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ARTICLE

Salt-induced recruitment of specific root-associated bacterial consortium capable of enhancing plant adaptability to salt stress

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Abstract

Salinity is a major abiotic stress threatening crop production. Root-derived bacteria (RDB) are hypothesized to play a role in enhancing plant adaptability to various stresses. However, it is still unclear whether and how plants build up specific RDB when challenged by salinity. In this study, we measured the composition and variation in the rhizosphere and endophyte bacteria of salt-sensitive (SSs) and salt-resistant (SRs) plants under soil conditions with/without salinity. The salt-induced RDB (both rhizobiomes and endophytes) were isolated to examine their effects on the physiological responses of SSs and SRs to salinity challenge. Moreover, we examined whether functional redundancy exists among salt-induced RDB in enhancing plant adaptability to salt stress. We observed that although SSs and SRs recruited distinct RDB and relevant functions when challenged by salinity, salt-induced recruitment of specific RDB led to a consistent growth promotion in plants regardless of their salinity tolerance capacities. Plants employed a species-specific strategy to recruit beneficial soil bacteria in the rhizosphere rather than in the endosphere. Furthermore, we demonstrated that the consortium, but not individual members of the salt-induced RDB, provided enduring resistance against salt stress. This study confirms the critical role of salt-induced RDB in enhancing plant adaptability to salt stress.

Introduction

Soil salinity is one of the major abiotic stresses adversely affecting crop growth and yield [1]. It has been estimated that salinity affects approximately 1 billion hectares of soils (*c*. 7.5% of the world's land area) across 100 countries [2]. Particularly, in agricultural systems, about 45 million hectares of irrigated soils (*c*. 19.5%) and 32 million hectares of dryland soils (*c*. 2.1%) were affected by salinity [3]. More seriously, salinity-affected agricultural soils are increasing at a rate as high as 10% per year, due to poor agricultural practices (e.g., excessive fertilization and saline water irrigation), climate change (e.g., reduced precipitation and enhanced surface evaporation), and industrial pollution [4–6]. High soil salinity often leads to ionic and osmotic stresses, and further induces oxidative stress, nutritional disorders, and organ senescence, in plants [7]. Since most crop plants are salt sensitive [8], it is extremely important to identify effective strategies applied by plants to adapt to salt stress, and to further develop potential approaches to improve plant performance under salinity conditions.

Because of their sessile nature, plants must directly face various environmental challenges and thus have to develop effective mechanisms to cope with biotic and/or abiotic stresses [9]. When grown under salinity stress, plants may employ several defensive tactics to protect themselves, e.g., forming salt-excreting glands or trichomes [10], re-establishing cellular ionic, osmotic, and reactive oxygen species equilibrium [11], and regulating critical developmental processes such as flowering time [12]. The physiological and molecular basis of salt adaptation in plants has been sufficiently proven [13]. There is no doubt that salt-tolerant plants are more adaptable to salt stress than salt-sensitive (SS) ones.

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Although salt adaptation in plants is often considered to be driven by genetic differentiation [14], the microbiota has recently been interpreted as a key factor in plant stress tolerance [15, 16]. Plant-associated microbiota is referred to as the plant's second genome [17], because it is not only important for regulating plant metabolism [18], but also critical for plant immune system [19, 20]. In plant-associated microbiota, root-derived bacteria (RDB), including rhizobiome and endophytes, have been extensively studied to decipher their roles in plant adaptation to salinity for the past decade [21–23]. Several studies have focused much interest on plant growth-promoting rhizobacteria capable of enhancing salt tolerance in plants [24–26], while others have measured the composition of RDB in various plant species under salinity conditions [27–29]. To date, however, very few studies have attempted to examine whether and how plants build up specific RDB when exposed to salt stress, or whether the capacity of RDB to alleviate salt stress in salt-tolerant plants is different from that in SS ones.

It has been demonstrated that specific strains of RDB can improve plant performance under salinity conditions, through several beneficial processes, such as mediating ion homeostasis, producing phytohormones, favoring osmolyte accumulation, improving antioxidant activity, and enhancing nutrient absorption [30]. Since root exudates shape the activity and diversity of RDB [31, 32] and since the composition of root exudates can be altered by various abiotic stresses [33], we hypothesize that once exposed to salinity conditions, plants may recruit specific RDB that enhance plant tolerance to salt stress. In addition, it has been documented that the composition of RDB is plant species-specific, because different plant species host specific RDB when grown on the same soil and because the same plant species can develop distinct RDB in different soils [17]. Therefore, we also hypothesize that RDB in salt-tolerant plants may be more capable of attenuating plant salt stress than those in SS ones. Verifying these two hypotheses could allow us to better understand the ecological importance of RDB for plant performance in response to abiotic stress.

To test these hypotheses, we focused on *Curcurbitaceae*, a plant family with a large variation in salt stress-resistant capacity. We examined the physiological responses of 85 cultivated varieties to salt treatments, and screened out 6 SS and 6 salt-resistant (SR) varieties, which were further used to measure the composition and variation in the rhizosphere (Rh) and endophyte bacteria under soil conditions combined with/without salt treatments. RDB (both rhizobiomes and endophytes) under salt treatments were also isolated to examine their effects on the physiological responses of SSs and SRs to salt treatments. Moreover, we examined whether functional redundancy exists among RDB in enhancing plant adaptability to salt stress, through a removal experiment by inoculating sterile soil microcosms with serial dilutions of the rhizospheric and endophytic bacterial suspension [34]. The objectives of this study were to examine (i) whether plants can recruit specific RDB to enhance plant tolerance to salt stress, (ii) whether RDB in salt-tolerant plants are more capable of enhancing plant adaptability to salt stress than those in SS ones, and (iii) whether functional redundancy exists among RDB in alleviating plant salt stress.

Materials and methods

Plant, soils, and experimental design

The plant materials used in this study consisted of 85 varieties belonging to the family *Curcurbitaceae* (for more information see Supplementary Table S1). This plant family was selected because it exhibits a large variation in salt stress-resistant capacity. The plant species used included *Cucumis sativus*, *Cucurbita moschata*, *Cucurbita maxima*, *Cucurbita ficifolia*, and *Lagenaria siceraria* (Supplementary Table S1). The soils used were collected from a forest site (forest type: temperate deciduous forest) free of pesticide and fertilizer (Beijing, China; 40°40′98″N, 115°89′70″E) according to the method described in Niu et al. [35], with some modifications. Briefly, the surface layer (0–10 cm) of the forest soil was removed and the 10–25 cm layer was collected. The selected soil (10–25 cm layer) was classified as sandy clay (55.64% sand, 6.43% silt, and 37.93% clay) according to the US textural classification triangle. It contained 22.6 g kg^{-1} of organic matter, 0.86 g kg^{-1} of total N, 0.41 g kg^{-1} of total P, 8.87 g kg^{-1} of total K, 8.33 g kg^{-1} of total Ca, 2.90 g kg^{-1} of total Mg, 0.17 g kg^{-1} of total S, and 0.04 cmol kg^{-1} Na^+, and had a bulk density of 1.18 g cm^{-3}, a water holding capacity of 40.75%, a pH of 7.39, and an electrical conductivity (EC) of 0.18 mS cm^{-1} (for more information see Supplementary Table S2). The collected soils were air-dried for 7 days at room temperature, and then homogenized and sieved with 5- and 2-mm meshes. To improve soil conditions, the air-dried soil was mixed 4:1 volume with a mature compost (the characteristics of compost, prepared from maize straw and cow manure, are listed in Supplementary Table S2), and subsequently stored at 4 °C in the dark until use. The selected characteristics of the compost-amended soil are listed in Supplementary Table S2. All the following experiments were carried out using the compost-emended soil. There was no salt stress or drought condition in either the native forest soil or the compost-amended soil, because their EC (native forest soil: 0.18 mS cm^{-1}; compost-amended soil: 0.72 mS cm^{-1}) and Na^+ concentration (native forest soil: 0.04 cmol kg^{-1}; compost-amended soil: 0.38 cmol kg^{-1}) were within the

range of non-salinization (EC: 0–2 mS cm^{-1}; Na^+: 0–1 cmol kg^{-1}) as defined by Vargas et al. [36]. Moreover, we also evaluated salt stress in soils by a bioassay test, which showed insignificant influence of salinity on the fresh and dry weights of all tested plants in the soils used for growing plants in the following experiments (Supplementary Fig. S1). Characteristics of bacterial and fungal communities in the forest soil, compost, and compost-amended soil are shown in Fig. 1. Clearly, the operational taxonomic unit (OTU) richness and abundance (based on quantitative PCR) of bacterial community were much higher than those of fungal community in all samples (Fig. 1c).

