Microbiome of the Rhizosphere: from Structure to Functions

Ning Ling (nling@njau.edu.cn)
Nanjing Agricultural University https://orcid.org/0000-0003-1250-4073
Tingting Wang
Nanjing Agricultural University
Yakov Kuzyakov
Georg-August University of Göttingen https://orcid.org/0000-0002-9863-8461

Article

Keywords: Bacterial communities and functions, Rhizosphere properties and processes, Microbial r and K strategies; Bacterial dormancy, Microbial hotspots and habitats

Posted Date: March 18th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-274143/v1

Version of Record: A version of this preprint was published at Nature Communications on February 11th, 2022. See the published version at https://doi.org/10.1038/s41467-022-28448-9.

Abstract

Microbial composition and functioning in the rhizosphere are among the most fascinating but hidden topics in microbial ecology. We generalized bacterial traits regarding community diversity, composition and functions using published 16s rDNA amplicon sequences of 584 pairs of bulk soils vs rhizosphere of crops. The lower bacterial diversity in the rhizosphere (-7% richness) compared to root-free soil reflects the excess of available organic substances near the root. The rhizosphere is enriched by Bacteroidetes, Proteobacteria and Cyanobacteria as well as other copiotrophic bacteria (*r* strategists). Complex but unstable bacterial networks in rhizosphere reflect tight microbial interactions and adaptations to fluctuating conditions common for *r* strategists. The dominant dormancy strategy in the rhizosphere is the toxin-antitoxin system, while sporulation is common in bulk soil. Function prediction analysis showed that the rhizosphere is strongly enriched (50–115%) in methanol oxidation, ureolysis, cellulolysis, chitinolysis and nitrogen fixation, but strongly depleted in functions related to N-cycling.

Introduction

The rhizosphere harbors a great abundance of diverse microbes, many of which undoubtedly benefit plants by suppressing pathogenic invasion and assisting in nutrient acquisition from the soil [1, 2]. Understanding the taxonomic and functional components of the rhizosphere microbiome and their differences from bulk soils (here: soil without direct root effects) are crucial for manipulating them for sustainable crop production. The recent development of next-generation sequencing enabled great progress in deciphering the microbiomes in specific crop rhizospheres. The diversity and composition of the rhizosphere bacterial community depends on both the plant species and soil properties [3, 4, 5]. Although plant species, or even genotypes within species, tend to assemble distinct rhizobacterial communities [6, 7, 8], these communities can be highly similar even in different environments [9]. A growing number of studies demonstrate that plants have selective effects on rhizobacterial assemblages in the bulk soil reservoir for acquiring specific functional traits related to plant fitness [8, 10, 11]. The result is that the rhizosphere microbiome considerably extends the functional repertoire of the plant [12]. Despite the different nutrient requirements, disease suppression mechanisms and edaphic habitats of plants, the rhizosphere conditions (with a surplus of available carbon) provide a similar set of conditions for microbial life. All crops are mineral resource-limited organisms and frequently suffer from pathogen invasions. Accordingly, there could be a generalized selective effect – targeted for nutrient acquisition and pathogen suppression – across all crops regardless of their soil environment or climate. Such potential general patterns for rhizosphere versus bulk soils in terms of the taxonomic and functional profiles of bacterial communities remain largely unexplored. Such information, however, is crucial to help understand and manage microbial functions in agroecosystems in support of enhancing global agriculture in the future.

Currently, amplicon sequence analysis of marker genes, typically 16S rRNA in the case of bacteria, helps to characterize the relative abundance of species in soil [13], and many studies have been conducted to

characterize root-associated microbial communities [9]. This has yielded extensive sequencing data targeting the rhizosphere because almost all the published studies are required to deposit their raw data in public gene banks. These high-resolution nucleic acid-based molecular techniques provide excellent insights into the specific microbiome members living in soil habitats. Research priorities for harnessing rhizosphere microbiomes for sustainable agriculture include determining the functional mechanisms mediating plant–microbiome interactions and defining the core microbiome of crops [14, 15]. The methodological progress made for these research priorities has provided massive data for an integrative analysis and subsequent synthesis. This opens the door for investigating the general principles of rhizosphere microbiome selection from bulk soils. We therefore tackle the challenge to infer the community compositions in rhizosphere and bulk soils, to determine specific rhizosphere microbiome properties common for the broad range of plant and environmental conditions, and to bridge the general rhizobacterial assemblages with their functions as related to community-aggregated dormancy potential, heterotrophic strategy, and individual nutrient cycling processes.

Herein, to clarify the general differences, we collected the 16s rRNA amplicon-based sequencing data from all available gene banks, and then synthetically analyzed the data using state-of-the-art bioinformatic methods. Bulk soil serves as a microbial reservoir for the rhizo-microbiome [16] and harbors considerable microbial diversities that are distinctly shaped by the environmental factors of the respective microhabitats [17, 18]. We thus hypothesized that i) as the rhizobacterial assemblages are predominantly recruited from the corresponding bulk soil, but are preselected by an excess of released root carbon, the bacterial diversity is generally lower and bacterial network displays less complexity in the rhizosphere. Relative to bulk soil, the rhizosphere can fuel its microbiota by providing rich and available energy and carbon resources [19, 20], and root-free soil is frequently carbon limited for microbes (Kuzyakov and Xu, 2013). We therefore further hypothesized that ii) richer copiotrophic bacteria are harbored in the rhizosphere relative to the bulk soil. To this end, we evaluated the community weighted mean 16s rRNA gene copies (rRNA operons) because copiotrophs are assumed to have more rRNA operons than oligotrophs [21, 22]. Based on the general pattern of N depletion in the rhizosphere versus bulk soil [23, 24] and on the more intensive N transformations in N-richer conditions, we further hypothesized that iii) the functions responsible for the N cycling-related processes are less represented in the rhizosphere. Plants deposit a significant proportion of their photosynthates into the rhizosphere as rhizodeposits and root litter. The rhizodeposits, including amino acids, organic acids, and polymeric carbohydrates such as cellulose and hemicellulose [25], are not only critical carbon and energy resources for rhizobacteria but are also an important trigger for attracting plant pathogens. We finally hypothesized that v) the functions related to ligocellulose degradation and plant pathogens are overrepresented in the rhizosphere. In this work, we analyzed and synthesized the very broad range of taxonomic and functional features of the microbiome of the rhizosphere as compared to the bulk soil separately from other drivers of compositional variation such as the environment. This yielded general principles of microbial selection around living roots, laying a foundation for harnessing the microbiome for sustainable agricultural production.

Results

Bacterial diversity and community composition in rhizosphere and bulk soils

The dataset was collected from the public databases with published papers across continents. Synthesis of the bacterial sequences from cropland paired bulk vs rhizosphere soils under the vegetation of Gramineae, Leguminosae, Solanaceae and Cucurbitaceae (Fig. S1) provided a generalized pattern of alpha-diversity. Bacterial diversity is depleted in the rhizosphere compared to the bulk soil in terms of richness (-7%), Shannon index (-3.9%), evenness (-2.8%) and phylogenetic diversity (-5.2%) (Fig. 1). These alpha-diversity indices lost the significant difference under nitrogen (N) addition, indicating a N fertilization-induced compensation of common N depletion in the rhizosphere compared to bulk soil. The absence of significances for Gramineae and C4 plants shows similarities in their rhizosphere independent of photosynthesis type. Intriguingly, significantly higher phylogenetic diversity was detected in rice rhizosphere, probably reflecting the strongly changing redox environment near rice roots.

Three bacterial phyla – Bacteroidetes, Proteobacteria and Cyanobacteria – were enriched in the rhizosphere, whereas Planctomycetes, Chloroflexi, Acidobacteria, Gemmatimonadetes and Nitrospirae were more abundant in the bulk soils (Fig. 2 and Fig. S3). Even though the relative abundance of Proteobacteria was higher in the rhizosphere, the genera Haliangium, Anaeromyxobacter, Acidovorax and Nitrosospira, belonging to Proteobacteria, were much richer in the bulk soils (Fig. 2). Although there was no difference in the relative abundance of Firmicutes, *Bacillus* sp. is more common in rhizosphere and *Romboutsia* sp. prefers the bulk soils. The relative abundance of the phylum Actinobacteria was also similar in the two soils, but some of its genera including Streptomyces, Glycomyces, Arthrobacter, Microbacterium and Lechevalieria are much richer in the rhizosphere, whereas genera including Gaiella, Aeromicrobium, Blastococcus and Solirubrobacter were mainly localized in the bulk soils.

