 0.05$, [Supplemental Table S5](#)). *Alphaproteobacteria* dominated the topology of the stressed bacterial

networks in both spineless and spiny root endosphere than controls (up to 27% of the total degree of connection in RS vs. 14.6% in RC; and 31.4% of the total degree of connection in GS vs. 16.9% in GC), while *Bacteroidetes* showed two times decrease in both stressed networks as compared to controls. In the spineless network, connectivity among the

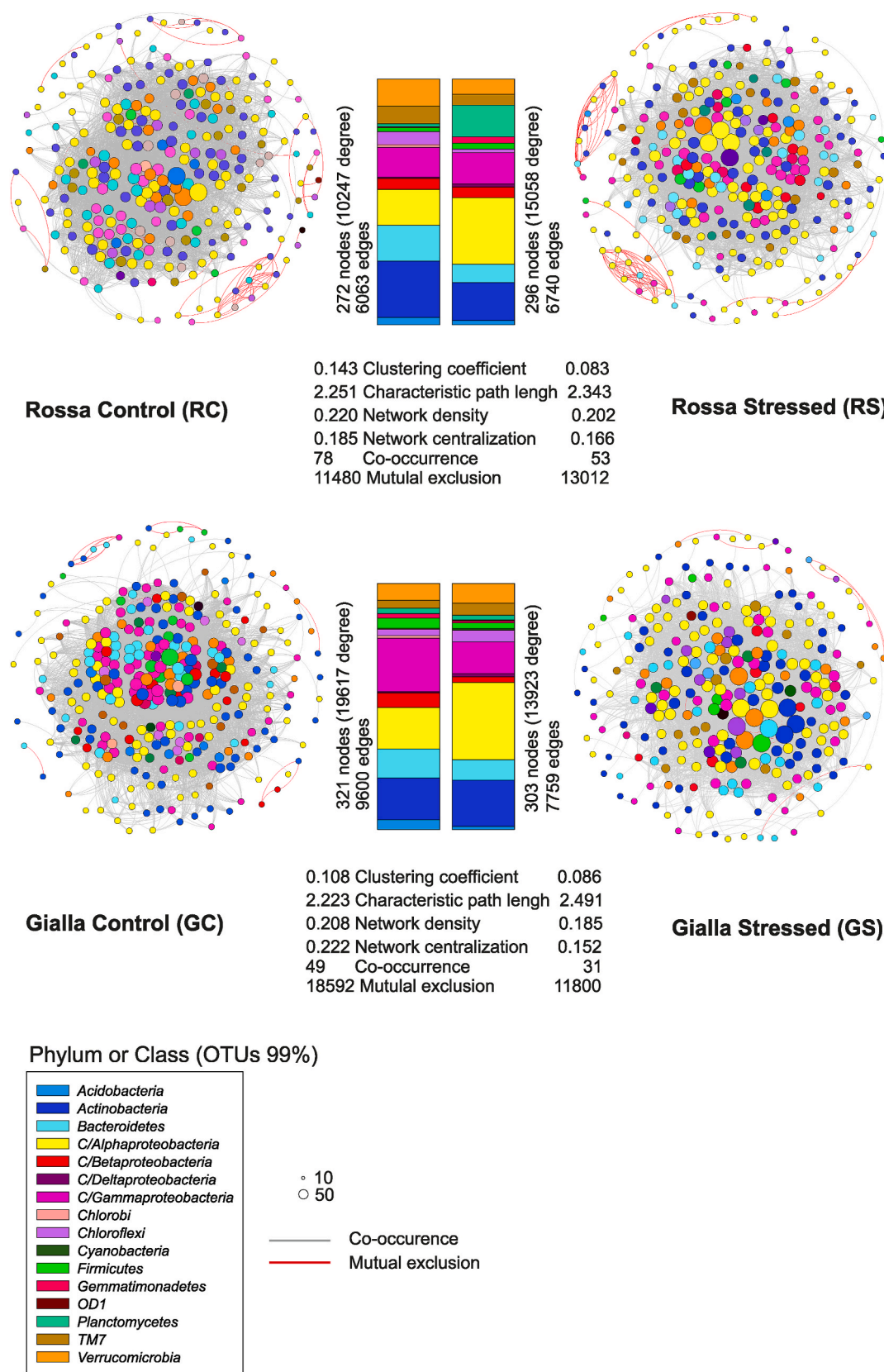


Fig. 7. Network topology plots of bacterial communities associated with root systems of spiny and spineless Opuntia. Subnetworks represent interaction among the OTUs in control and stressed roots of Rossa and Gialla cultivars. The nodes of each network are colored according to phylum or class affiliation (97%) and sized according to degree of connection. The edges connecting the nodes are represented by gray lines to indicate co-occurrence interactions and by red for mutualistic exclusions. Degree of connection is reported at the phylum/class level in the bar chart for each root treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Effect of salt stress on the phylum/class connection within root bacterial community networks of spineless and spiny cactus. Four treatments were defined as: Gialla control (GC), Rossa control (RC), Gialla stressed (GS), and Rossa stressed (RS).

Distribution of degree of connection	RC			RS			GC			GS		
	Tot	(-)	(+)	Tot	(-)	(+)	Tot	(-)	(+)	Tot	(-)	(+)
	%	%	%	%	%	%	%	%	%	%	%	%
<i>Acidobacteria</i>	3.0	100	0	1.9	100	0	4.1	100	0	1.3	100	0
<i>Actinobacteria</i>	22.9	100	0	15.3	100	0	16.9	100	0	18.8	100	0
<i>Bacteroidetes</i>	14.6	99	1	7.5	99	1	11.7	100	0	8.3	100	0
<i>C/Alphaproteobacteria</i>	14.6	99	1	27.0	99	1	16.9	100	0	31.4	100	0
<i>C/Betaproteobacteria</i>	4.5	100	0	4.4	99	1	5.9	99	1	2.3	99	1
<i>C/Deltaproteobacteria</i>	0.4	100	0	1.1	100	0	0.5	100	0	1.3	100	0
<i>C/Gammaproteobacteria</i>	12.2	98	2	12.8	100	0	21.8	100	0	12.8	100	0
<i>Chlorobi</i>	0.9	100	0	0.0	0	0	1.1	100	0	0.0	100	0
<i>Chloroflexi</i>	5.4	98	2	1.4	100	0	2.6	100	0	4.7	99	1