Four experiments were conducted. In Expt. 1, we aimed to examine whether soil bacterial communities play a pivotal role in alleviating plant salt stress. First, we measured the physiological responses of 85 varieties to salt treatments, and divided them into two groups (SR and SS) based on the screening-concentration of NaCl. For the SR group, all varieties could survive under high-salinity condition (150 mM NaCl). With respect to the SS group, however, all varieties withered and died under 150 mM NaCl but could survive under low-salinity condition (50 mM NaCl). Within each group, we further screened out the top 3 relatively resistant varieties and the top 3 relatively sensitive varieties under their corresponding screening conditions, based on the plant salt-tolerance index (PSTI). The PSTI was calculated by integrating key plant salt tolerance indicators that could effectively reflect plant physiological responses to salt stress. The PSTI was calculated according to the equation $PSTI=\sum_{i=1}^{n} S_i W_i$, where *Wi* is the PC weighting factor and *Si* presents the indicator score for variable *i*. It was assumed that higher PSTI meant better plant salt tolerance. The steps regarding how PSTI has been developed are shown in Supplementary Materials and Methods. Together, a total of 12 varieties were screened out for further study (Fig. 1a). For the SS group, the varieties screened out included JY4 (Chinese *Cucumis sativus* L. cv. Jinyou No. 4), JY1 (Chinese *Cucumis sativus* L. cv. Jinyan No. 108_2), CY (Chinese *Cucumis sativus* L. cv. Chiyu No. 8), JM (European *Cucumis sativus* L. cv. Jinmei No.3), JC (Chinese *Cucumis sativus* L. cv. Jinchun No.2), and JYM (European *Cucumis sativus* L. cv. Jinyanmini No.5). For the SR group, the varieties screened out included LZ (Japanese *Cucurbita moschata* Duch cv. Lizhiyuan), HM (Japanese *Cucurbita moschata* Duch cv. Hemei No.3), BN (Chinese *Cucurbita moschata* Duch cv. Beinongliangzhen), XL (Chinese *Cucurbita maxima* Duch. Xili), CF (Chinese *Lagenaria siceraria* Standl. Chaofengkangshengwang), and XH (Korean *Lagenaria siceraria* Standl. Xuanhe). Second, we conducted a three-level factorial experiment (soil sterilization × plant variety × salt treatment) to investigate whether soil bacteria (unsterilized vs. sterilized) can influence the physiological responses of SRs and SSs to salt stress. The soil sterilization treatments considered were (i) unsterilized soils and (ii) γ-irradiation (at 60 kGy) sterilized soils [37], while the salt treatments considered were (i) 0 mM NaCl and (ii) 75 mM NaCl. Together, this resulted in a total of 48 experimental treatments (2 soil sterilization treatments × 12 plant varieties × 2 salt treatments). For each treatment, there were four replicates with 16 seedlings per replicate. The concentration of 75 mM NaCl (moderate salt stress) was chosen through a concentration gradient (0–300 mM NaCl) test. Further details of materials and methodology for Expt. 1 are provided in Supplementary Materials and Methods. The efficiency of soil sterilization by γ-irradiation is closely associated with the γ-ray dose [37]. Here, three methods were used to check for the efficiency of sterilization by γ-irradiation at 60 kGy: (i) the DNA extracted from unsterilized and γ-sterilized samples was used as a template for PCR with the universal primers for bacterial 16S rRNA gene and fungal ITS gene amplifications, (ii) the DNA extracted was used as a template for quantitative PCR, and (iii) soil solutions were spread onto R2A (bacteria) and PDA (fungi) plates, and incubated at 30 °C (Supplementary Fig. S2). Clearly, γ-irradiation at 60 kGy was sufficient in soil sterilization, because no microbial DNA and live microbes were observed under γ-sterilized treatments (Supplementary Fig. S2).

In Expt. 2, we aimed to examine whether SS and salt-tolerant plants can recruit specific root-associated bacteria under salt stress. We conducted a two-level factorial experiment (plant variety × salt treatment) to measure the composition and variation in the Rh and endophyte bacteria of SRs and SSs under unsterilized soil conditions. The salt treatments considered were (i) 0 mM NaCl and (ii) 75 mM NaCl. There were a total of 24 experimental treatments (12 plant varieties × 2 salt treatments). For each treatment, there were four replicates with 16 seedlings per replicate. The bulk soil, Rh, and endophyte bacteria were characterized by using pyrosequencing of bacterial 16S rRNA genes. Further details of materials and methodology for Expt. 2 are provided in Supplementary Materials and Methods.

In Expt. 3, we aimed to examine whether specific root-associated bacteria, recruited by plants under salt stress, are capable of enhancing plant salt tolerance. First, we selected the top 3 relatively resistant varieties (XL: Chinese *Cucurbita maxima* Duch. Xili; CF: Chinese *Lagenaria siceraria* Standl. Chaofengkangshengwang; XH: Korean *Lagenaria siceraria* Standl. Xuanhe) within the SR group and the top 3 relatively sensitive varieties (JY4: Chinese *Cucumis sativus* L. cv. Jinyou No. 4; JY1: Chinese *Cucumis sativus* L. cv. Jinyan No. 108_2; CY: Chinese *Cucumis sativus* L. cv. Chiyu No. 8) within the SS group for further study and isolated their bacterial consortia (BC) from the Rh and endosphere (En) under salt stress. The BC–Rh and BC–En were characterized by using pyrosequencing of

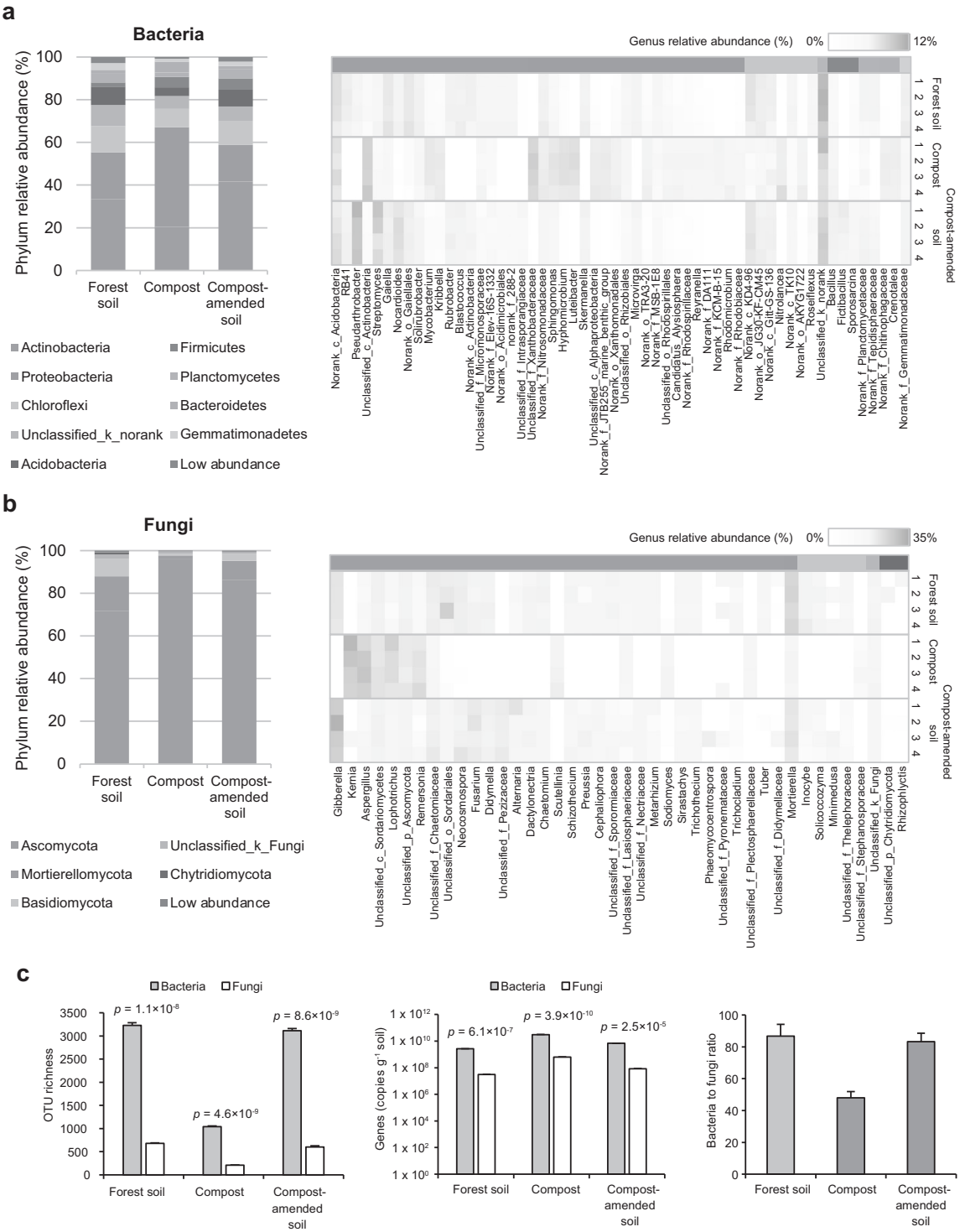


Fig. 1 Characteristics of bacterial and fungal communities in the forest soil, compost, and compost-amended soil. a Bacterial community composition at the phylum (left) and genus (right) levels. Genera of all samples with greater than 0.5% abundance are listed. **b** Fungal community composition at the phylum (left) and genus (right) levels. Genera of all samples with greater than 0.5% abundance are listed. **c** OTU richness, bacterial and fungal abundance (based on quantitative PCR), and the bacterial to fungal ratio. Data bars represent means and error bars represent the standard error of the mean. Three technical replicates showed high reproducibility (mean SEM <0.6% of mean), so only biological replicates ($n = 3$) were run.

bacterial 16S rRNA genes. Second, we conducted a three-level factorial experiment (BC × plant variety × salt treatment) under γ-irradiation sterilized soil conditions to evaluate the effects of self BCs on plant salt tolerance (i.e., plant varieties were treated by their own BCs). The BC treatments considered were (i) germ-free, (ii) self BC–Rh, and (iii) self BC–En, while the salt treatments considered were (i) 0 mM NaCl and (ii) 75 mM NaCl. Together, this resulted in a total of 36 (3 BC treatments × 6 plant varieties × 2 salt treatments) experimental treatments. Finally, we investigated whether self BCs were more effective than non-self BCs in enhancing plant salt tolerance. To meet this need, we used two representative plant varieties, XH (Korean *Lagenaria siceraria* Standl. Xuanhe; the most SR plant variety) and JY4 (Chinese *Cucumis sativus* L. cv. Jinyou No. 4; the most SS plant variety), and conducted a three-level factorial experiment (BC × plant variety × salt treatment). The BC treatments considered were (i) germ-free, (ii) self BC–Rh, (iii) non-self BC–Rh, (iv) self BC–En, and (v) non-self BC–En, while the salt treatments considered were (i) 0 mM NaCl and (ii) 75 mM NaCl. Together, this resulted in a total of 20 experimental treatments (5 BC treatments × 2 plant varieties × 2 salt treatments). Further details of materials and methodology for Expt. 3 are provided in Supplementary Materials and Methods.

