



# Decoding the microbiome for sustainable agriculture

Kai Sun<sup>1</sup>, Wei Zhang<sup>1</sup>, Xiaolin Wang<sup>2</sup>✉, Chuan-Chao Dai<sup>1</sup>✉

<sup>1</sup> Jiangsu Key Laboratory for Pathogens and Ecosystems, Jiangsu Engineering and Technology Research Center for Industrialization of Microbial Resources, College of Life Sciences, Nanjing Normal University, Nanjing 210023, China

<sup>2</sup> Guangdong Provincial Key Laboratory of Plant Molecular Breeding, State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, South China Agricultural University, Guangzhou 510642, China

Received: 8 April 2024 / Accepted: 16 April 2024

**Abstract** Root-associated microbiota profoundly affect crop health and productivity. Plants can selectively recruit beneficial microbes from the soil and actively balance microbe-triggered plant-growth promotion and stress tolerance enhancement. The cost associated with this is the root-mediated support of a certain number of specific microbes under nutrient limitation. Thus, it is important to consider the dynamic changes in microbial quantity when it comes to nutrient condition-induced root microbiome reassembly. Quantitative microbiome profiling (QMP) has recently emerged as a means to estimate the specific microbial load variation of a root microbiome (instead of the traditional approach quantifying relative microbial abundances) and data from the QMP approach can be more closely correlated with plant development and/or function. However, due to a lack of detailed-QMP data, how soil nutrient conditions affect quantitative changes in microbial assembly of the root-associated microbiome remains poorly understood. A recent study quantified the dynamics of the soybean root microbiome, under unbalanced fertilization, using QMP and provided data on the use of specific synthetic communities (SynComs) for sustaining crop productivity. In this editorial, we explore potential opportunities for utilizing QMP to decode the microbiome for sustainable agriculture.

**Keywords** Quantitative microbiome profiling, Microbial load, Unbalanced fertilization, SynComs, Sustainable agriculture

To feed a growing global human population, new solutions provided by microorganisms are required (Jansson et al. 2023). Plant roots assemble microbial communities, both inside the roots and in their rhizosphere, and the presence of beneficial root microbiomes plays pivotal roles in plant productivity (Berendsen et al. 2012). The assembly of root-associated microbiota can vary by host genotypes (Wagner et al. 2016), plant developmental stages (Zhang et al. 2018) and soil properties (Philippot et al. 2013). With respect to soil properties, sub-optimal or unbalanced distribution of mineral

nutrients in the soil is common in agroecosystems, due either to a lack of or inappropriate fertilizer application. However, the mechanism(s) by which different fertilizer regimes trigger alterations in root microbial reassembly to influence plant development remains unexplored.

In a recent study, Wang et al. (2024) used quantitative microbiome profiling (QMP) to reveal the temporal dynamics of the root-associated microbiome in response to unbalanced fertilization, and identified several low-nitrogen (N)-enriched microbes that could promote soybean growth, when grown on low-N soil. This study offered new insights into the dynamic assembly of root-associated microbiomes and highlights the potential for harnessing stress-tailored microbiota to enhance plant crop adaptation.

✉ Correspondence: xlwang@scau.edu.cn (X. Wang), daichuan-chao@njnu.edu.cn (C.-C. Dai)

In a 40-year field trial, conducted under different fertilization regimes (namely exclusion of N, phosphorous (P) or potassium (K)), these various nutrient conditions were shown to differentially affect soybean performance. Here, it is noteworthy that N deficiency did not significantly reduce soybean yield and even showed a slight yield-enhancement in 2020. In this same year, rhizosphere soil and root samples of soybean were collected to monitor the dynamics of the root-associated microbiome.