<i>Cyanobacteria</i>	0.0	0	0	0.0	0	0	0.4	100	0	0.6	100	0
<i>Firmicutes</i>	1.6	100	0	2.4	99	1	4.1	100	0	2.3	100	0
<i>Gemmatimonadetes</i>	0.4	100	0	2.6	100	0	1.9	100	0	0.7	100	0
<i>OD1</i>	0.0	0	0	0.0	0	0	0.0	0	0	0.4	100	0
<i>Planctomycetes</i>	1.3	100	0	12.8	100	0	2.1	100	0	1.9	100	0
<i>TM7</i>	7.1	100	0	4.5	100	0	3.1	100	0	5.0	100	0
<i>Verrucomicrobia</i>	11.2	99	1	6.3	100	0	7.0	99	1	8.1	99	1

Quantitative report of taxa connection as a percentage (%) of the total degree (Tot), specifying the percentage of co-occurrence (+) and mutual exclusion (–) interaction observed for each taxon.

bacterial community was particularly driven by an increase of *Planctomycetes* (12.8% in RS vs. 1.3% in RC) and of *Gemmatimonadetes* (2.6% in RS vs. 0.4% in RC) followed by a significant decrease of *Actinobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *TM7*, *Acidobacteria*, and *Chloroflexi* in RS vs. RC. However, in the spiny network, connectivity among the bacterial community was shaped by the increase of *Actinobacteria* (18.8% in GS vs. 16.9% in GC), *TM7* (5.0% in GS vs. 3.1% in GC) and *Chloroflexi* (4.7% in GS vs. 2.6% in GC) followed by a decrease of *Gammaproteobacteria*, *Betaproteobacteria*, *Firmicutes*, *Acidobacteria*, and of *Gemmatimonadetes*. It is worth to mention that there is not a significant difference between the key phylum/classes driving the taxonomy of connectivity between the two stressed networks RS and GS, except the highest % connectivity of *Planctomycetes* in RS vs. GS (12.8% vs. 1.9% respectively) and the increase of % connectivity of *Chloroflexi* in GS vs. RS (4.7% vs. 1.4% respectively). Because of that we intended to identify specific bacterial keystone taxa that might function as hubs under salinity stress. Phylogenetic network analysis identified 20 hubs distinctly different between the four treatments (3 hubs in RC network, 5 hubs in RS, 1 hub in GC, and 11 hubs in GS) (Table 3), suggesting an increased competition between network hubs under salinity stress. Hubs are mainly dominated by