In Expt. 4, we aimed to examine whether functional redundancy exists among RDB in enhancing plant salt tolerance. To meet this need, we conducted a removal experiment by inoculating sterile soil microcosms with serial dilutions of self BC suspension [34]. We used two representative plant varieties, XH (Korean *Lagenaria siceraria* Standl. Xuanhe; the most SR plant variety) and JY4 (Chinese *Cucumis sativus* L. cv. Jinyou No. 4; the most SS plant variety), for further study. For either BC–Rh or BC–En, five levels of dilution (10^0, 10^{-1}, 10^{-3}, 10^{-5}, and 10^{-7} for BC–Rh, and 10^0, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} for BC–En) of the BC suspension were used as inocula to create a gradient of BC diversity. For all dilution levels, the bacterial diversity was characterized by using pyrosequencing of bacterial 16S rRNA genes. Seedlings were sown in γ-irradiation sterilized soils re-inoculated with self BCs having different diversities but globally similar abundances. After the seedlings developed two true leaves, the seedlings were continuously irrigated with the nutrient solution containing 0 or 75 mM NaCl for 14 days. Further details of materials and methodology for Expt. 4 are provided in Supplementary Materials and Methods.

Surface sterilization and gemination of plant seeds

All seeds used in this study were surface sterilized by soaking in 70% ethanol for 1 min, 3% sodium hypochlorite for 10 min, and rinsed three times with sterile distilled water. Then, 250 µl of water was taken from the third rinse and used to check for contamination as described in Niu et al. [35]. The surface-sterilized seeds were placed in sterile petri dishes lined with two layers of moist sterile filter paper, and then incubated in the dark at 28 °C until the seeds germinated.

Measurements of plant parameters associated with salt tolerance

To effectively reflect plant physiological responses to salt stress, several indices were calculated. Those indices were the relative decrease in plant biomass (RDPB), the relative decrease in plant fresh weight, the relative decrease in plant height, the relative decrease in plant water content, plant K^+ decrease rate, plant Na^+ increase rate (IR_Na^+), the ratio of K^+ to Na^+ in plant, the salt injury index, and the death rate of plant. To comprehensively evaluate plant salt tolerance, a PSTI was calculated by integrating key indices that could effectively reflect plant physiological responses to salt stress. In addition, to verify the efficiency of PSTI in evaluating the plant salt tolerance, linear regressions were also performed between PSTI and salt tolerance-related parameters (Supplementary Figs. S3 and S4). Nutrient elements N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn, and Mo were determined and then used to calculate the specific absorption rate (SAR) of a nutrient element, which was visualized as a radar chart. Further details regarding this section are provided in Supplementary Materials and Methods.

Isolation of salt-induced bacterial consortia from the rhizosphere and endosphere

BC associated with the roots of salt-affected plants grown under salinity conditions were evaluated for their effects on plant salt tolerance. The BC was recovered from the Rh and En of salt-affected plant roots [35], by resuspending and homogenizing the Rh and the smashed root (En) samples in 1×PBS buffer (1.0 g sample per 5 ml of buffer). Then, homogenates were used as inocula for enrichment cultures of BC by using the R2A liquid medium (at 28 °C for 36 h on a rotary shaker at 180 rpm). The R2A medium was selected because of its high efficiency in recovering RDB [38, 39]. Each type of BC was enriched in ten replicates and combined.

In addition, the Rh and root components obtained from salt-affected plants were also used for bacterial isolations by colony picking as well as 16S rRNA gene profiling as described in Bai et al. [38]. Briefly, the Rh and the smashed root samples were resuspended and homogenized in PBS buffer. Homogenates were serially diluted and applied to plating on five different bacterial growth media (R2A). Isolates were picked from the plates containing less than 20

colony-forming units after a maximum of 2 weeks of incubation.

Sampling, DNA extraction, PCR amplification, and sequencing

The bulk soil (S), Rh, and root compartments (En) were sampled using the procedure as described in Niu et al. [35]. Genomic DNA was extracted and purified from S, Rh, and En samples by using the PowerSoil DNA Isolation Kit (QIAGEN Inc., CA, USA) following the manufacturer's recommendations. To universally amplify and sequence the V4 region of the 16S rRNA gene (bacteria), we used forward primer 515 F (5′-GTGCCAGCMGCCGCGGTAA-3′) and reverse primer 806 R (5′-GGACTACHVGGGTWTC TAAT-3′) containing a variable 12 bp barcode sequence [35]. To universally amplify and sequence the ITS gene (fungi), we used forward primer ITS1 F (5′-CTTGGTCA TTTAGAGGAAGTAA-3′) and reverse primer ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′). The PCR was performed under the following conditions: an initial denaturation step at 95 °C for 3 min, followed by 27 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 10 min. Sample libraries for sequencing were prepared according to the MiSeq Reagent Kit Preparation Guide (Illumina, San Diego, CA, USA) as described previously [40], and subjected to a single sequencing run on the MiSeq platform (Illumina, Inc., San Diego, CA, USA). Further details about this section are provided in Supplementary Materials and Methods.

Sequencing analyses and microbiome statistical analyses

Analysis of 16S rRNA (bacteria) and ITS (fungi) gene sequences was performed as described in [41]. After removing chimeric sequences [42, 43], the remaining sequences were binned into OTUs with 97% similarity [44] and the representative sequence for each OTU was taxonomically classified via the Ribosomal Database Project's classifier [45] and the SILVA database (version 128) [46]. All OTUs identified as belonging to chloroplast and mitochondria were removed from the data set. Then, the representative sequences for each OTU were aligned using PyNAST [47] in QIIME [48].

OTU richness, Shannon index, Chao1 estimator, and Simpson index were applied to directly compare the α-diversity of the samples with differing sampling efforts. Both principal coordinate analyses (PCoA) and non-metric multidimensional scaling were used to assess how β-diversity could be partitioned into variation attributable to salt treatments and plant types [49, 50]. The "capscale" function was implemented in the vegan R library to constrain the variable of interest [51]. Statistical significance was determined by using the permutation-based ANOVA test ("anova.cca" R package with 5000 permutations) [51]. Linear discriminant analysis (LDA) of effect size (LEfSe) was applied on the OTU table to identify the differentially abundant bacterial taxa (at genus to phylum levels) that significantly change after salt treatments in bulk soil (S), Rh, and root compartments (En) of both SS and SR groups [51]. Wilcoxon rank-sum test for pairwise comparison (false discovery rate (FDR) adjusted $p < 0.05$) and the absolute LDA score (>3.25) were used to analyze the statistical significance and strength, respectively [52]. To estimate the potential function of bacteria, the database for the functional annotation of prokaryotic taxa (FAPROTAX; a database for converting microbial community profiles into putative functional profiles based on current literatures on cultivated strains) based on 16S rRNA gene sequencing (http://www.zoology.ubc.ca/louca/FAPROTAX) was referred to analyze several ecologically important functional groups related to plant growth (e.g., carbon, nitrogen and sulfur metabolisms, and photosynthesis) that change after salt treatments in bulk soil (S), Rh, and root compartments (En) of both SS and SR groups [41, 53].

Results

Soil bacteria alleviate salt stress in both salt-resistant and salt-sensitive plants

Based on the evaluation of PSTI, the varieties JY4, JY1, CY, JM, JC, and JYM were selected from the SS group, while LZ, HM, BN, XL, CF, and XH were selected from the SR group for further study (Fig. 2a). To explore whether soil bacteria can enhance the salt-tolerance in plants, we sowed these 12 plant varieties in unsterilized or γ-sterilized soils, and further challenged them with/without salinity (Fig. 2b). Interestingly, almost all of plant varieties (except BN), regardless of plant types (SS and SR), exhibited significantly lower salt-induced RDPB in unsterilized soils than in γ-sterilized soils ($p < 0.05$; Fig. 2c), indicating the importance role played by soil bacteria in enhancing plant adaptability to salt stress. Direct challenge of plants by salinity will generally result in an increased level of Na^+ in plants. However, similar to RDPB, for both SR and SS plants, salt-induced IR_Na^+ in plants was significantly lower in unsterilized soils than in γ-sterilized soils (Fig. 2d), demonstrating the ability of soil bacteria to reduce Na^+ accumulation in plants. Because salt stress generally restricts nutrient absorption by roots, the SAR of nutrients was also measured to test the effects of soil bacteria on nutrient uptake in plants challenged by salinity.