Co-occurrence patterns in rhizosphere and bulk soils

Networks of bacterial communities in the rhizosphere (Fig. 3a) and bulk (Fig. 3b) soils have topological differences. The number of nodes and edges in bulk soil are 362 and 3032, whereas the number of nodes and edges of the rhizosphere network are 410 and 2369, respectively (Fig. 3 and Table S1). Although the rhizosphere network features many more modules (Table S1), the bacteria community network in the bulk soils is much more robust. This is reflected by the always higher natural connectivity upon removal of any level of nodes (Fig. 3c). In both networks, Proteobacteria and Actinobacteria were dominant in each of the modules (Fig. 3d and 3e). However, the species highly connecting the nodes among modules differ (Fig. 3f): in rhizosphere network, the main connectors are affiliated with *Lacibacter* sp., *Terrimonas* sp., *Nocardioides* sp., *Steroidobacter* sp., *Diaphorobacter* sp., *Ralstonia* sp., *Afipia* sp. and *Arachidicoccus* sp., whereas the role of the between-module connectors in the bulk soil network was played by the species *Blastococcus* sp., *Streptomyces* sp., *Devosia* sp., *Acidibacter* sp. and *Bacillus* sp..

Dormancy potential and heterotrophic strategies at bacterial community level

Dormancy potential, characterized by genes involved in toxin-antitoxin and sporulation, was evaluated to characterize bacterial community-aggregated dormancy strategies in the rhizosphere and bulk soil.

Generally, a 42% higher toxin-antitoxin functional potential is common in the former (Fig. 4a), while a12% higher functional potential for sporulation was determined in the latter (Fig. 4b). Consequently, the bacteria inhabiting the rhizosphere employed distinctly different dominant strategies to enter dormancy than those inhabiting bulk soils. These differences in functional potential regarding toxin-antitoxin were removed by N fertilization, which compensated the N depletion in the rhizosphere. Paddy soil conditions eliminated the bacteria community with a well-developed toxin-antitoxin system in the rhizosphere, revealing a strong effect of land use on the bacterial dormancy potential.

The heterotrophic strategy was characterized by the rRNA operon copy number. Bacteria with more rRNA operon counts are able to sustain a higher maximum growth rate and respond faster to resources. The 6.9% more rRNA operon counts in the rhizosphere (Fig. 4c) indicate that much more fast-growing bacteria (r-strategists) colonize the rhizosphere than the bulk soil. The heterotrophic strategy at the bacterial community-level was also impacted by the land-use regime: the rhizosphere effect on the heterotrophic strategy was weaker in paddies than in upland soils.

Functional signatures in rhizosphere and bulk soils

All bacterial metabolic and ecologically relevant functions are predicted by the FAPROTAX based on the sequences (Fig. 5). Rhizosphere-inhabiting bacteria have increased functional potentials related to plant pathogens (+ 196%), methanol oxidation (+ 116%), ureolysis (+ 97%), cellulolysis (+ 76%), methylotrophy (+ 73%), chitinolysis (+ 62%) and nitrogen fixation (+ 52%) compared to bulk soil. Significant differences in these functions (except for chitinolysis) were absent in paddy soils. Nitrogen fertilization decreased these rhizosphere effects on function potentials related to cellulolysis, chitinolysis, methylotrophy, and methanol oxidation. In contrast, the bulk soils hosted a bacterial community with 26–44% richer functional potentials related to nitrite respiration, nitrate denitrification, nitrite denitrification, nitrous oxide denitrification, denitrification, aerobic ammonia oxidation, iron respiration, nitrification and aerobic nitrite oxidation (Fig. 5). The plant types, land use, and N addition can modify the effect sizes between rhizosphere and bulk soils. For instance, the effects on N cycling-related functions including nitrite respiration, nitrate denitrification, nitrite denitrification, nitrous oxide denitrification, and denitrification become non-significant in Gramineae and under N fertilization. Again, paddy soils showed a specific response with the absence of the rhizosphere effects on such functions as nitrification, nitrite respiration, aerobic nitrite oxidation and iron respiration.

Discussion

Bacterial diversity and composition in rhizosphere and bulk soil

This meta-analysis is based on pairwise data (rhizosphere vs bulk soils) from amplicon sequencing approaches characterizing the taxonomic and functional features. This yielded fundamental insights into crop rhizosphere microbiomes on an across-continent scale and onto the plant-driven microbial taxa and their functional properties in this unique but unified habitat. Our design enabled testing the general influence of crops on their rhizosphere bacterial community across soil and climate environments. This

highlights the benefit of using sequencing data in soils to synthesize general microbiome patterns and to indicate specialized functions and life strategies of microbial taxa based on niche differences between rhizosphere and bulk soils.

The fact that rhizosphere microbiota differ from bulk soil microbiota is well documented [11, 15, 26, 27, 28], and this is attributed to significant differences in physico-chemical attributes that result in niche differentiation [4, 20, 29, 30]. In addition to the between-niche difference in terms of the environment, the generalized contrast between bulk soil and rhizosphere was the most important source of the distinction in microbiota composition [11, 31]. We observed an overall lower bacterial richness, Shannon index, evenness and phylogenetic diversity for the rhizosphere vs bulk soil microbiota in all environments. Consequently, bacterial diversity decreases with increasing substrate availability, the conditions common in the rhizosphere. The general view is that the rhizosphere microbiota, a subset of the community in bulk soil, possess certain similar traits across plants, within varied environments. This underlines the selective effect of the rhizosphere, which to a certain extent has generalized consequences for the rhizobacterial assemblages across crops. This even holds true for bacteria belonging to various classes, orders and families. Though the effect size differs between crop groups (Fig. 1), we stress that, even after accounting for genotypic and environmental differences, the selection of microorganisms common for the rhizosphere still displayed certain similar traits [4, 8, 32].

Of course, the specific environmental conditions fundamentally shift the rhizosphere effect size: the rice rhizosphere, for example, harbors phylogenetically broader bacteria than its corresponding bulk soil. Paddy soils commonly develop anaerobic conditions, and rice plants therefore exhibit a developed aerenchyma. Due to the oxygen release around roots, the Eh and oxygen content in the rice rhizosphere are much higher than in the bulk soil [33, 34] and fluctuate strongly. Thus, a broader phylogeny of both anaerobic and aerobic bacteria colonize the rice rhizosphere. These results indicate that environmental heterogeneity, such as root exudates, Eh and oscillating moisture, interact to shape the rhizosphere selective effect.

The particular microbial taxa recruited to the rhizosphere from the soil microbial reservoir can apparently form a particular core microbiome [11, 13]. The core microbiome around roots contributes to plant growth and fitness [16]. To date, the core microbiome of plants, regardless of whether in the rhizosphere, endosphere or phyllosphere, has been defined mostly based on taxonomic markers. We, however, emphasize that more attention should be paid to identifying microbes having common functions that are selected for in a general rhizosphere setting. Accordingly, a function-based definition of the microbiome should facilitate efforts to manipulate communities for useful purposes. Our comprehensive analysis revealed a few predominant taxa that are consistently enriched in the rhizosphere, for example the phyla Bacteroidetes and Proteobacteria (Fig. 2). This result underscores the fact that these phyla are generally adapted to C-rich conditions (common in the rhizosphere) [30, 35], and are consequently very similar across diverse plant species. This finding is not surprising because Bacteroidetes and Proteobacteria utilize labile carbon sources for high metabolic activity, fast growth and propagation- They are generally

considered to be r-selected, or weedy fast-growing microbiota whose populations fluctuate opportunistically [28]. In contrast to the rhizosphere, the bulk soil is generally enriched by other dominant phyla including Acidobacteria (Fig. 2b), which are oligotrophs [36, 37]. Interestingly, a few phylum-level taxa are similar between rhizosphere and bulk soil, but finer taxonomic resolution reveals differences (Fig. 2). A case in point is Firmicutes: its class Bacilli is richer in the rhizosphere, whereas the class Clostridia is more abundant in the bulk soils. Similarly, Actinobacteria is richer in the rhizosphere, but its class Thermoleophilia is more abundant in the bulk soils. Consequently, the resulting generalized pattern that emerges based on the selective effect of the rhizosphere depends on the taxonomic resolution and on the fundamental niches at the level of classes and families.