Alphaproteobacteria and *Verrucomicrobia* whether in the presence and absence of saline stress, stressing the importance of such bacterial groups in the structure of the microbiome. In the control bacterial networks, *Acidobacteria* was identified as a specific keystone taxa in spineless community, while *Firmicutes* were specifically associated to the spiny networks. We also noticed that hubs are members of *Deltaproteobacteria* dominated in the presence of saline stress and specifically in spineless root networks. However, spiny’s hubs under salt stress were dominantly represented by members of *Actinobacteria*, *Bacteroidetes*, and *Chloroflexi*, indicating clear distinct keystone taxa within the spineless and spiny communities under salinity stress condition. Overall, these findings suggested that NaCl treatment greatly promotes species persistence by increasing reconnection opportunities in spiny than in spineless cultivar.

4. Discussion

In the face of climate change, there is an urgent need to focus on growing crop plants capable of adapting and thriving sustainably in arid regions. To address this issue, it is possible to optimize plant growth

Table 3
The bacterial hubs for the two sub-networks displayed by spineless and spiny cactus under salt stress. The degree of each hub per treatment is presented.

OTU#	RC	RS	GC	GS	Taxonomy (Phylum/Class)	Closet relative's species (NCBI accession number)	Similarity (%)
2790	94				<i>Acidobacteria</i>	<i>Stenotrophobacter terrae</i> (NR_146023)	97
3770	94				<i>Alphaproteobacteria</i>	<i>Sphingomonas rhizophila</i> (NR_159352)	98
2774	94				<i>Verrucomicrobia</i>	<i>Alterococcus agarolyticus</i> (NR_036763)	92
2966696		94			<i>Alphaproteobacteria</i>	<i>Dongia rigui</i> (NR_133740)	98
695260		94			<i>Alphaproteobacteria</i>	<i>Shinella zoogloeoides</i> (NR_119062)	98
996		94			<i>Alphaproteobacteria</i>	<i>Martellella endophytica</i> (NR_171494)	92
500203		94			<i>Deltaproteobacteria</i>	<i>Litorimonas haliclona</i> (NR_159917)	94
1527		94			<i>Verrucomicrobia</i>	<i>Pelagicoccus croceus</i> (NR_041583)	85
314			130		<i>Firmicutes</i>	<i>Bacillus haynesii</i> (NR_157609)	97
249571				97	<i>Actinobacteria</i>	<i>Actinoplanes lichenis</i> (NR_147781)	97
4458015				97	<i>Actinobacteria</i>	<i>Actinomadura adrarensis</i> (NR_144570)	96
1084157				97	<i>Actinobacteria</i>	<i>Janibacter alkaliphilus</i> (NR_109453)	97
220715				97	<i>Alphaproteobacteria</i>	<i>Microvirga aerilata</i> (NR_114298)	99
1655				97	<i>Alphaproteobacteria</i>	<i>Rhodoligotrophos jinshengii</i> (NR_134155)	78
2207				97	<i>Bacteroidetes</i>	<i>Chryseolinea soli</i> (NR_165708)	93
1444				97	<i>Chloroflexi</i>	<i>Pelotomaculum terephthalicum</i> (NR_040948)	85
3968				97	<i>Firmicutes</i>	<i>Tumebacillus ginsengisoli</i> (NR_112564)	96
931				97	<i>Verrucomicrobia</i>	<i>Chthoniobacter flavus</i> (NR_115225)	90
3474				97	<i>Verrucomicrobia</i>	<i>Pelagicoccus albus</i> (NR_041556)	93
3460				97	<i>Verrucomicrobia</i>	<i>Luteolibacter gellanilyticus</i> (NR_158117)	95