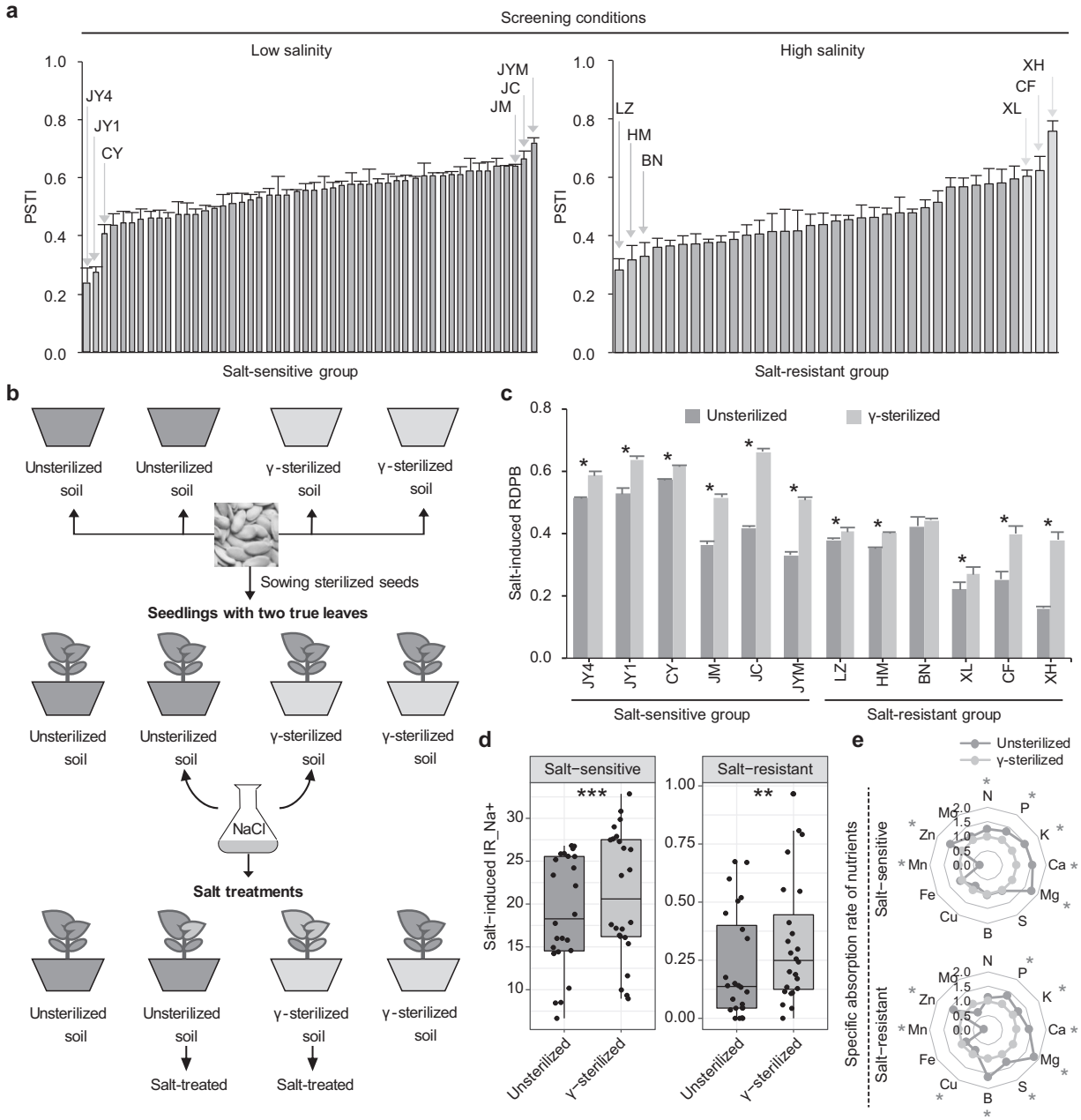


Fig. 2 Efficiency of soil microbial communities in alleviating plant salt stress. a The plant salt-tolerance index (PSTI) of salt-sensitive and salt-resistant groups. Within each group, the top 3 relatively resistant varieties and the top 3 relatively sensitive varieties were screened out under their corresponding screening conditions, based on the PSTI. Data bars represent means and error bars represent the standard error of the mean. **b** Schematic representation of the experimental design to assess the efficiency of soil microbial communities in alleviating plant salt stress. **c** The salt-induced relative decrease in plant biomass (RDPB) of salt-sensitive and salt-resistant groups under unsterilized and γ-sterilized conditions. Data bars represent means and error bars represent the standard error of the mean. An asterisk indicates statistically supported differences ($*p < 0.05$). **d** The salt-induced plant Na^+ increase rate (IR_Na^+) of salt-sensitive and salt-resistant groups under unsterilized and γ-sterilized conditions. The horizontal bars within boxes represent medians. The tops and bottoms of boxes represent the 75th and 25th percentiles, respectively. The upper and lower whiskers extend to data no more than 1.5× the interquartile range from the upper edge and lower edge of the box, respectively. Asterisks indicate statistically supported differences ($**p < 0.01$, $***p < 0.001$). **e** The specific absorption rate (SAR) of nutrients of salt-sensitive and salt-resistant groups under unsterilized and γ-sterilized conditions. Asterisks indicate statistically supported differences ($*p < 0.05$). JY4: Chinese *Cucumis sativus* L. cv. Jinyou No. 4; JY1: Chinese *Cucumis sativus* L. cv. Jinyan No. 108_2; CY: Chinese *Cucumis sativus* L. cv. Chiyu No. 8; JM: European *Cucumis sativus* L. cv. Jinmei No.3; JC: Chinese *Cucumis sativus* L. cv. Jinchun No.2; JYM: European *Cucumis sativus* L. cv. Jinyanmini No.5; LZ: Japanese *Cucurbita moschata* Duch cv. Lizhiyuan; HM: Japanese *Cucurbita moschata* Duch cv. Hemei No.3; BN: Chinese *Cucurbita moschata* Duch cv. Beinongliangzhen; XL: Chinese *Cucurbita maxima* Duch. Xili; CF: Chinese *Lagenaria siceraria* Standl. Chaofengkangshengwang; XH: Korean *Lagenaria siceraria* Standl. Xuanhe.

Clearly, soil bacteria (unsterilized vs. γ-sterilized) induced an overall enhancement in the SAR of nutrients of plants grown under salt conditions (Fig. 2e). Together, these results suggest that soil bacteria can alleviate salt stress in plants regardless of their salinity tolerance capacities.

Salt induces recruitment of specific bacterial consortium in roots

To characterize salinity-induced variation in root-associated bacteria, we built the 16S rRNA amplicon libraries, followed by Illumina sequencing. A total of 15,038,123 high-quality sequences were obtained from 288 samples (average, 52,215; range, 30,652–93,233 reads per sample). Analysis of the valid OTUs (Supplementary Table S3) showed that the differences in root En and Rh bacteria were significant and detectable at the phylum level, either between plant types (SRs vs. SSs) or between salt treatments (NaCl vs. control) (Fig. 3a). Measurement of α-diversity revealed that the En bacteria of both SSs and SRs had lower diversity under salinity conditions (NaCl vs. control; Fig. 3b), indicating that both SS and salt-tolerant plants recruited specific bacterial species when challenged by salinity. Interestingly, however, a significant reduction in the α-diversity of Rh bacteria due to salinity challenge was observed for SSs, but not for SRs (Fig. 3b), implying that SR plants had stronger ability to maintain stable bacteria in the Rh than SS plants. Despite that, measurement of β-diversity showed that the composition of both En and Rh bacteria differed in salt treatments (NaCl vs. control) for either SRs or SSs (Fig. 3c and Supplementary Figs. S5 and S6). Constrained PCoA (CPCoA) revealed that although SRs and SSs separated along CPCoA1 in the same direction, significant and consistent differences were observed between salt treatments (NaCl vs. control) for both En and Rh bacteria along CPCoA2 (Fig. 3c), indicating the salt-induced recruitment of specific root-associated BC.

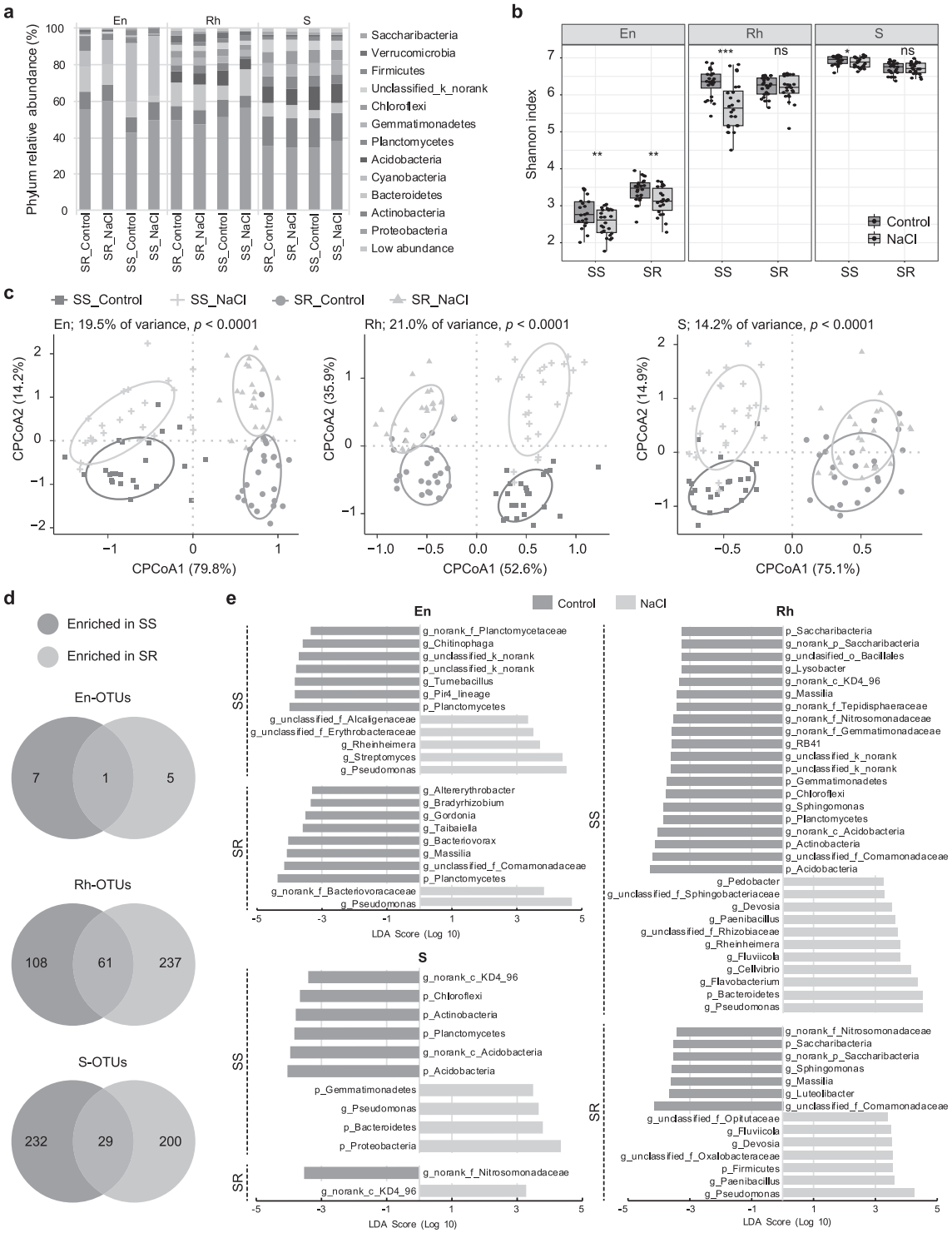
Next, we examined salinity-induced variation in root-associated bacteria at the OTU level (Fig. 3d and Supplementary Table S4). Specifically, OTUs enriched in SSs and SRs accounted for 87.5% (7 out of 8 OTUs) and 83.3% (5 out of 6 OTUs) in En, 63.9% (108 out of 169 OTUs) and 79.5% (237 out of 298 OTUs) in Rh, and 88.9% (232 out of 261 OTUs) and 87.3% (200 out of 229 OTUs) in S, respectively (Fig. 3d). It was noted that OTUs enriched in SSs did not show significant overlap with those enriched in SRs (Fig. 3d), emphasizing that SS and SR plants recruited distinct bacterial species, when challenged by salinity. Furthermore, the LefSe analysis showed that the number of genera, strongly enriched under salinity condition, was higher in SSs than in SRs (FDR-adjusted $p < 0.05$, Wilcoxon rank-sum test, the absolute LDA score >3.25; Fig. 3e, Supplementary Table S5, and Supplementary Figs. S7–S9), and that less than half of genera enriched in SSs (En: 20%, 1 out of 5; Rh: 40%, 4 out of 10) were consistent with those enriched in SRs (Fig. 3e). Notably, in either En or Rh, the genus Pseudomonas was enriched in both SSs and SRs (Fig. 3e).