Microbiome formation: from structuring to functions

In microbial networks, highly interconnected species are grouped into a module, within which species interactions are more frequent and intensive than with the remaining community. The rhizosphere bacterial network modularity is higher than that in the bulk soil (Fig. 3 and Table S1). One potential explanation is more pronounced niche differentiations – both spatially and temporally – in the rhizosphere [25] because modules can be interpreted as microbial niches [38, 39]. Modularity is one of the main organizing principles of biological networks [40], and the higher modularity in the rhizosphere indicates a more complex topological structure. Nevertheless, the rhizosphere bacterial co-occurrence network is less robust (Fig. 3c). This generalization is not always valid, for example in the case of a single wheat rhizosphere network that was more stable than that in the bulk soil [26]. This is attributable to the fact that the networks (Fig. 3) are constructed across plant species. Moreover, that particular rhizosphere is characterized by very high temporal dynamics as compared to the more static conditions in the bulk soil [30, 41]. Plant species selectively enrich specific microorganisms by investing in root exudates to feed their rhizosphere microbiota [1, 42]. The rhizosphere indigenous microbial community structure often differs remarkably across host species [43]. In a soybean cultivation, the microbial community in the rhizosphere was selected via niche filtering, whereas the bulk soil community arose via neutral (stochastic) processes [44]. The rhizosphere network allocates more network modules for executive functions, but fewer for network robustness, which partly reflects the fast element cycling in the rhizosphere [45, 46].

Rhizosphere and bulk soil are characterized by different dominant strategies of microbial dormancy: sporulation factors and toxin–antitoxin systems (Fig. 4a). The sporulation factors were more abundant in the bulk soil, whereas the toxin–antitoxin systems were enriched in the rhizosphere (Fig. 4b). During plant growth, roots actively and passively release a broad range of organic compounds into the rhizosphere. These compounds are the driving force for microbial density and activity [29, 47, 48]. The sporulation factor was abundant in the bulk dryland soils but played nonsignificant role in paddies (Fig. 4b). Hence, bacterial sporulation is more common in upland soils because the environmental conditions in paddies are more stable and homogeneous, and paddies are always moist. The less role of sporulation played in the rhizosphere (as compared to bulk soil) indirectly confirmed the buffered amplitude of moisture variation, which involves mucilage swelling conditions [49, 50, 51]. The genes related to

dormancy/sporulation strongly increase with aridity [52]. Toxin–antitoxin systems are composed by the genes that encode a toxin protein that inhibits cell growth and an antitoxin that counteracts the toxin [53].

More copiotrophs (e.g. Bacteroidetes and Proteobacteria) inhabited the rhizosphere, as confirmed by their rRNA operon counts, which were considerably higher there (Fig. 4c). Copiotrophs have more operon counts than oligotrophs [54]. The lower rRNA operon copy number is common for *K*-selected microbiota, and leads to slower growth rates and a more stable population. The bacterial functional trait of rRNA operon copy numbers increases with resource availability [22]. Organisms with multiple operon counts tend to be r-strategists, which are dominant in resource-abundant conditions and respond more rapidly to nutrient inputs [55, 56, 57]. Therefore, copiotrophs are dominant when resources are abundant, such as in the rhizosphere habitat.

Genes related to nitrification and denitrification were higher in the bulk soil (Fig. 5) because the rhizosphere is nutrient depleted due to root uptake [30]. This is indirectly confirmed by the fact that the rhizosphere affects nearly all these N cycle-related functions, whereby this depletion is alleviated by N fertilization (Fig. 5g-q). Nevertheless, cellulolysis, ureolysis and chitinolysis were more intensive in the rhizosphere, reflecting the increased abundance of bacteria degrading these substances. Members of Lysobacter (affiliated to Gammaproteobacteria) were abundant in the rhizosphere (Fig. 2) and are known to be chitinolytic bacteria [58]. The methanol oxidation and methylotrophy genes are much more abundant in the rhizosphere than in the soil (except paddies). Methylotrophy is higher in the rhizosphere, but this difference is equalized in paddy soils because of the aerobic microenvironment around rice roots and because the available reduced C is strongly diluted in water excess.

In nature, specifically in the rhizosphere, plants are constantly challenged by thousands of microbial populations, including commensals, pathogens and symbionts. Plant pathogens and N-fixers (e.g. *Rhizobium* sp., etc.) are enriched in the rhizosphere (Fig. 5) because their reproduction and functioning are dependent on a plant host supply with organics. Although the rhizosphere is a fluctuating environment in which the microbiome rapidly evolves in space and time, the accumulating evidence verifies that plants shape their rhizosphere microbiome to their own benefit, making sophisticated use of the microbial functional repertoire [12]. Nonetheless, it remains a challenge to clarify the equilibrium conditions for maintaining plant fitness, which involves establishing a balance between the passive attacks of pathogens and the active recruitment of beneficial bacteria.

By integrating sequencing data from several studies, we generalized the main differences in the microbiomes of rhizosphere and bulk soil regarding bacterial diversity, composition, selection of specific groups, co-occurrence network, and a very broad range of functions (Fig. 6). Bacterial diversity in the rhizosphere is reduced by 2.6-7% and represents a subset of the communities in bulk soil. The rhizosphere community composition is highly enriched with copiotrophic bacteria such as Proteobacteria and Bacteroidetes, while it is strongly depleted in Chloroflexi, Acidobacteria and Nitrospirae. As the rhizosphere has an organic C surplus and circulates nutrients quickly, the quicker-growing bacteria are overrepresented with functions related to lignocellulose degradation and plant pathogens, but depleted in

the functions responsible for N cycling-related processes (except N fixation). The indirect proof of the generalizations presented here is that nitrogen fertilization alleviated nearly all rhizosphere effects on bacterial diversity and functions. This also confirms the common mineral N depletion against the background of excess C around roots. In conclusion, the selective effects of the rhizosphere in shaping microbial communities to some extent overrides the differences between soils, crops and climate. This makes the rhizosphere the strongest factor in forming the composition, structure and functions of the soil microbiome and, thus, a key factor in the cycling of biogenic elements.

Methods

Data collection

An extensive literature survey was conducted through the Web of Science database (http://apps.webofknowledge.com/) from 2015 until March 2020. The key words of the literature search were “bulk” and “rhizosphere” and “bacteria”. A total of 1081 samples from 29 publications including field and greenhouse experiments were collected (Fig. S1), comprising 584 pairs of bulk soils vs rhizosphere (Appendix A). These studies examined the bacterial communities by high-throughput sequencing, including Illumina and 454 platforms.

The following criteria were used to select appropriate studies: (1) experiments in agricultural soil were included, studies in grassland, forest, or desert ecosystems excluded; (2) articles with sequencing data in one to-one correspondence between rhizosphere and bulk soil were retained. Nine primer pairs were identified from the study metadata (Table S1), with most samples using the primer set of 515F and 806R [28]. Besides the sequencing data, we also collected the following parameters: plant type (C3, C4), plant family (including Gramineae, Leguminosae, Solanaceae and Cucurbitaceae), soil texture and pH, location (i.e., latitude and longitude), mean annual temperature (MAT), mean annual precipitation (MAP) and the nitrogen, phosphorus and potassium fertilization rate.

Bioinformatic analysis and taxonomical annotation

We downloaded the raw sequence data according to the Accession Number from NCBI, and then FASTQ files were obtained for each study. First, raw paired-end sequences of each study were merged with the *fastq_mergepairs* command and quality filtered with the *fastq_filter* command in USEARCH software [59]. Sequences with a quality score below 20 and shorter than 200 base pairs were filtered. In a next step, the *fastx_uniques* command was implemented to remove the redundant sequences, and the denoising was conducted by Unosie3 to remove the chimeras and obtain the single nucleotide precision fragments. To integrate studies that used different 16S rRNA gene regions, we adopted a closed-reference workflow, which is a database-dependent approach employing a predefined set of reference sequences with known taxonomy, to obtain representative sequences and assign taxonomy (Fig. S2). Then, the *closed_ref* command (sequence identity ≥ 97%) in USEARCH was used to map the above-obtained fragments to non-redundant full-length 16S rRNA sequences with the Silva database (http://www.drive5.com/usearch/manual/sintax_downloads.html). The fragments that could not be

mapped were assigned to unknown sequences. After that, the OTU table was constructed according to the mapped full-length 16S sequences. Finally, the mapping OTU result of each data set in Usearch table format was directly imported to R software (version 3.6.0, R Core Team, 2019) and merged into an integrated OTU table for further analyses. All samples were rarefied to the same sequencing depth (10,000 reads per sample) for the downstream analysis.