Based on QMP profiling, Wang et al. (2024) established that the composition of root-associated bacteria strongly changed with plant development and the total number of bacteria significantly increased at the later stages. Particularly, members of the phylum, Bacteroidetes, which have been reported to facilitate nutrient uptake of the host plant (Pan et al. 2023), were enriched in the root microbiome, and were associated with enhanced-soybean fitness. Thus, plant development allows roots to harbor increased microbial quantity and drives expansion of specific bacterial groups targeting plant-growth enhancement. Further analyses revealed that rhizosphere bacteria were more sensitive to nutrient status compared to endosphere bacteria. Notably, the rhizosphere microbiome exhibited a unique succession pattern with multiple bacterial taxa showing increased abundance in the absence of N fertilization, whereas the lack of P fertilizer hindered the development of the rhizosphere microbiome and generally decreased the total load of microbial taxa. Importantly, the symbiosis between soybean and rhizobia was enhanced under low-N conditions, which could provide an additional N source to support plant growth. These findings suggest that nutrient-stress environments can force plants to actively manipulate the microbial population within the rhizosphere and prioritize allocation of limited exudate resources to distinct functional taxa and increase their biomass.

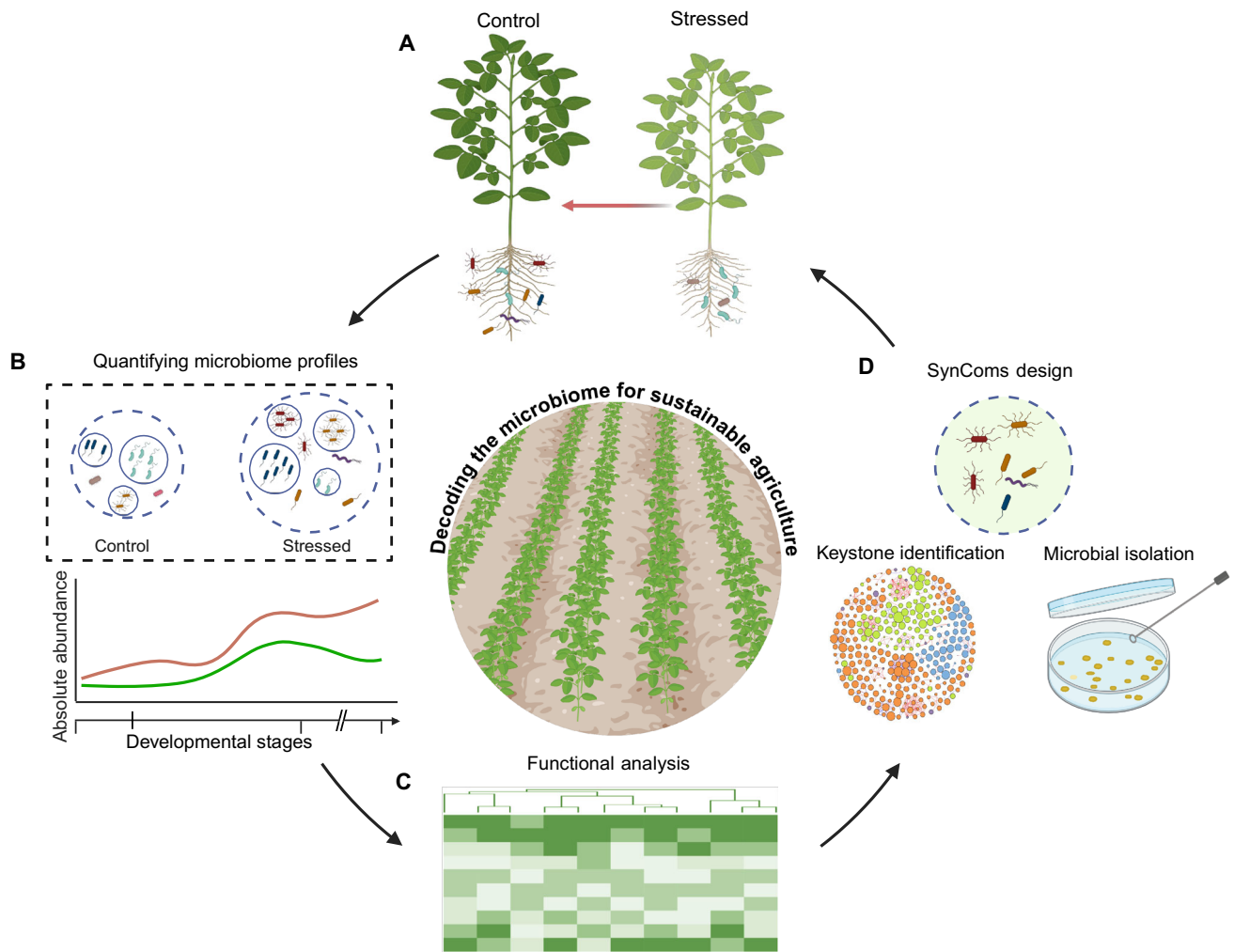
Metagenomic analysis revealed the functional adaptation of rhizosphere microbiomes under different nutrient conditions. Specifically, N mineralization-associated genes were significantly enriched in N-treatment conditions. Rhizosphere N mineralization is triggered by a microbial biomass increase or community reassembly regarding the microbial priming effect (Valadares et al. 2020). And in legumes, N fixation by rhizobia enhances the rhizosphere priming effect by promoting photosynthesis and rhizodeposition (Henneron et al. 2020). Thus, a two-step process of microbial N supply for soybean, under N limitation, can be verified. First, N deficiency increases the abundance of rhizobia, which benefits N fixation; second, adequate carbon and N, afforded through N fixation, allows functional microbes