Salt induces shifts in metabolic and ecological functions of root-associated bacteria

To further explore the response of bacterial functionality to salinity challenge, we performed the FAPROTAX analysis. The dominant functions were chemoheterotrophy (16.1–42.4%) and aerobic chemoheterotrophy (13.4–39.1%) (Supplementary Table S6), both of which were significantly enriched in Rh for either SSs or SRs (FDR-adjusted $p < 0.05$, Wilcoxon rank-sum test; Fig. 4a). Nevertheless, most functions were significantly depleted in Rh after suffering salinity. Although nearly half of the functions in Rh (40.0%, 16 out of 40 functions) were consistently depleted in both SSs and SRs, distinct depletion was observed: 11 functions (27.5%) were depleted only in SSs; 7 functions (17.5%) were depleted only in SRs. For instance, some functions related to nitrogen metabolism (e.g., nitrogen fixation and nitrate reduction) were significantly depleted only in SSs, while others (e.g., nitrite respiration, denitrification, nitrous oxide denitrification, nitrate denitrification, and nitrite denitrification) were depleted only in SRs. Unexpectedly, in contrast to Rh, the En samples exhibited only a few functions that showed significant shifts under salinity condition (Fig. 4a). Despite that, the function related to fermentation was depleted only in SSs, while those related to ureolysis, aromatic hydrocarbon degradation, aliphatic non-methane hydrocarbon degradation, and aromatic compound degradation were depleted only in SRs. Moreover, the distinction between salt treatments (NaCl vs. control) based on function was significant and detectable in Rh (Fig. 4b). Together, these results confirmed the salt-induced shifts in bacterial functionality, which occurred primarily in the Rh and differed between SSs and SRs.

Salt-induced bacterial consortium promotes plant growth under salinity condition

To test the ability of salt-induced BC to enhance plant salt tolerance, we cultivated plants in γ-sterilized soils re-inoculated with salt-induced BC (i.e., plant varieties were treated by self BCs) and then challenged them with salinity (Supplementary Fig. S10a). The dominant



bacteria (the relative abundance >1%) in salt-induced BC were identified and classified into 4 bacterial phyla (i.e., Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria; Supplementary Fig. S10b), 13 bacterial genera (Fig. 5a and Supplementary Tables S7 and S8), and 19 bacterial species (Fig. 5b). Although significant differences in the composition of salt-induced BCs existed between En and Rh and between SSs and SRs (Fig. 5a–c), almost all of the plant varieties, regardless of plant types (SS and SR), exhibited significantly stronger growth under BC–En and BC–Rh than under germ-free conditions when challenged by salinity ($p < 0.05$; Fig. 5d

◀ **Fig. 3 Specific root-associated bacteria recruited by plants when challenged by salinity. a** Phylum-level distribution of the root microbiota of salt-sensitive (SS) and salt-resistant (SR) plants under non-salinity (control) and salinity (NaCl) conditions (En endosphere, Rh rhizosphere, S bulk soil). **b** Shannon index of the root bacteria of SS and SR plants under non-salinity (control) and salinity (NaCl) conditions. The horizontal bars within boxes represent medians. The tops and bottoms of boxes represent the 75th and 25th percentiles, respectively. The upper and lower whiskers extend to data no more than 1.5× the interquartile range from the upper edge and lower edge of the box, respectively. Asterisks indicate statistically supported differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; ns not significant, $p > 0.05$). **c** Constrained principal coordinate analyses (CPCoA) with Bray–Curtis distance showing the distinction of the root bacteria of SS and SR plants under non-salinity (control) and salinity (NaCl) conditions. Ellipses cover 68% of the data for each treatment. **d** Venn diagrams showing the overlap of OTUs significantly enriched in SS and SR plants under salinity conditions (FDR-adjusted $p < 0.05$, Wilcoxon rank-sum test). **e** The linear discriminant analysis (LDA) scores to identify the salinity shaped taxa at the phylum (p) and genus (g) levels, detected by the LDA of effect size (LEfSe) analysis. Only taxa with the absolute LDA score >3.25 are shown. For the SS group, the plant genotypes used included JY4 (Chinese *Cucumis sativus* L. cv. Jinyou No. 4), JY1 (Chinese *Cucumis sativus* L. cv. Jinyan No. 108_2), CY (Chinese *Cucumis sativus* L. cv. Chiyu No. 8), JM (European *Cucumis sativus* L. cv. Jinmei No.3), JC (Chinese *Cucumis sativus* L. cv. Jinchun No.2), and JYM (European *Cucumis sativus* L. cv. Jinyanmini No.5). For the SR group, the plant genotypes used included LZ (Japanese *Cucurbita moschata* Duch cv. Lizhiyuan), HM (Japanese *Cucurbita moschata* Duch cv. Hemei No.3), BN (Chinese *Cucurbita moschata* Duch cv. Beinongliangzhen), XL (Chinese *Cucurbita maxima* Duch. Xili), CF (Chinese *Lagenaria siceraria* Standl. Chaofengkangshengwang), and XH (Korean *Lagenaria siceraria* Standl. Xuanhe). All of these plant genotypes were grown in non-sterile soils and treatments considered were non-salinity (control) and salinity (NaCl).

and Supplementary Figs. S10c and S11), emphasizing the capacity of salt-induced BCs in enhancing plant adaptability to salt stress. Notably, for SSs, BC–Rh was more effective than BC–En, while for SRs, BC–En was more effective than BC–Rh (Fig. 5d). Both SR and SS plants showed a significant reduction in salt-induced IR_Na^+ and an overall enhancement in the SAR of nutrients under BC–En and BC–Rh as compared to germ-free conditions (Fig. 5e,f). Similar to previous observation, chemoheterotrophy and aerobic chemoheterotrophy, which were dominant in En and Rh (Supplementary Table S6), were also the major functions in the salt-induced BCs (Supplementary Fig. S12 and Supplementary Table S9). Together, these results confirmed that salt-induced BCs were capable of enhancing plant adaptability to salt stress.

To further test whether self BCs were more effective than non-self BCs in enhancing plant salt tolerance, we cultivated plants in γ-sterilized soils re-inoculated with self BCs or non-self BCs (both of which were salt-induced) and then challenged them with salinity (Supplementary Fig. S13a). Interestingly, both self BCs and non-self BCs were capable of enhancing plant growth under salinity (Fig. 6a and Supplementary Figs. S13b and S14). However, only for Rh, self BCs were more effective than non-self BCs ($p < 0.05$), and this trend was consistent for both SS (JY4) and SR (XH) plants (Fig. 6a). In addition, a significant reduction in salt-induced IR_Na^+ and an overall enhancement in the SAR of nutrients were observed under all BC-related treatments as compared to germ-free (Fig. 6b, c). Together, these results emphasized that salt-induced BCs could alleviate salt stress in plants regardless of their salinity tolerance capacities, and that the plant species-specific effect occurred primarily in the Rh rather than in the En.