Statistical analysis

Calculation of the response ratio

Declarations

Data availability

The sequences obtained in this study are previously published and publicly available, which were downloaded from the NCBI Sequence Read Archive. All the accession numbers were listed in Appendix A.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31772398, 41977080).

Footnotes

Author contributions: NL and YK designed research; NL, TTW and YK performed research; NL, TTW and YK analyzed data; and NL, TTW and YK wrote the paper.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E. V. L., Schulze-Lefert, P. Structure and Functions of the Bacterial Microbiota of Plants. *Annu. Rev. Plant. Biol.* **64**, 807–838 (2013).
2. Leach, J. E., Triplett, L. R., Argueso, C. T., Trivedi, P. Communication in the Phytobiome. *Cell* **169**, 587–596 (2017).
3. Vorholt, J. A., Vogel, C., Carlstrom, C. I., Muller, D. B. Establishing Causality: Opportunities of Synthetic Communities for Plant Microbiome Research. *Cell Host Microbe* **22**, 142–155 (2017).
4. Jiang, Y. J., *et al.* Plant cultivars imprint the rhizosphere bacterial community composition and association networks. *Soil Biol. Biochem.* **109**, 145–155 (2017).
5. Garbeva, P., van Elsas, J. D., van Veen, J. A. Rhizosphere microbial community and its response to plant species and soil history. *Plant Soil* **302**, 19–32 (2008).
6. Li, Y., *et al.* Rhizobacterial communities of five co-occurring desert halophytes. *Peerj* **6**, e5508 (2018).

7. Matthews, A, Pierce, S., Hipperson, H., Raymond, B. Rhizobacterial Community Assembly Patterns Vary Between Crop Species. *Front. Microbiol.* **10**, 581 (2019).
8. Perez-Jaramillo, J. E., Mendes, R., Raaijmakers, J. M. Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Mol. Biol.* **90**, 635–644 (2016).
9. Xu, J., *et al.* The structure and function of the global citrus rhizosphere microbiome. *Nat. Commun*, **9**, 4894 (2018).
10. Howard, M. M., Munoz, C. A., Kao-Kniffin, J., Kessler, A. Soil Microbiomes From Fallow Fields Have Species-Specific Effects on Crop Growth and Pest Resistance. *Front. Plant Sci.* **11**, 1171 (2020).
11. Yan, Y., Kuramae, E. E., de Hollander, M., Klinkhamer, P. G., van Veen, J. A. Functional traits dominate the diversity-related selection of bacterial communities in the rhizosphere. *ISME J.* **11**, 56–66 (2017).
12. Bakker, P. A. H. M., Berendsen, R. L., Doornbos, R. F., Wintermans, P. C. A., Pieterse, C. M. J. The rhizosphere revisited: root microbiomics. *Front. Plant Sci.* **4**, 165 (2013).
13. Lundberg, D. S., *et al.* Defining the core Arabidopsis thaliana root microbiome. *Nature* **488**, 86-+ (2012).
14. Busby, P. E., *et al.* Research priorities for harnessing plant microbiomes in sustainable agriculture. *Plos Biol*, **15**, e2001793 (2017).
15. de Vries, F. T., Griffiths, R. I., Knight, C. G., Nicolitch, O., Williams, A. Harnessing rhizosphere microbiomes for drought-resilient crop production. *Science* **368**, 270-+ (2020).
16. Hamonts, K., *et al.* Field study reveals core plant microbiota and relative importance of their drivers. *Environ. Microbiol.* **20**, 124–140 (2018).
17. Xu, Q., *et al.* Long-Term Chemical-Only Fertilization Induces a Diversity Decline and Deep Selection on the Soil Bacteria. *mSystems* **5**, e00337-20 (2020).
18. Richter, A., Schöning, I., Kahl, T., Bauhus, J., Ruess, L. Regional environmental conditions shape microbial community structure stronger than local forest management intensity. *Forest Ecol. Manag.* **409**, 250–259 (2018).
19. Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., Vivanco, J. M. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* **57**, 233–266 (2006).
20. Wallenstein, M. D. Managing and manipulating the rhizosphere microbiome for plant health: A systems approach. *Rhizosphere* **3**, 230–232 (2017).
21. Roller, B. R. K., Stoddard, S. F., Schmidt, T. M. Exploiting rRNA operon copy number to investigate bacterial reproductive strategies. *Nat. Microbiol.* **1**, 16160 (2016).
22. Wu, L. W, *et al.* Microbial functional trait of rRNA operon copy numbers increases with organic levels in anaerobic digesters. *Isme J.* **11**, 2874–2878 (2017).
23. Schöttelndreier, M., Falkengren-Grerup, U. Plant induced alteration in the rhizosphere and the utilisation of soil heterogeneity. *Plant Soil* **209**, 297–309 (1999).
24. Peng, Y., *et al.* Temporal and spatial dynamics in root length density of field-grown maize and NPK in the soil profile. *Field Crop. Res.* **131**, 9–16 (2012).

25. Nuccio, E. E, *et al.* Niche differentiation is spatially and temporally regulated in the rhizosphere. *Isme J.* **14**, 999–1014 (2020).
26. Fan, K. K., Weisenhorn, P., Gilbert, J. A., Chu, H. Y. Wheat rhizosphere harbors a less complex and more stable microbial co-occurrence pattern than bulk soil. *Soil Biol. Biochem.* **125**, 251–260 (2018).
27. Fan, K. K., *et al.* Rhizosphere-associated bacterial network structure and spatial distribution differ significantly from bulk soil in wheat crop fields. *Soil Biol. Biochem.* **113**, 275–284 (2017).
28. Peiffer, J. A, *et al.* Diversity and heritability of the maize rhizosphere microbiome under field conditions. *P. Natl. Acad. Sci. USA* **110**, 6548–6553 (2013).
29. Baudoin, E., Benizri, E., Guckert, A. Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. *Soil Biol. Biochem.* **35**, 1183–1192 (2003).
30. Kuzyakov, Y., Razavi, B. S. Rhizosphere size and shape: Temporal dynamics and spatial stationarity. *Soil Biol. Biochem.* **135**, 343–360 (2019).
31. Ren, Y., *et al.* Functional compensation dominates the assembly of plant rhizospheric bacterial community. *Soil Biol. Biochem.* **150**, 107968 (2020).
32. Chen, Y., Sun, R. B., Sun, T. T., Liang, Y. T., Jiang, Y. J., Sun, B. Organic amendments shift the phosphorus-correlated microbial co-occurrence pattern in the peanut rhizosphere network during long-term fertilization regimes. *Appl. Soil Ecol.* **124**, 229–239 (2018).
33. Atulba, S. L., *et al.* Evaluation of rice root oxidizing potential using digital image analysis. *J. Korean Soc. Appl. Bi.* **58**, 463–471 (2015).
34. Schmidt, H., Eickhorst, T., Tippkotter, R. Monitoring of root growth and redox conditions in paddy soil rhizotrons by redox electrodes and image analysis. *Plant Soil* **341**, 221–232 (2011).
35. Pausch, J., Zhu, B., Kuzyakov, Y., Cheng, W. Plant inter-species effects on rhizosphere priming of soil organic matter decomposition. *Soil Biol. Biochem.* **57**, 91–99 (2013).
36. Finn, D., Kopittke, P. M., Dennis, P. G., Dalal, R. C. Microbial energy and matter transformation in agricultural soils. *Soil Biol. Biochem.* **111**, 176–192 (2017).
37. Jones, R. T., Robeson, M. S., Lauber, C. L., Hamady, M., Knight, R., Fierer, N. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *Isme J.* **3**, 442–453 (2009).
38. Zhao, S., *et al.* Biogeographical distribution of bacterial communities in saline agricultural soil. *Geoderma* **361**, 114095 (2020).
39. Eiler, A., Heinrich, F., Bertilsson, S. Coherent dynamics and association networks among lake bacterioplankton taxa. *Isme J.* **6**, 330–342 (2012).
40. Zhou, J., Xiao, G. X., Wong, L., Fu, X. J., Ma, S., Cheng, T. H. Generation of arbitrary two-point correlated directed networks with given modularity. *Phys. Lett. A* **374**, 3129–3135 (2010).
41. Herron, P. M., Gage, D. J., Pinedo, C. A., Haider, Z. K., Cardon, Z. G. Better to light a candle than curse the darkness: illuminating spatial localization and temporal dynamics of rapid microbial growth in the rhizosphere. *Front.Plant Sci.* **4**, 323 (2013).