to increase biomass and initiate organic N mineralization (Henneron et al. 2020; Valadares et al. 2020). These processes co-ordinately contribute to the sustained soybean productivity and implicate an economic strategy for legume plants to respond to N shortage. For P-treatment, inorganic P solubilization and P starvation-associated genes were specifically enriched in the rhizosphere so as to increase available P sources for soybean growth.

Through co-occurrence network analysis of core bacterial amplicon sequence variants (ASVs), bacterial isolation and synthetic community (SynCom) construction, Wang and colleagues identified and matched seven core ASVs with bacterial strains in low-N-enriched cluster (LNE cluster) and designated as SynCom7 (i.e., *Rhodococcus*, *Lysobacter*, *Terrabacter*, *Arthrobacter*, *Phyllobacterium*, *Bosea* and *Aeromicrobium*) and SynCom5 (excluding *Bosea* and *Aeromicrobium* from SynCom7). The SynComs from the LNE cluster were able to improve soybean growth. Interestingly, this process did not rely on the presence of rhizobia since the formation of root nodules was not observed and the growth-promoting effects of SynCom5 showed no difference from SynCom7. This result may provide a valuable implication that apart from rhizobial symbiosis, the capability of other rhizosphere microbes in rescuing host N nutrition should be emphasized for legume plants, and also for non-legume plants. It has been demonstrated that the use of microbial consortia as eco-friendly inoculants can provide functional benefits over single-strain inoculants (Kaminsky et al. 2019). Thus, the beneficial consortia that integrates symbiotic rhizobia with low-N-enriched rhizosphere microbes may have more extensive application prospects for reducing N input during soybean production.

Overall, Wang and colleagues describe a distinctive dynamic pattern in soybean root-associated microbiomes using QMP and establish a framework for decoding microbial SynComs to rescue crop productivity under nutrient shortage (Fig. 1). Future exploration will be required to comprehend the molecular mechanisms by which the LNE-specific SynComs interact with soybean to facilitate plant N nutrition absorption, in a non-N<sub>2</sub>-fixing manner. In addition, apart from root-bacterial interactions, the cooperations between other functional microbiomes (including fungi and archaea) are common in the rhizosphere zone (Sun et al. 2023). Therefore, the potential cross-kingdom communications among rhizobionts and their effects on plant fitness are of significant interest to advance sustainable agriculture.

The strategy of restructuring root microbiota in response to nutrient deprivation by enhanced root exudation of specific metabolites has been



**Fig. 1** A framework for decoding the microbiome for sustainable agriculture. **A** Initially, plants are exposed to specific stress conditions to rewild the high stress plant microbiomes. After obtaining a stable stress-induced phenotype, the agronomic traits and soil properties related to the stressed conditions, and especially the yield, are assessed. **B** Subsequent microbiome variation among comparative samples, or over time, are quantified using QMP to estimate the specific load of different microbial groups. **C** Subsequent to quantifying the microbial dynamics, metagenomic analysis is used to identify the functional adaptive changes of the microbiome under the imposed stress conditions. **D** Co-occurrence network analysis is subsequently employed to identify the stress-enriched microbial ecological clusters and further identify the keystone taxa. The microbial strains are then isolated, identified, and tested for their plant-growth-promotion effects. SynComs are then designed after matching the core taxa with the isolated microbial strains. The effects of the SynComs on plant growth can be tested and evaluated under the chosen stress condition. An assessment of specific SynComs on crop growth and yield will ultimately identify stress-enriched microbiomes for the development of sustainable agriculture. Created with Biorender.com