under stress conditions by using the beneficial rhizosphere microbiome in conservation agriculture practices (Paliwoda and Mikiciuk, 2020). In their natural habitat, inland prickly-pear cacti are rarely subjected to salt stress; however, they are cultivated in soils that may become salinized, due to the increased rate of evaporation in arid areas which in turn affects the growth and development of cacti. To our knowledge, this study is the first that evaluate the influences of the cactus-coastal rhizosphere microbiota of *Opuntia littoralis* and soil salinity on the development of two inland cactus cultivars (Rossa as spineless cultivar and Gialla as spiny cultivar) widely used by farmers in arid areas for their numerous benefits. Most previous studies have focused on the morphological variability of prickly pear cultivars between or within these two forms (spineless and spiny) (Adli et al., 2017; Amani et al., 2019) and on assessing the diversity, composition, and abundance of the spineless cactus microbiome under arid conditions (Flores-Nunez et al., 2020; Fonseca-García et al., 2016; Gargouri et al., 2021; Karray et al., 2020). However, limited knowledge exists on how bacterial microbiomes from coastal saline environments influence the physiology and root-endosphere microbiota of inland cacti under salinity. Our findings indicate that the microbiome plays a pivotal role in promoting Rossa's growth under non-stressed conditions, while significantly enhancing salt tolerance in the Gialla cultivar, which demonstrated greater resilience to salinity compared to Rossa.

To link the physiological modifications to the microbiome community alterations, we focused on the analysis of the network complexity as well as on the co-occurrence and mutual-exclusion patterns of bacterial groups harbored by roots of both cultivars under non-stressed and salinity conditions (Fig. 7). Under non-stressed condition and in the spiny plant control (Gialla), *Bacillus haynesii* emerged as the unique hub, suggesting a potential role in supporting plant growth and development. Indeed, a recent study reported that *B. haynesii* was involved in the production of gibberellic acid, indole-3-acetic acid, and other beneficial compounds which could promote plant growth and alleviate salinity stress (Peng et al., 2023). Furthermore, *B. haynesii* was also documented to contribute to increase the phosphate availability in agricultural soils via the increase of root surface area and rock phosphate solubilization (Abdelgalil et al., 2022), which could be one of the explanation of the increased root length of spiny control plants as compare to the spineless cultivar. The spineless plant control (Rossa) hubs were dominated by two genera: *Stenotrophobacter* and *Sphingomonas*. *Stenotrophobacter* is known for its ability to tolerate desiccation (Huber et al., 2022) and to play a crucial role in enhancing plant growth in hydroponic systems (Sato et al., 2023). *Sphingomonas* genus was identified to have many potential benefits for plant growth including the production of phytohormones, enhancement of plant nutrition, promotion of root growth, and increase in plant biomass (Asaf et al., 2020; Lombardino et al., 2022; Sultana et al., 2024; Wang et al., 2022). This, suggest the involvement of *Sphingomonas* genus in the increase of root biomass observed specifically for the spineless control plants. Together, these findings suggest a distinct root physiological adaptation under non-stressed conditions between spiny and spineless cacti and which it is most likely controlled by distinct bacteria genera.

Under salinity condition, it was shown that the root/shoot dry-weight ratio registered a significant decrease when the salinity reached 150 mM NaCl and that the decrease of roots growth was usually more important than the decrease of shoots growth (Berry and Nobel, 1985; Freire et al., 2018; Gersani et al., 1993; Inglese et al., 2017; Murillo-Amador et al., 2001; Nerd et al., 1991; Nobel, 1988; Silva-Ortega et al., 2008). Our findings indicate a notable reduction in shoot growth in the stressed spiny cactus (Gialla), while in the roots we didn't register any significant decrease. However, it is interesting to note that the spineless cultivars (Rossa) showed sensitivity to salinity in both shoot and root growth, highlighting the variability of the impact of salinity depending on the cactus cultivar. This observation could be due to the strategies adopted by the spiny cultivars to avoid salt stress, strategies based on the exclusion of salt from the stem at root level, retaining

sodium in the roots (Fig. 3), and also by the reduced growth of absorptive fine roots during periods of stress (Nobel, 1984; NOBEL, 1983; Yadav et al., 2014). This could be one of the explanations why we noticed an increase in the number of damaged cladodes after applying salinity to the spineless cultivar, in contrast to the spiny cultivar (Table 1). Some studies have reported that water salinity has adverse effects on cladodes. For instance, Freire et al. (2018) found that a salinity level of 3.6 dS m⁻¹ resulted in a significant reduction in thickness, width and number of cladodes. Similarly, Silva-Ortega et al. (2008), observed a 25% decrease in the thickness of young cladode in *O. streptacantha* when the plant was exposed to 250 and 350 mM of NaCl. Conversely, in our study the spiny cultivars did not exhibit any significant differences in this regard which suggest that the influence of salinity on cladodes is dependent on the cacti cultivar.