Salt-induced bacterial consortium enhances plant adaptability to salt stress through synergistic regulation

We successfully created a broad diversity gradient in salt-induced BCs (Fig. 7a–c and Supplementary Fig. S15). When challenged by salinity, plant biomass declined strongly with reductions in bacterial diversity and simplification of salt-induced BCs (Fig. 7d), implying that plant adaptability to salt stress is driven by the diversity and species composition of various groups of salt-induced BCs. The changes in the diversity and composition of salt-induced BCs also influenced bacterial functionality (Fig. 7e and Supplementary Fig. S16). Changes in bacterial functionality were tightly linked to the ability of salt-induced BCs to regulate plant adaptability to salt stress (Fig. 7f). These results highlight that the consortium, but not individual members of the salt-induced BCs, provides enduring resistance against salt stress, and that synergistic regulation, rather than individual effect, is the underlying mechanism employed by salt-induced BCs to enhance plant adaptability to salt stress.

Discussion

This study shows that salinity challenge in plant roots leads to recruitment of specific root-associated BC capable of enhancing plant adaptability to salt stress. Although SR plants were more adaptable to salinity than SS ones, the salt-induced BC enhanced salt tolerance in plants regardless of their salinity tolerance capacities. As a consortium, the salt-induced bacteria in either Rh or En are beneficial to the plants as together they induce resistance against salinity but also enhance plant growth. Together these findings provide in-depth insights into the potential functional significance of stress-induced recruitment of specific root-associated bacteria in plants suffering from abiotic stresses.

These findings are in agreement with the most recent comparative studies confirming that biotic stresses, such as pathogen infection and insect attack, can lead to the

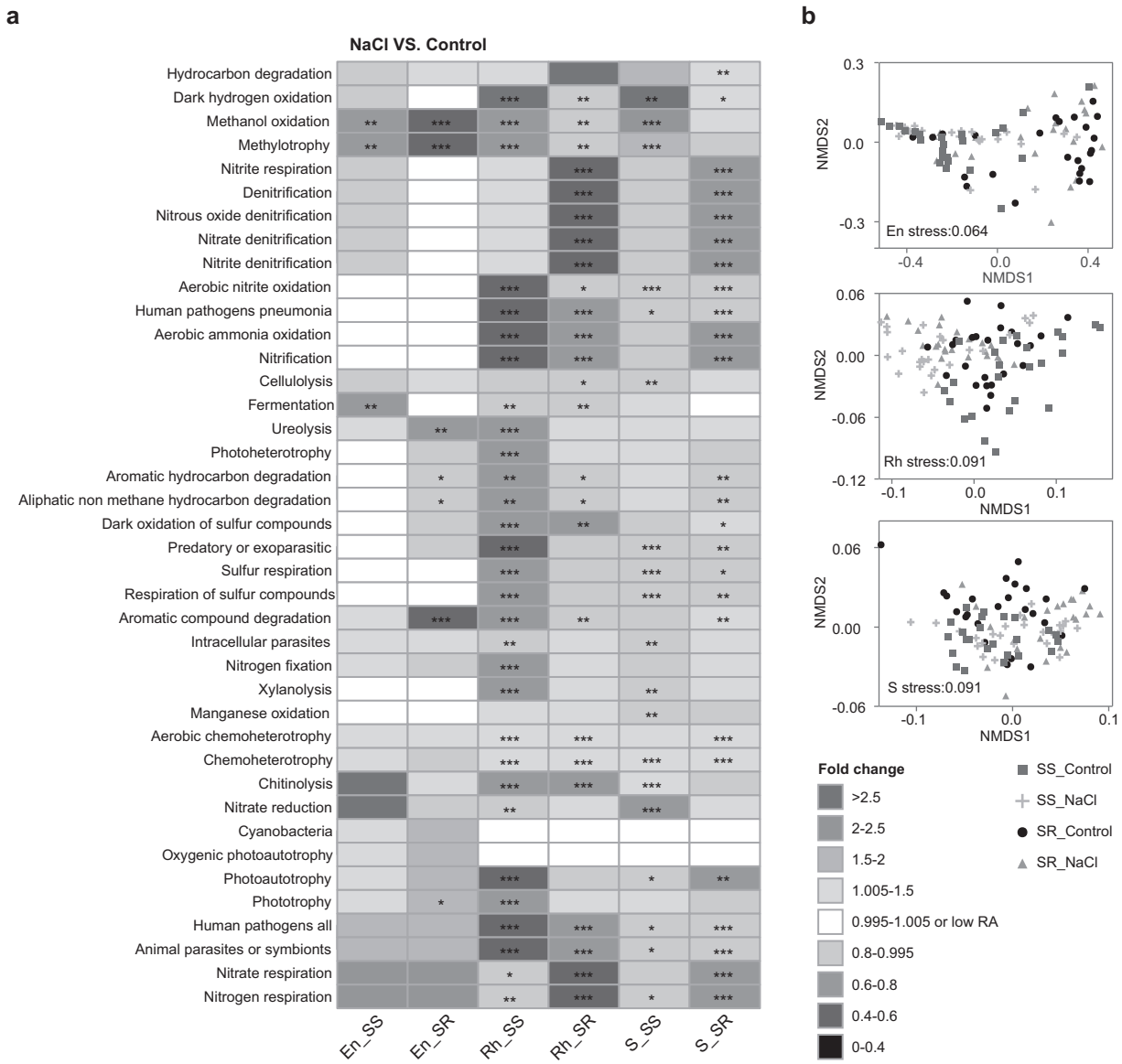
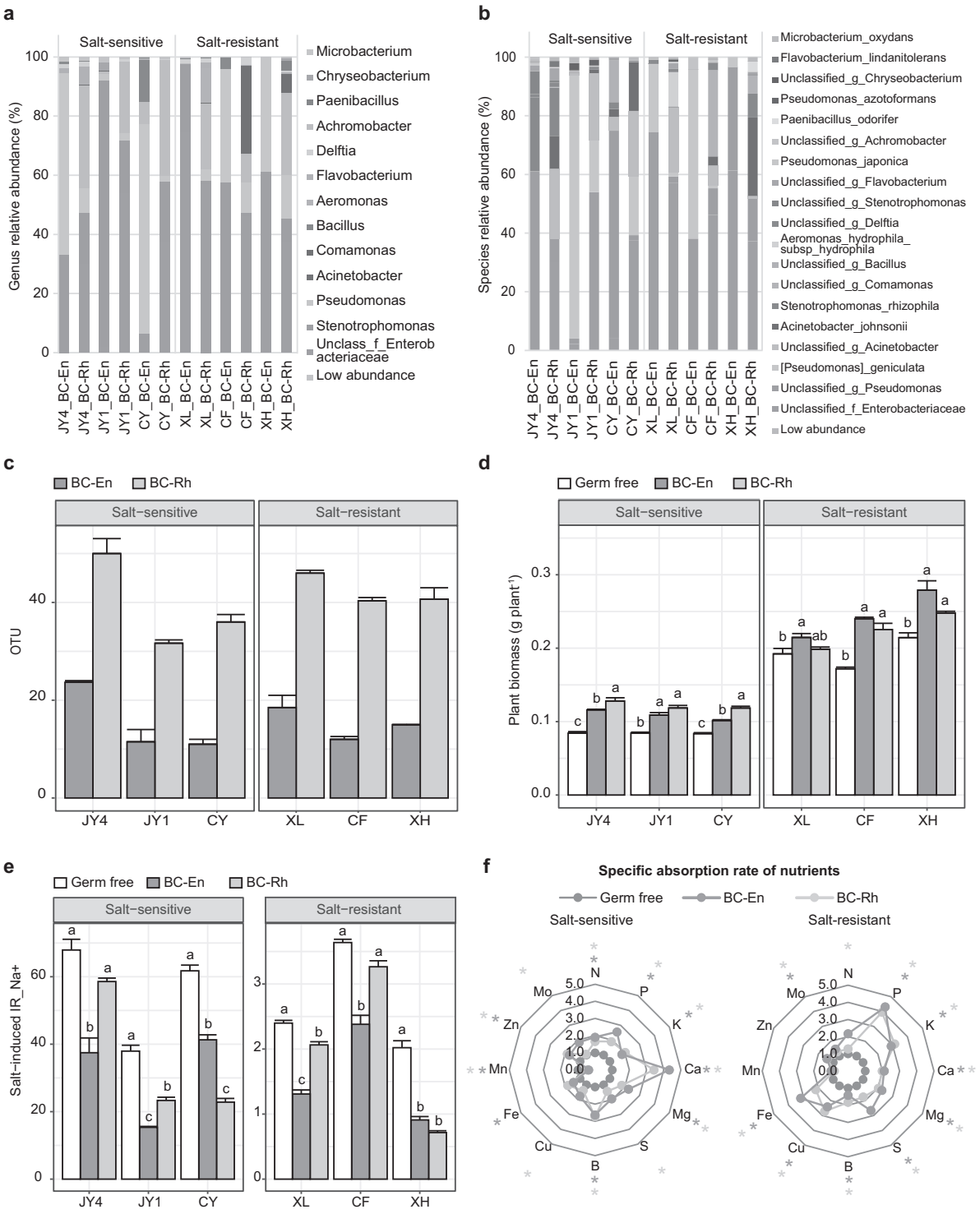


Fig. 4 Metabolic and ecological functions of root-associated bacteria. a Fold change of relative abundance (RA) of functional bacterial communities in roots of salt-sensitive (SS) and salt-resistant (SR) plants due to salinity challenge (En endosphere, Rh rhizosphere, S bulk soil). Control, non-salinity; NaCl, salinity. Asterisks indicate statistically supported differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). **b** Non-metric multidimensional scaling (NMDS) plots performed to visualize data associated with metabolic and ecological functions of root-associated bacteria. For the SS group, the plant genotypes used included JY4 (Chinese *Cucumis sativus* L. cv. Jinyou No. 4), JY1 (Chinese *Cucumis sativus* L. cv. Jinyan No. 108_2), CY (Chinese *Cucumis sativus* L. cv. Chiyu No. 8), JM (European *Cucumis sativus* L. cv. Jinmei No.3), JC (Chinese *Cucumis sativus* L. cv. Jinchun No.2), and JYM (European *Cucumis sativus* L. cv. Jinyanmini No.5). For the SR group, the plant genotypes used included LZ (Japanese *Cucurbita moschata* Duch cv. Lizhiyuan), HM (Japanese *Cucurbita moschata* Duch cv. Hemei No.3), BN (Chinese *Cucurbita moschata* Duch cv. Beinongliangzhen), XL (Chinese *Cucurbita maxima* Duch. Xili), CF (Chinese *Lagenaria siceraria* Standl. Chaofengkangshengwang), and XH (Korean *Lagenaria siceraria* Standl. Xuanhe). All of these plant genotypes were grown in non-sterile soils and treatments considered were non-salinity (control) and salinity (NaCl).