demonstrated in various plant species, such as maize (Yu et al. 2021) and rice (Liu et al. 2023). These root microbiomes promote plant nutrient acquisition by multiple ways, including modulating lateral root development (Yu et al. 2021) and increasing the bioavailability of soil available nutrients (Liu et al. 2023). Therefore, specific root-associated microbes can be harnessed to sustain plant growth by serving as bio-fertilizers, thereby reducing the need of chemical fertilizers (Jansson et al. 2023). Apparently, an innovatively designed microbiome approach for global assessment of the variation and function of the root-associated

microbiome is urgently needed (Singh et al. 2020). Although relative abundance of microbial taxa has been widely used to describe microbiome variation, its drawbacks have gradually emerged. For example, in the studies of the gut microbiome, relative abundance data have been found to misrepresent the microbiome heritability (Bruijning et al. 2023) and failed to characterize the core microbiota alterations-associated with host health (Vandeputte et al. 2017). Similarly, in the studies of the plant microbiome, relative abundance approaches are limited in their ability to reveal links between microbial load and plant physiologic parameters (Guo

et al. 2020; Wang et al. 2020). Thus, without absolute quantitation, the genuine interplay patterns between host and microbial groups may be masked by relative profiling. Fortunately, QMP has recently been proposed to estimate the specific load of microbial groups that occur between individuals or over time (Tkacz et al. 2018; Wang et al. 2020) and have been used to clarify the variation in root-associated microbiomes of legume plants (Wang et al. 2020, 2021, 2022). The utilization of QMP in unlocking the microbiome for nutrient-stressed crops remained explored until Wang and colleagues (2024) provide experimental evidence for decoding LNE-specific SynComs to increase soybean fitness.

More broadly, the targeted design of SynComs, on the basis of QMP datasets, can open new perspectives for meeting the challenges of multiple environmental stresses. In addition to microbial selection for SynComs, the inoculum size applied in the rhizosphere is also important to further enhance plant growth (Moreira et al. 2016). The temporal dynamics of specific microbial load, obtained through QMP, may provide reliable guidance in optimizing inoculum size for different plant-development stages. On the other hand, integrating multi-omics technologies is another promising method for elucidating the environment-mediated interaction between plant performance and plant microbiomes (Wang et al. 2021). Thus, further combining QMP with quantitative plant omics (e.g., digital RNA sequencing, quantitative proteomics, quantitative metabolomics, and field phenomics) is required to integrate quantitative information on microbiota assembly, plant genetic, physiologic, and phenotypic changes to accelerate decoding of microbiomes for crop breeding.

Another important aspect that needs to be addressed is how to structure the microbiota for sustainable production, as the density of beneficial microbes for modern crops is reduced by agricultural practices, including fertilization and irrigation (Porter and Sachs 2020). It has been proposed that rewilding plant microbiomes could offer a way to obtain functional plant microbiomes (Raaijmakers and Kiers 2022). One of the effective rewilding approaches is to expose plants to specific stresses (e.g., drought, salt, or nutrient deprivation), which can expedite the search for specific beneficial consortia, via the host-derived selective pressures on the microbiomes (Raaijmakers and Kiers 2022). That is to say, the functional microbiomes could be domesticated from stressed plant hosts. In addition, these plant microbiomes should be viewed in a long-term context, as plants appear to take time (several years) to cultivate typical microbes (Kulmatiski and Beard 2011). Encouragingly, the work of Wang et al. (2024) rewilded the soybean microbiomes by exposing

plants to long-term nutrient-stressed conditions and successfully obtained the low-N-enriched taxa for plant-growth enhancement. Furthermore, a holistic consideration of stress conditions and plant genotype may maximize the efficiency in obtaining a specific microbiome for sustainable agriculture (He et al. 2024).

In future, rewilding crop microbiomes, under multiple stresses, may become one of the most important topics in the efforts to develop sustainable agroecosystems. By quantifying the dynamics of these microbiomes and identifying the core microbial taxa, a stress-tolerant “microbial resource bank” could finally be established (Fig. 1). These efforts would not only broaden our understanding of the development of microbial inoculants but also provide a promising strategy for transforming the mode of agricultural production and mitigating global climate change.