42. Blagodatskaya, E., Blagodatsky, S., Anderson, T. H., Kuzyakov, Y. Microbial Growth and Carbon Use Efficiency in the Rhizosphere and Root-Free Soil. *Plos One* **9**, e93282 (2014).
43. Berendsen, R. L., Pieterse, C. M. J., Bakker, P. A. H. M. The rhizosphere microbiome and plant health. *Trends Plant Sci.* **17**, 478–486 (2012).
44. Mendes, L. W., Kuramae, E. E., Navarrete, A. A., van Veen, J. A., Tsai, S. M. Taxonomical and functional microbial community selection in soybean rhizosphere. *Isme J.* **8**, 1577–1587 (2014).
45. Hinsinger, P. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* **237**, 173–195 (2001).
46. Kuzyakov, Y., Blagodatskaya, E. Microbial hotspots and hot moments in soil: Concept & review. *Soil Biol. Biochem.* **83**, 184–199 (2015).
47. Loeppmann, S., Blagodatskaya, E., Pausch, J., Kuzyakov, Y. Substrate quality affects kinetics and catalytic efficiency of exo-enzymes in rhizosphere and detritusphere. *Soil Biol. Biochem.* **92**, 111–118 (2016).
48. Ma, X. M., Zarebanadkouki, M., Kuzyakov, Y., Blagodatskaya, E., Pausch, J., Razavi, B. S. Spatial patterns of enzyme activities in the rhizosphere: Effects of root hairs and root radius. *Soil Biol. Biochem.* **118**, 69–78 (2018).
49. Kroener, E., Zarebanadkouki, M., Kaestner, A., Carminati, A. Nonequilibrium water dynamics in the rhizosphere: How mucilage affects water flow in soils. *Water Resour. Res.* **50**, 6479–6495 (2014).
50. Carminati, A. Rhizosphere wettability decreases with root age: a problem or a strategy to increase water uptake of young roots? *Front. Plant Sci.* **4**, 298 (2013).
51. Holz, M., Zarebanadkouki, M., Kaestner, A., Kuzyakov, Y., Carminati, A. Rhizodeposition under drought is controlled by root growth rate and rhizosphere water content. *Plant Soil* **423**, 429–442 (2018).
52. Tripathi, B. M., Moroenyane, I., Sherman, C., Lee, Y. K., Adams, J. M., Steinberger, Y. Trends in Taxonomic and Functional Composition of Soil Microbiome Along a Precipitation Gradient in Israel. *Microb. Ecol.* **74**, 168–176 (2017).
53. Harms, A., Brodersen, D. E., Mitarai, N., Gerdes, K. Toxins, Targets, and Triggers: An Overview of Toxin-Antitoxin Biology. *Mol. Cell* **70**, 768–784 (2018).
54. Klappenbach, J. A., Dunbar, J. M., Schmidt, T. M. rRNA operon copy number reflects ecological strategies of bacteria. *Appl. Environ. Microb.* **66**, 1328–1333 (2000).
55. Nemergut, D. R., *et al.* Decreases in average bacterial community rRNA operon copy number during succession. *Isme J.* **10**, 1147–1156 (2016).
56. Cui, J., *et al.* Carbon and nitrogen recycling from microbial necromass to cope with C:N stoichiometric imbalance by priming. *Soil Biol. Biochem.* **142**, 107720 (2020).
57. Blagodatskaya, E. V., Blagodatsky, S. A., Anderson, T. H., Kuzyakov, Y. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. *Appl. Soil Ecol.* **37**, 95–105 (2007).

58. Lupatini, M., Korthals, G. W., de Hollander, M., Janssens, T. K. S., Kuramae, E. E. Soil Microbiome Is More Heterogeneous in Organic Than in Conventional Farming System. *Front. Microb.* **7**, 2064 (2017).
59. Edgar, R. C. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **10**, 996-+ (2013).
60. Hedges, L. V., Gurevitch, J., Curtis, P. S. The meta-analysis of response ratios in experimental ecology. *Ecology* **80**, 1150–1156 (1999).
61. Liu, S. W., *et al.* Response of soil carbon dioxide fluxes, soil organic carbon and microbial biomass carbon to biochar amendment: a meta-analysis. *GCB Bioenergy* **8**, 392–406 (2016).
62. Noble, W. S. How does multiple testing correction work? *Nat. Biotechnol.* **27**, 1135–1137 (2009).
63. Lima-Mendez, G., *et al.* Determinants of community structure in the global plankton interactome. *Science* **348**, (2015).
64. Luo, F., Zhong, J. X., Yang, Y. F., Scheuermann, R. H., Zhou, J. Z. Application of random matrix theory to biological networks. *Phys. Lett. A* **357**, 420–423 (2006).
65. Shannon, P., *et al.* Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res.* **13**, 2498–2504 (2003).
66. Peng, G. S., Wu, J. Optimal network topology for structural robustness based on natural connectivity. *Physica A* **443**, 212–220 (2016).
67. Ruan, Y., Wang, T. T., Guo, S. W., Ling, N., Shen, Q. R. Plant Grafting Shapes Complexity and Co-occurrence of Rhizobacterial Assemblages. *Microb. Ecol.* **80**, 643–655 (2020).
68. Newman, M. E. J. Modularity and community structure in networks. *P. Natl. Acad. Sci. USA* **103**, 8577–8582 (2006).
69. Deng, Y., Jiang, Y. H., Yang, Y. F., He, Z. L., Luo, F., Zhou, J. Z. Molecular ecological network analyses. *BMC Bioinformatics* **13**, (2012).
70. Guimera, R., Amaral, L. A. N. Functional cartography of complex metabolic networks. *Nature* **433**, 895–900 (2005).
71. Olesen, J. M., Bascompte, J., Dupont, Y. L., Jordano, P. The modularity of pollination networks. *P. Natl. Acad. Sci. USA* **104**, 19891–19896 (2007).
72. Ling, N., *et al.* Insight into how organic amendments can shape the soil microbiome in long-term field experiments as revealed by network analysis. *Soil Biol. Biochem.* **99**, 137–149 (2016).
73. Louca, S., Parfrey, L. W., Doebeli, M. Decoupling function and taxonomy in the global ocean microbiome. *Science* **353**, 1272–1277 (2016).
74. Douglas, G. M., *et al.* PICRUSt2: An improved and customizable approach for metagenome inference. *bioRxiv*, 672295 (2020).
75. Kearns, P. J., Shade, A. Trait-based patterns of microbial dynamics in dormancy potential and heterotrophic strategy: case studies of resource-based and post-press succession. *Isme J.* **12**, 2575–2581 (2018).

76. Lennon, J. T., Jones, S. E. Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nat. Rev. Microbiol.* **9**, 119–130 (2011).