assemblage of a group of stress resistance-inducing and growth-promoting beneficial bacteria in plant roots [54–57]. Interestingly, although SS and SR plants recruited distinct bacterial species and relevant functions when challenged by salinity (Figs. 3 and 4), a consistent growth promotion by salt-induced BCs was observed under salinity conditions (Figs. 5 and 6). This could be explained by the fact that soils contain highly diverse bacterial communities [58], most of which can directly or indirectly influence plant growth, nutrition, and health [59]. Furthermore, it also emphasizes



that in addition to the genetic differentiation-driven stress adaptation in plants [60], stress-induced recruitment of specific root-associated bacteria is also an effective mechanism that cannot be ignored, because plants must directly face various biotic and/or abiotic stresses due to their sessile nature [9].

Salinity is a major abiotic stress threatening global agricultural production [3]. Despite the fact that the physiological and molecular basis of salt adaptation in plants has been sufficiently proven and some stress-tolerant plants developed [13], it is still a big challenge to enhance plant salt tolerance under field conditions [61]. Our findings

◀ **Fig. 5 Plant salt adaptation as affected by salt-induced bacterial consortium associated with roots. a** Genus-level distribution of the salt-induced bacterial consortium (BC) isolated from the root endosphere (En) and rhizosphere (Rh) of salt-sensitive and salt-resistant plants challenged by salinity. **b** Species-level distribution of the salt-induced BC isolated from the root En and Rh of salt-sensitive and salt-resistant plants challenged by salinity. **c** OTU number of the salt-induced BC isolated from the root En and Rh of salt-sensitive and salt-resistant plants challenged by salinity. **d**, **e** The plant biomass and salt-induced plant Na^+ increase rate (IR_Na^+) of salt-sensitive and salt-resistant plants grown under salinity conditions as affected by salt-induced BCs. Data bars represent means and error bars represent the standard error of the mean. Different letters indicate statistically significant differences between treatments as determined by one-way ANOVA with post hoc Tukey HSD test ($p < 0.05$). **f** The specific absorption rate (SAR) of nutrients of salt-sensitive and salt-resistant plants as affected by salt-induced BCs. Asterisks indicate statistically supported differences ($*p < 0.05$). JY4: Chinese *Cucumis sativus* L. cv. Jinyou No. 4; JY1: Chinese *Cucumis sativus* L. cv. Jinyan No. 108_2; CY: Chinese *Cucumis sativus* L. cv. Chiyu No. 8; XL: Chinese *Cucurbita maxima* Duch. Xili; CF: Chinese *Lagenaria siceraria* Standl. Chaofengkangshengwang; XH: Korean *Lagenaria siceraria* Standl. Xuanhe.

provide a clear pathway for the development of salt adaptability in plants through querying opportunistic mutualisms with prokaryotes that help plants solve context-specific challenges (Figs. 2–7). Our experiments, on two plant types (SS and SR), have revealed some novel plant responses to salinity challenge. Metagenomics and functional genomics, combined with plant growth tests, demonstrated that the salt-induced BC rescued both SS and SR plants from the salinity challenge (Figs. 2–6). This means that, notwithstanding the significance of genetic differentiation, plant roots seem to display a broadly similar response profile in the face of salinity, i.e., recruitment of specific root-associated BC that is capable of enhancing plant adaptability to salt stress. This can be partly supported by the results reported by Rodriguez et al. [62], who found that native plants from coastal (saline–alkaline) habitats require symbiotic microorganisms for salt tolerance.

Metagenomics and functional genomics data from Figs. 3 and 4 led to a second novel result: notwithstanding the consistent growth promotion by salt-induced BCs, SS and SR plants recruited distinct bacterial species and relevant functions when challenged by salinity. This implies that plants employ a species-specific strategy to recruit beneficial soil bacteria that can help them solve salinity challenge. Indeed, plants are powerful drivers and selective forces in the evolutionary history of native microorganisms [63, 64]. For instance, we found that the phylum Bacteroidetes was strongly enriched in the Rh of SSs, while the phylum Firmicutes was strongly enriched in the Rh of SRs (Fig. 3e). Interestingly, both Bacteroidetes and Firmicutes are dominant halophilic/halotolerant phylotypes in saline soils [65]. Furthermore, our functional genomics data revealed that these two phyla were characterized by chemoheterotrophy, fermentation, and sulfate respiration, all of which are the functions closely associated with nutrient cycling [66, 67]. It has been demonstrated that microorganisms can mitigate salt stress through enhancing the ability of plants to absorb nutrient [68]. Similar trends were observed in this study (Figs. 2e, 5f, and 6c). Despite the distinction between plant types based on bacterial taxonomy, we also noted that for either SS plants or SR plants, the genus Pseudomonas was enriched in both En and Rh under salinity conditions (Fig. 3e). This suggests that Pseudomonas had high adaptability to salt environment and also affinity to plant roots, and thereby high potential to enhance plant adaptability to salt stress. Indeed, recent studies have documented that most species belonging to the genus Pseudomonas have the ability to ameliorate salinity stress in plants [69], through the production of stress alleviating metabolites such as exopolysaccharides, gibberellins, ACC deaminase, and indole acetic acid [70]. Taken as a whole, these findings validated our first hypothesis, that is, that when challenged by salinity, plants can recruit specific RDB that enhance plant tolerance to salt stress.

Unexpectedly, results from Figs. 5 and 6 did not confirm our second hypothesis that RDB in SR plants might be more capable of attenuating plant salt stress than those in SS ones. We calculated the plant response to salt-induced BC (PRSIBC) under salinity conditions, and found that the PRSIBC for En–BC was not significantly different between SS and SR plants ($p = 0.824$), but the PRSIBC for Rh–BC was significantly higher in SS plants than in SR plants ($p = 0.033$) (Supplementary Fig. S17). This implies that the plant species-specific effect occurred primarily in the Rh rather than in the En, which is consistent with the results from Fig. 6a, and that the salt-induced bacteria in the Rh were more beneficial to the SS plants than the SR plants. Indeed, the root surface has been increasingly recognized as a frontier for plant microbiome research [71]. Nevertheless, salt-induced BCs enhanced stress adaptation in plants regardless of their salinity tolerance capacities. Together, irrespective of the degree of salt tolerance, plants can recruit microbes capable of protecting plants against salt stress in the En and Rh. However, theoretically speaking, root-associated bacteria of SS plants may play a limited role in alleviating salt stress in plants, because SS plants are less adaptable to salt stress than SR ones. Despite this, it does not mean that the root-associated bacteria of SS plants do not protect plants against salt stress. If there was no bacterium existing in the En and Rh, the SS plants would be more vulnerable to salinity (Fig. 5d–f). Indeed, the salt-induced bacteria

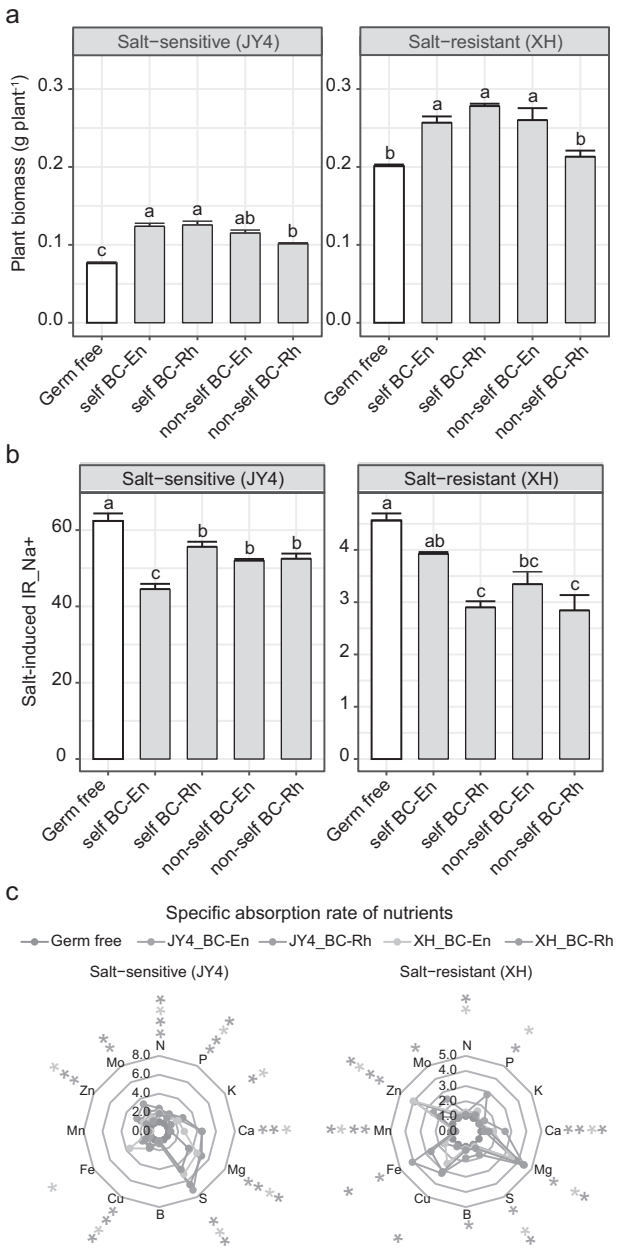


Fig. 6 Comparison of the effectiveness of self and non-self bacterial consortiums in enhancing plant salt adaptation. a, **b** The plant biomass and salt-induced plant Na^+ increase rate (IR_Na^+) of salt-sensitive and salt-resistant plants grown under salinity conditions as affected by self and non-self bacterial consortiums (BCs) that were induced by salinity (En endosphere, Rh rhizosphere). Data bars represent means and error bars represent the standard error of the mean. Different letters indicate statistically significant differences between treatments as determined by one-way ANOVA with post hoc Tukey HSD test ($p < 0.05$). **c** The specific absorption rate (SAR) of nutrients of salt-sensitive and salt-resistant plants as affected by self and non-self BCs that were induced by salinity. Asterisks indicate statistically supported differences (*$p < 0.05$). JY4: Chinese *Cucumis sativus* L. cv. Jinyou No. 4; XH: Korean *Lagenaria siceraria* Standl. Xuanhe. Genus- and species-level distributions, and OTU number of the salt-induced bacterial consortium (BC) isolated from the root En and Rh of JY4 and XH are shown in Fig. 5a–c. For JY4, non-self BCs were the BCs isolated from XH. For XH, non-self BCs were the BCs isolated from JY4.