In addition to the impact of salinity on growth parameters, plants may adjust osmotic potential via different minerals to resist salinity stress (Huang et al., 2007). In our study, we observed an important accumulation of Na⁺ ions in cladodes and roots of the cultivar Rossa, whereas in the cultivar Gialla Na⁺ accumulation was only observed in the roots (Fig. 3). This highlighted that the spiny cultivar has developed a protection mechanism against the oxidative stressor at root level. The presence of Na⁺ was exclusively detected in the root tissue of the spiny cacti, indicating that the Gialla cultivar exhibits salinity tolerance compared to the Rossa cultivar. (Su et al. (2015), reported that salinity tolerant genotypes can maintain high cytosolic K⁺/Na⁺ ratio through excluding Na⁺ from shoots. In fact, Na⁺ accumulation in plant cells was evaluated by the ion-exchange activity of Na⁺ influx-efflux (Apse and Blumwald, 2007). Under salt stress, the sodium ion is recirculated through ion channels. Na⁺ influx occurs through a high-affinity K⁺ transporter "HKT" concomitantly with a non-selective cation channels (Berthomieu et al., 2014), whereas Na⁺ efflux was rather mediated by SOS1 (salt overly sensitive 1) Na⁺/H⁺ antiporter (Wu, 2018). This observation needs to be further consolidated via kinetic studies of ion accumulation in cells. The osmotic balance employed by the cultivar Gialla could be ascribed to a strategy of *Opuntia* that favors survival rather than vegetative growth and, therefore, makes it more adapted to adverse conditions.

One key objective motivating this research was to dissect the link between microbial community alterations of spiny and spineless cacti and plant salt tolerance. Our findings suggest that the prokaryotic patterns in *Opuntia* align with those reported for prokaryotic communities associated with cacti and agave plants (Coleman-Derr et al., 2016; Fonseca-García et al., 2016; Puente et al., 2004). This would imply that the plant-associated compartment exhibits a weak selection among succulent plants sharing a core group of soil- and root-associated microorganisms. When salt stress was applied, both cultivars exhibited a predominance of bacteria belonging to the *Alphaproteobacteria* phylum in their roots. Similarly, Zhang et al. (2019), Zhao et al. (Q. Zhao et al., 2020a,b), and Zhao et al. (S. Zhao et al., 2020a,b) noted an enrichment of bacteria belonging to *Alphaproteobacteria* and *Gammaproteobacteria* under salt stress conditions.

Intriguingly, the spiny and spineless cultivar had a different distribution of *Bacteroidetes* and *Firmicutes* in roots under salt stress. These two phyla were lower in roots of Gialla and higher in roots of Rossa in presence of salt. *Bacteroidetes* tended to be a dominant phyla within the salinized soil microbiota (Hou et al., 2021). They are well known as degraders of polymeric organic matter, especially in the form of polysaccharides (Fierer et al., 2007; Thomas et al., 2011). *Firmicutes* phylum members were also reported to be predominant in salinized soils because of their Gram-positive cell wall and ability to form spores under these stressed conditions (Hashmi et al., 2020). Overall, members of these two bacterial phyla are pivotal and unique predictors of soil salinization as they are highly resistant to salinity (Hidri et al., 2016; Hou et al., 2021; Keshri et al., 2013; Zheng et al., 2017), suggesting that the inoculated spineless cultivar is recruiting more salt-tolerant bacteria to bestow their salinity resistance which is not the case of the inoculated spiny cultivar.

This latter most likely does not fully rely on microbes to optimize its fitness and salt resistance. Taken together, the physiological distinction correlates with differences in microbial community composition between the two cultivars. Under salt stress, Gialla's root microbiome exhibited a higher abundance of *Alphaproteobacteria*, a group known for enhancing plant salt tolerance. In contrast, the spineless Rossa cultivar recruited more *Bacteroidetes* and *Firmicutes*, phyla associated with organic matter degradation and salinity resistance. This suggests that Rossa may rely more on its microbiome for stress mitigation, potentially recruiting salt-tolerant bacteria to compensate for its lower inherent salt tolerance.