Figures

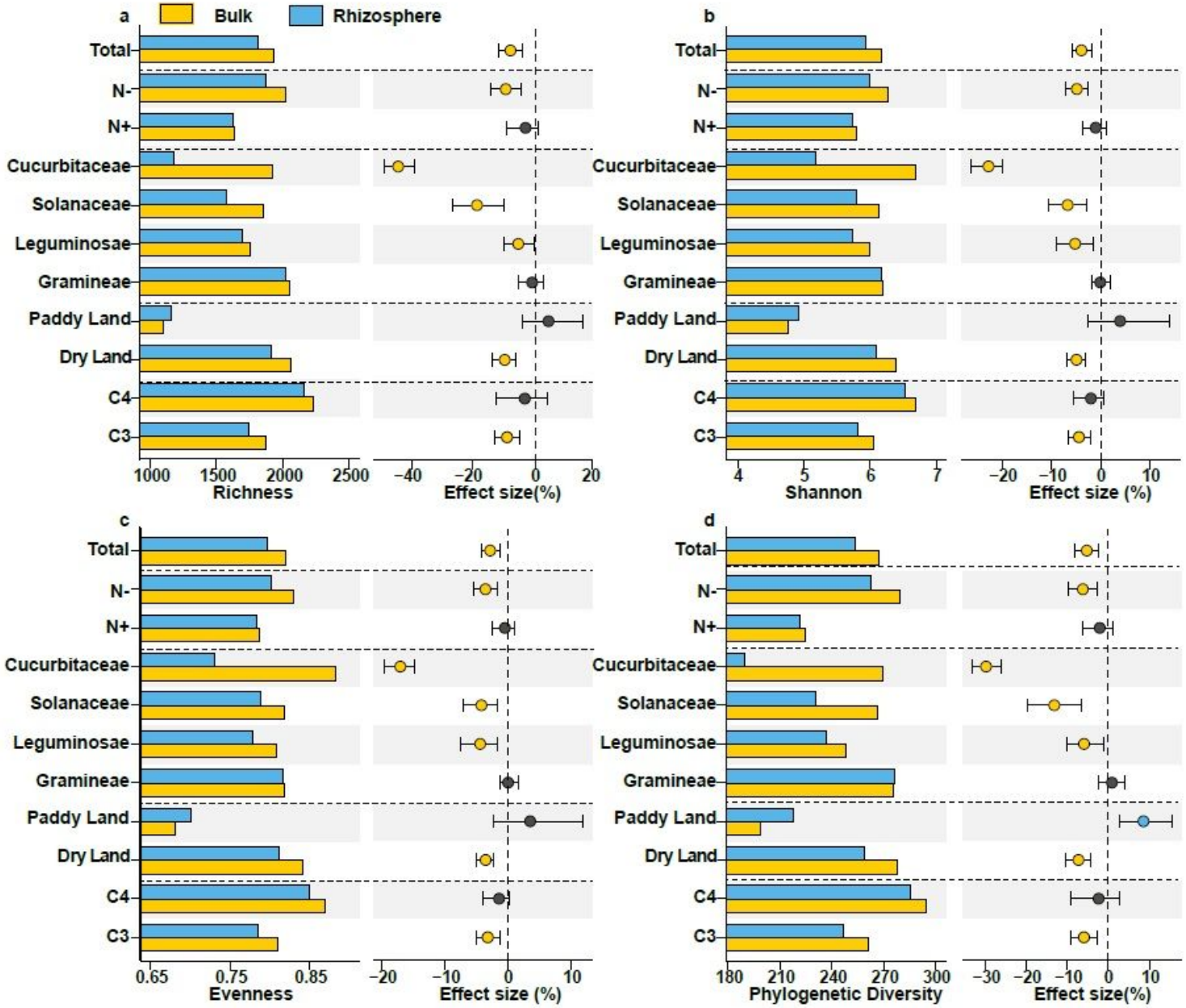

Figure 1

Diversity of bacterial communities: rhizosphere versus bulk soils: a, richness; b, Shannon indexes; c, evenness; d, phylogenetic diversity. All dots represent the percentage change of the effect size between bacterial diversity in rhizosphere and bulk soils with 95% confidence intervals (CIs). Mean values < 0 denote a higher diversity in the bacterial community of bulk soil (yellow dots; depletion in rhizosphere), whereas mean values > 0 reflect a significantly higher diversity in the rhizosphere bacterial community

(blue dots). The intersection of error bars with the zero line indicates absence of significant difference between bacterial communities in rhizosphere and bulk soils (black dots). N- and N+ denote samples without and with nitrogen fertilization, respectively.

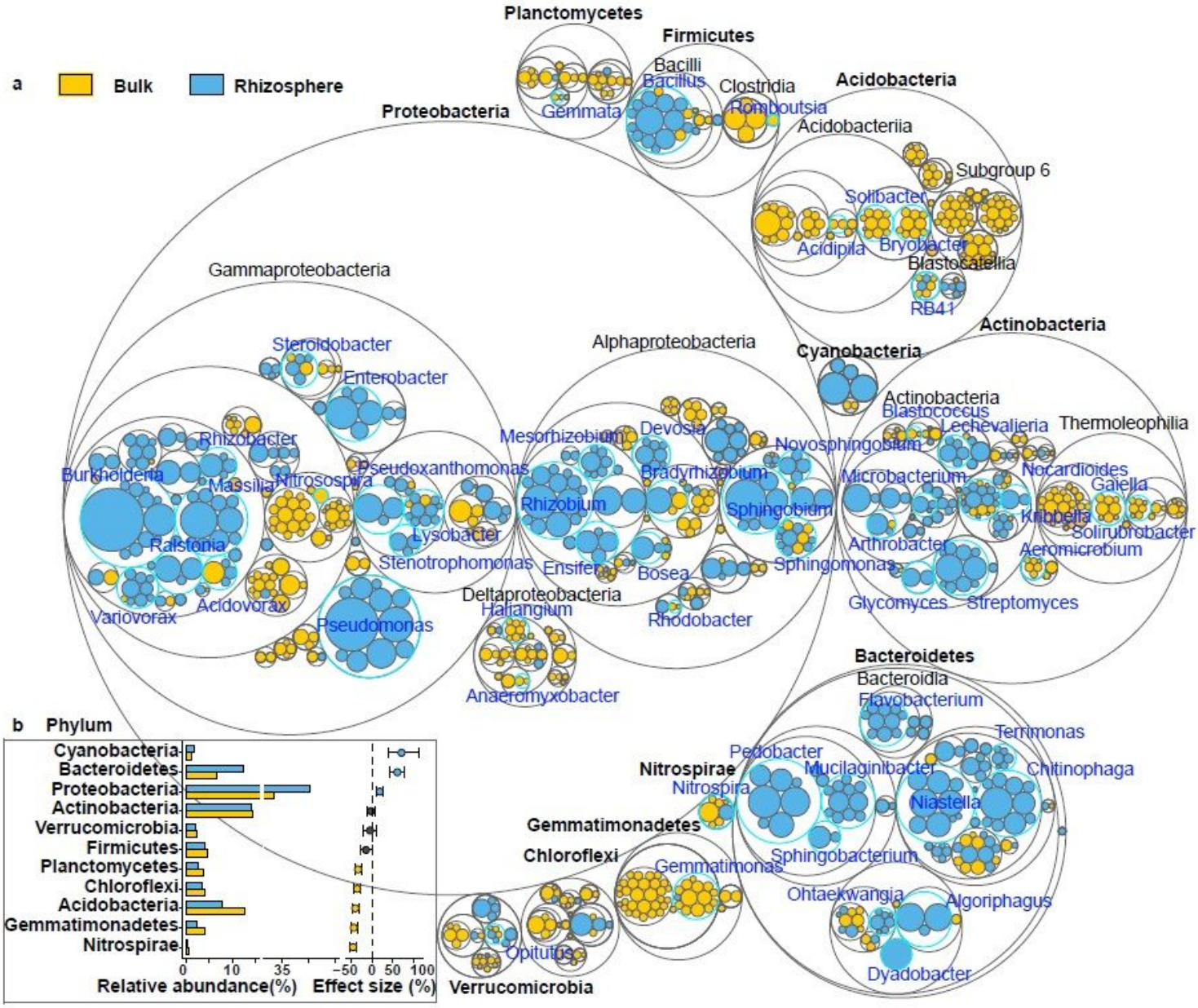

Figure 2

Relative abundance of bacterial taxa between rhizosphere and bulk soils: a, OTU relative abundance. The largest circles represent phylum level, the inner circles class, family and genus (blue outer circles). The yellow and blue bubbles represent OTUs with significant enrichment in rhizosphere and bulk soils, respectively. Bubble sizes represent the difference between the OTU relative abundances in rhizosphere and bulk soils; b, phyla differences between bacterial communities in rhizosphere and bulk soils in terms of relative abundance. Dots represent the percentage change of the effect size between phylum relative abundance in rhizosphere and bulk soils with 95% confidence intervals (CIs). Mean values < 0 denote a

higher phylum relative abundance bulk soil (yellow points: depletion in rhizosphere), while mean bar values > 0 reflect higher relative abundance in the rhizosphere (blue dots). Intersection of error bars with zero line indicates no significant difference between the phylum relative abundances in rhizosphere and bulk soils (black dots).