in either En or Rh were beneficial to both SS and SR plants (Fig. 5d–f and Supplementary Fig. S17).

Resistance to various environmental stresses represents a major life support function of plants due to their sessile nature [9]. Many recent reports have verified the ability of a single or a few strains to help plants solve context-specific challenges [54, 72]. However, the diversity of microbial, in particular bacterial, species in a single gram of Rh soil can be enormous [49, 73]. Furthermore, theoretical and experimental studies have indicated that microbial communities with high diversity are often less prone to being disturbed than simpler ones [74, 75]. Similarly, in the present study, results from Fig. 7 revealed that the synergistic regulation of salt-induced BCs determined plant adaptability to salt stress, whereby reduction in the diversity of salt-induced BCs resulted in lower plant biomass when challenged by salinity (Fig. 7). These findings are in agreement with some previous comparative studies confirming that microbial diversity exerts a positive effect on the alleviation of biotic stresses (e.g., pathogen infection and insect attack) in plants [76–78]. However, these findings somehow contradict studies where single microbes were able to protect plants against salt stress [79, 80]. This comes as no surprise, because synergistic interaction is an important mechanism for microbes to adapt to their living surroundings including Rh and En [74–78]. Moreover, microbes in nature mostly occur as part of complex communities rather than simple ones, and this has been noted since the time of van Leeuwenhoek [81]. Although most previous studies focused on the capacity of single microbes in alleviating salt stress in plants, our studies demonstrated the multispecies synergistic interactions of beneficial microbes in the Rh and En of plants.

In summary, this study provides support for the hypothesis that when challenged by salinity, plants can recruit specific RDB that enhance stress adaptation in plants, regardless of their salinity tolerance capacities. Despite the distinct bacterial species and relevant functions that existed between plant types (SS and SR), salt-induced recruitment of specific RDB led to a consistent growth promotion, implying that plants employ a species-specific strategy to recruit beneficial soil bacteria that can help them solve salinity challenge. The plant species-specific effect occurred primarily in the Rh rather than in the En. Notably, the consortium, but not individual members of the salt-induced RDB, provided enduring resistance against salt stress. While the positive effect of bacterial diversity on plant growth and health has previously only been well documented in biotic stresses (e.g., pathogen infection and insect attack), the present study extends our knowledge of the critical roles of bacterial diversity in alleviating abiotic stresses such as salinity.

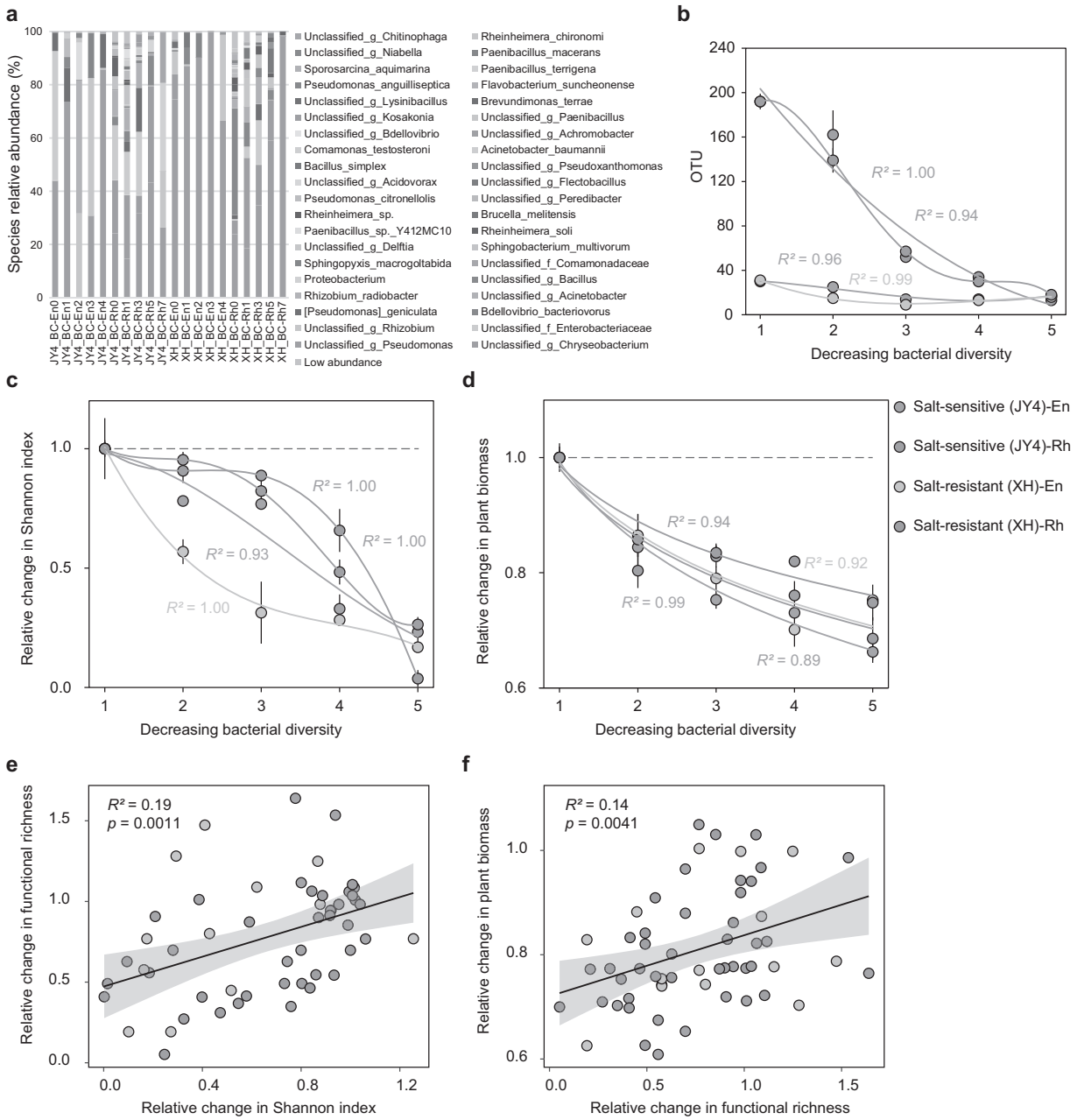


Fig. 7 Synergistic regulation of plant salt adaptation by salt-induced bacterial consortium. a Species-level distribution of the salt-induced bacterial consortium (BC) from the root endosphere (En) and rhizosphere (Rh) of salt-sensitive (JY4) and salt-resistant (XH) plants challenged by salinity. For either BC–Rh or BC–En, five levels of dilution (10^0, 10^{-1}, 10^{-3}, 10^{-5}, and 10^{-7} for BC–Rh, and10^0, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} for BC–En) of the BC suspension were used as inocula to create a gradient of BC diversity. Notably, these diluted BCs had different diversities but globally similar abundances. **b** OTU numbers of the salt-induced BC–En and BC–Rh with different diversities. Bacterial diversity gradient was established by serial dilutions of salt-induced BCs. Numbers greater than one (bacterial diversity 1) refer to higher levels of dilution and, thus, reduced bacterial diversity. Notably, these diluted BCs had different diversities but globally similar abundances. Lines highlight trends in the changes in OTU number across the decreasing gradient of bacterial diversity. **c**, **d** Relative changes in Shannon index in microbial communities and plant biomass challenged by salinity with increasing simplification of salt-induced BCs. For each BC, means ± standard errors of Shannon index and plant biomass are expressed as a ratio of the most complete salt-induced microbial consortium (bacterial diversity 1, dashed line) such that values below 1 represent a reduction in the Shannon index and plant biomass. Lines highlight trends in the changes in Shannon index and plant biomass across the decreasing gradient of bacterial diversity. **e** Functional richness in relation to the Shannon index in salt-induced BCs. **f** Plant biomass challenged by salinity in relation to the functional richness in salt-induced BCs. Shading indicates 95% confidence interval.

Data availability

All raw amplicon reads can be found in the NCBI database and the SRA accession numbers SRP267882, SRP268035, SRP268033, SRP268020, and SRP295317.

Author contributions HL, LG, and YT developed the study concept and experimental design. LG and YT supervised the project. HL, SL, and XZ performed laboratory work. HL, SL, and XZ collected the samples. HL and YT conducted data analysis. HL and YT wrote and revised the manuscript. All authors read and approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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