At family level, both cultivars showed higher abundance of *Kiloniellaceae* under salt stress conditions. This family is actually uniquely represented by the genus *Kiloniella* and the species *Kiloniella laminariae*. These bacteria are known for its salt tolerance, supporting the hypothesis that both cacti recruit salt-resistant bacteria to mitigate stress (Wiese et al., 2019). Interestingly, the spiny Gialla cultivar exhibited greater specificity in its microbial recruitment post-stress (Fig. 5b), suggesting a more selective and efficient microbial association to enhance stress resilience. This was reflected in the stronger bacterial community cohesion (Fig. 7) and the increased abundance of stress-tolerant bacteria in Gialla's roots (Fig. 6a), highlighting a distinct physiological and microbial strategy for managing salinity.

By contrast, the microbial network in Rossa became less connected after salt stress, indicating a breakdown in community stability under environmental pressure. This loss of network complexity is often observed under significant environmental changes, such as altered pH or prolonged stress (Zhou et al., 2024). Despite this, Rossa recruited endobacterial hubs which are associated with plant growth and pathogen suppression rather than direct stress mitigation. For instance, *Dongia* genus, is recognized for its potential role in plant morphogenesis (Visioli et al., 2018). Yang et al. (2023) have shown a higher relative abundance of *Dongia* in the rhizosphere soil of healthy plants, indicating its potential role in promoting plant health and suppressing pathogens. *Marteella endophytica* was identified as a species with inhibitory activity against oomycete plant pathogens (Bibi et al., 2013). *Litorimonas* genus, associated with healthy tissue in macroalgal microbiomes, played a key role in maintaining healthy microbial consortia associated with the algal surface (Paix et al., 2021). *Shinella Zoogloeoides* has been reported to exhibit a high potential for crude oil degradation within a short period, indicating its potential for bioremediation application (Goma-Tchimbakala et al., 2022; Zhang and Huang, 2020). This suggests that Rossa may prioritize growth-related microbial functions over stress tolerance, which could explain its lower resilience under salinity.

The microbial network analysis revealed that Gialla's root microbiota under salt stress maintained higher functional connectivity than Rossa's (Fig. 7), with more nodes and edges indicating greater resilience to environmental perturbations (Santolini and Barabási, 2018). In particular, Gialla recruited members of the *Actinobacteria* phylum, which have been found to play a role in mitigating the effects of salinity stress on plants, making them potential biofertilizers to improve crop production in saline soils (González et al., 2023; Ravi Kumar et al., 2023). Among spiny hubs, *Microvirga aerilata* have been reported to have a crucial role in the plant microbiome salinity tolerance. The *Microvirga* genus belongs to nitrogen-fixing bacteria that can colonize legume root nodules alongside other rhizobial species, which have the potential to adapt to desert conditions (Marasco et al., 2018; Martínez-Hidalgo and Hirsch, 2017), further supporting Gialla's adaptive strategy to salinity. Overall, our study reveals that while both spiny and spineless cultivars of *Opuntia* recruit salt-tolerant microbiomes under stress, their strategies differ significantly. Gialla's selective recruitment of stress-tolerant microbes and its maintenance of microbial network stability under salinity underscore its inherent and microbial-mediated resilience. In contrast, Rossa appears to rely more on generalist growth-promoting bacteria, which may limit its capacity to withstand salinity. These findings highlight the importance of plant-microbiome interactions in

improving plant salt tolerance and provide insights into developing microbial-based strategies for enhancing crop resilience in saline environments.

Funding

The research reported in this publication was funded by Collaborative Research Program (CRP)-ICGEB Research Grant (CRP/TUN 15-01-EC) from International Centre for Genetic Engineering and Biotechnology, Italy. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. This work was also published with the support of the Tunisian Ministry of Higher Education, Scientific Research and Technology.

CRediT authorship contribution statement

Ameni Ben Zineb: Writing – review & editing, Writing – original draft, Visualization, Software, Formal analysis, Data curation. **Mariem Zakraoui:** Writing – original draft, Methodology. **Imane Bahlouli:** Writing – review & editing, Visualization. **Fatma Karray:** Data curation. **Asma Ben Salem:** Methodology. **Ahmed Mliki:** Writing – review & editing. **Stephan Declerck:** Writing – review & editing. **Mahmoud Gargouri:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mahmoud Gargouri reports financial support was provided by International Centre for Genetic Engineering and Biotechnology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rhisph.2024.100984>.

Data availability

Data will be made available on request.

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