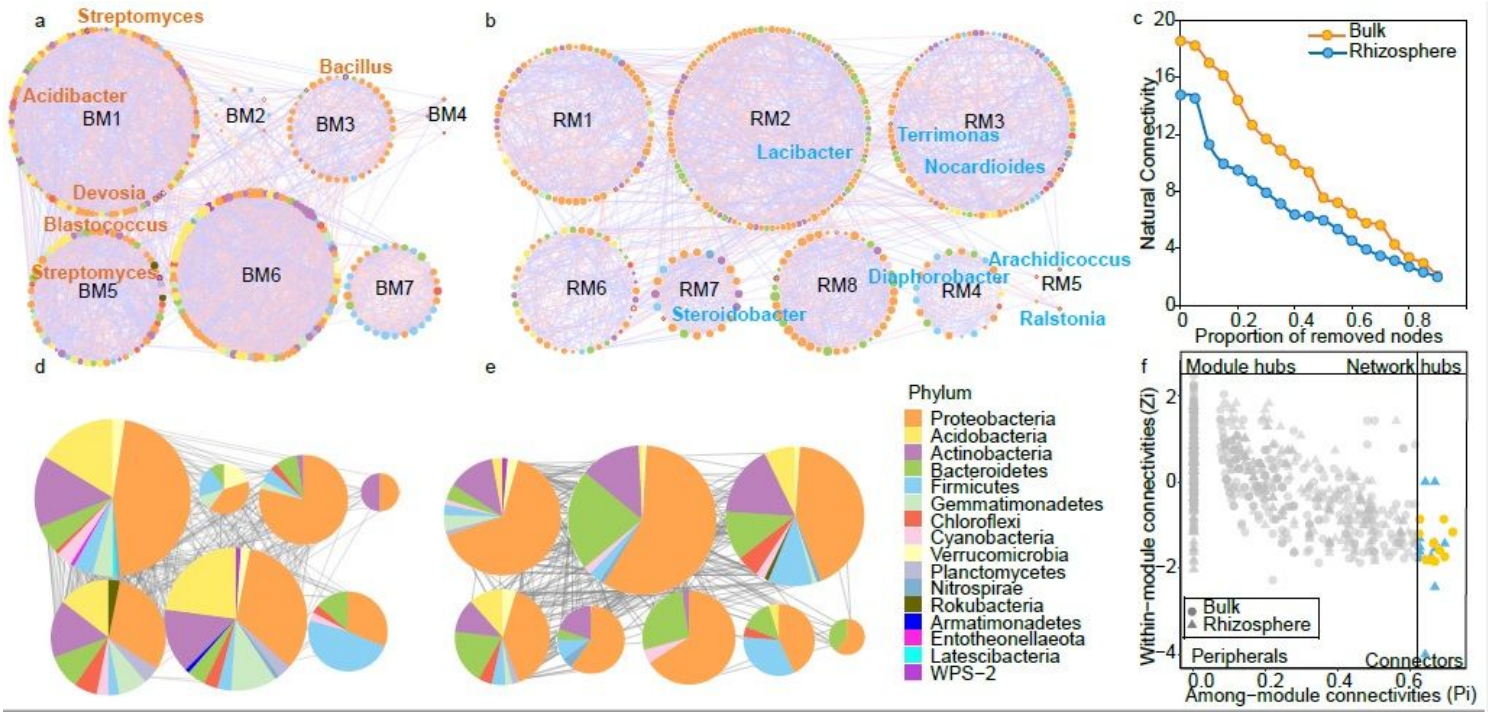

Figure 3

Co-occurrence networks of bacterial OTUs in rhizosphere and bulk soils: a, co-occurrence in bulk soil; b, co-occurrence in rhizosphere; BM and RM represente bulk and rhizosphere modules, respectively. Colors of nodes indicate different major phylum. c, robustness of bacterial network in bulk soil (yellow dots) and rhizosphere (blue dots); d, pie charts represent the phylum composition of modules in bulk soil; e, phylum composition of modules in rhizosphere; f, classification of nodes to identify keystone species within the bulk soil network and rhizosphere network. Taxonomy of connectors is labelled in yellow in bulk soil network (a) and in blue in rhizosphere network (b).

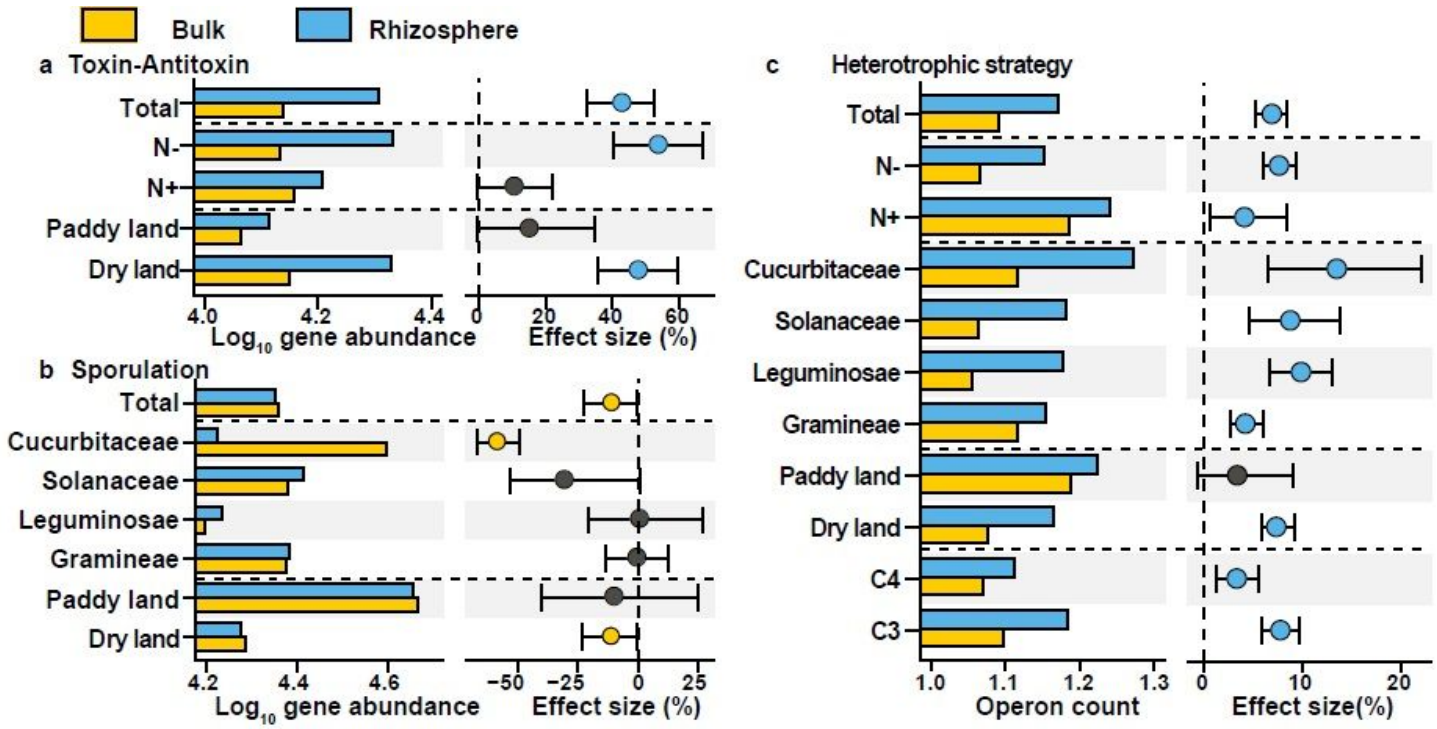

Figure 4

Dormancy potentials and heterotrophic strategies of bacterial communities in rhizosphere and bulk soils: a, differences in the abundances of toxin-antitoxin systems genes between communities in rhizosphere and bulk soils; b, differences in the sporulation factor abundances between community in rhizosphere and bulk soils; c, differences in the weighted mean ribosomal operon copy numbers between communities in rhizosphere and bulk soils. Dots represent the percentage change of the effect size between bacterial communities in rhizosphere and bulk soils with 95% confidence intervals (CIs). Mean values < 0 denote a higher dormancy potential or heterotrophic strategy in bulk soil (yellow dots: depletion in rhizosphere), while mean values > 0 reflect a higher dormancy potential or heterotrophic strategy in the rhizosphere (blue dots). Intersection of error bars with zero line indicates no significant difference between bacterial communities in rhizosphere and bulk soils (black dots). N- and N+ denote samples without and with nitrogen fertilization, respectively.

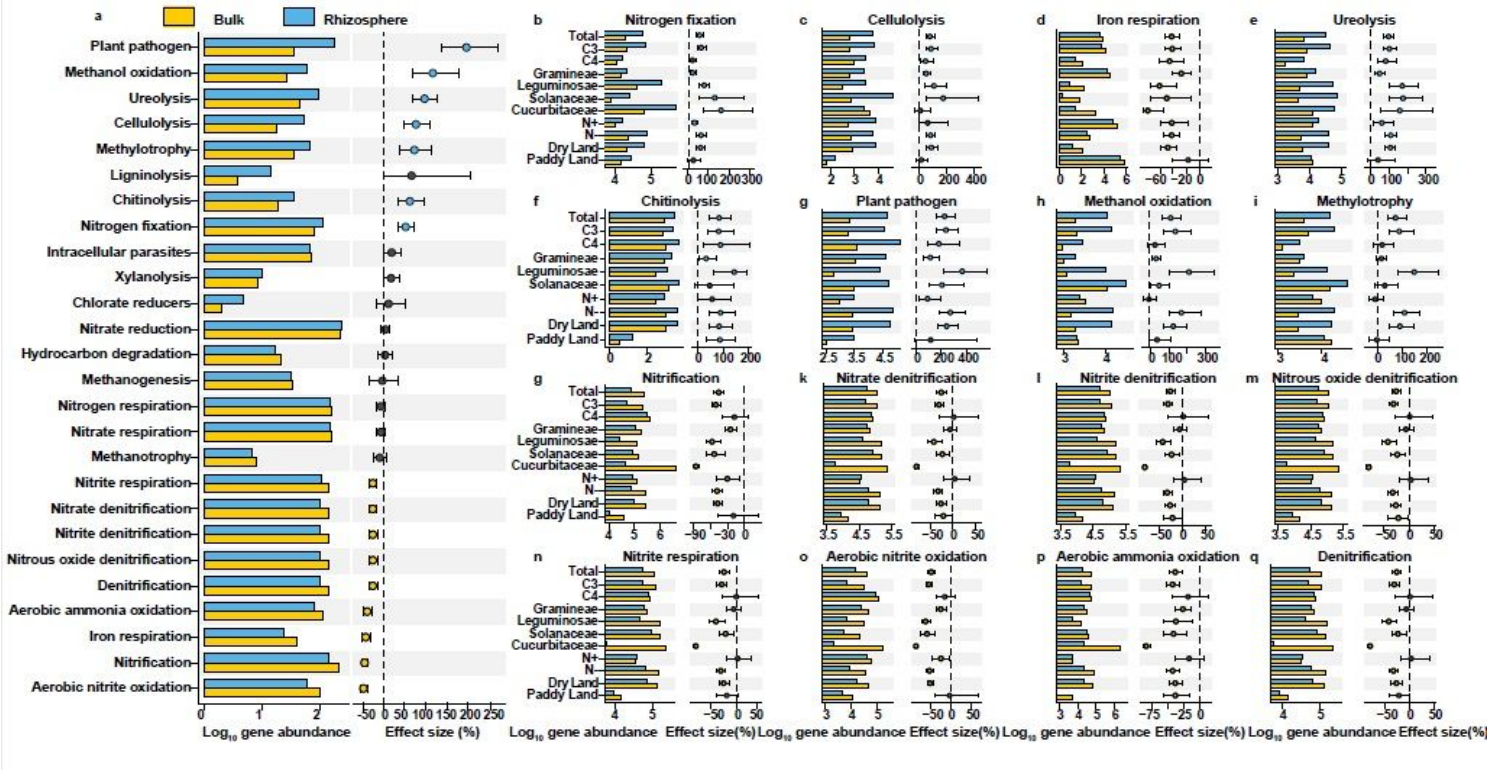

Figure 5

Functional potentials of bacterial communities in rhizosphere and bulk soils: a, the Faprotax annotated function differences; b to q, differences in functions related to nitrogen fixation, cellulolysis, iron respiration, ureolysis, chitinolysis, plant pathogens, methanol oxidation, methylotrophy, nitrification, nitrate denitrification, nitrite denitrification, nitrous oxide denitrification, nitrite respiration, aerobic nitrite oxidation, aerobic ammonia oxidation and denitrification. All dots represent the percentage change of the effect size between bacterial community function potentials in rhizosphere and bulk soils with 95% confidence intervals (CIs). Mean values < 0 denote a higher function in bulk soil (yellow dots: depletion in rhizosphere), while mean values > 0 reflect a higher function in the rhizosphere (blue circle). Intersection of error bar with zero line indicates no significant difference of the function between bacterial communities in rhizosphere and bulk soils (black dots). N- and N+ denote samples without and with nitrogen fertilization, respectively.

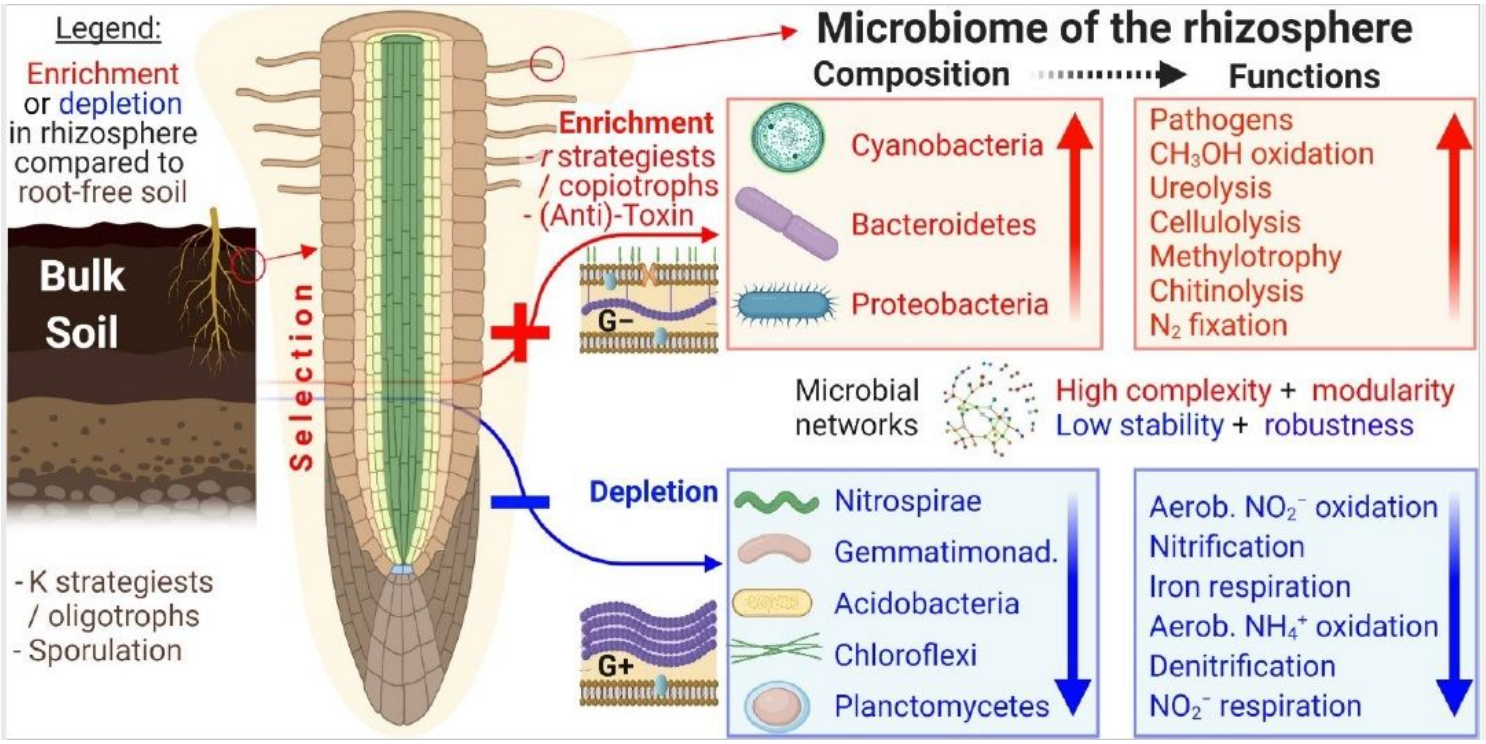

Figure 6

Conceptual figure showing enrichment (red) and depletion (blue) of taxa and functions of bacterial communities in rhizosphere compared to bulk soil. Intensity of changes corresponds to the arrows. G+ and G- are Gram-positive and Gram-negative bacteria, respectively. The light peach-colored area around the root reflects enrichment with available organics caused by exudates.

Supplementary Files

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

- AppendixA.xlsx
- Supplementary.docx
- nrreportingsumpdf.pdf
- PRISMA2009flowdiagram.pdf
- Supplementary.docx