**Acknowledgements** We are grateful to Xianan Xie (South China Agricultural University) and Jiayu Zhou (Institute of Botany, Jiangsu Province and Chinese Academy of Sciences) for proof-reading the manuscript.

**Author contributions** KS, XLW and C-CD wrote the manuscript and WZ contributed to the revision. All authors read and approved the final manuscript.

**Funding** This work was supported by grants from the National Natural Science Foundation of China (grant nos. 32301332, 32071638 and 32100227), and a project funded by the Priority Academic Program Development (PAPD) of the Jiangsu Higher Education Institutions of China.

**Data availability** Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Berendsen RL, Pieterse CMJ, Bakker PA (2012) The rhizosphere microbiome and plant health. *Trends Plant Sci* 17:478–486. <https://doi.org/10.1016/j.tplants.2012.04.001>
- Bruijning M, Ayroles JF, Henry LP, Koskella B, Meyer KM, Metcalf CJE (2023) Relative abundance data can misrepresent heritability of the microbiome. *Microbiome* 11:222. <https://doi.org/10.1186/s40168-023-01669-w>
- Guo X, Zhang X, Qin Y, Liu YX, Zhang J, Zhang N, Wu K, Qu B, He Z, Wang X, Zhang X, Hacquard S, Fu X, Bai Y (2020) Host-associated quantitative abundance profiling reveals the microbial load variation of root microbiome. *Plant Commun* 1:100003. <https://doi.org/10.1016/j.xplc.2019.100003>
- He XM, Wang DN, Jiang Y, Li M, Delgado-Baquerizo M, McLaughlin C, Marcon C, Guo L, Baer M, Moya YAT, von Wirén N, Deichmann M, Schaaf G, Piepho HP, Yang ZK, Yang JL, Yim B,



- Smalla K, Goormachtig S, de Vries FT, Hüging H, Baer M, Sawers RJH, Reif JC, Hochholdinger F, Chen XP, Yu P (2024) Heritable microbiome variation is correlated with source environment in locally adapted maize varieties. *Nat Plants*. <https://doi.org/10.1038/s41477-024-01654-7>
- Henneron L, Kardol P, Wardle DA, Cros C, Fontaine S (2020) Rhizosphere control of soil nitrogen cycling: a key component of plant economic strategies. *New Phytol* 228:1269–1282. <https://doi.org/10.1111/nph.16760>
- Jansson JK, McClure R, Egbert RG (2023) Soil microbiome engineering for sustainability in a changing environment. *Nat Biotechnol* 41:1716–1728. <https://doi.org/10.1038/s41587-023-01932-3>
- Kaminsky LM, Trexler RV, Malik RJ, Hockett KL, Bell TH (2019) The inherent conflicts in developing soil microbial inoculants. *Trends Biotechnol* 37:140–151. <https://doi.org/10.1016/j.tibtech.2018.11.011>
- Kulmatiski A, Beard KH (2011) Long-term plant-growth legacies overwhelm short-term plant growth effects on soil microbial community structure. *Soil Biol Biochem* 43:823–830. <https://doi.org/10.1016/j.soilbio.2010.12.018>
- Liu CY, Jiang MT, Yuan MM, Wang ET, Bai Y, Crowther TW, Zhou JZ, Ma ZY, Zhang L, Wang Y, Ding JX, Liu WX, Sun B, Shen RF, Zhang JB, Liang YT (2023) Root microbiota confers rice resistance to aluminium toxicity and phosphorus deficiency in acidic soils. *Nat Food* 4:912–924. <https://doi.org/10.1038/s43016-023-00848-0>
- Moreira H, Pereira SIA, Marques APGC, Rangel AOSS, Castro PML (2016) Selection of metal resistant plant growth promoting rhizobacteria for the growth and metal accumulation of energy maize in a mine soil-effect of the inoculum size. *Geoderma* 278:1–11. <https://doi.org/10.1016/j.geoderma.2016.05.003>
- Pan XY, Raaijmakers JM, Carrión VJ (2023) Importance of Bacteroidetes in host-microbe interactions and ecosystem functioning. *Trends Microbiol* 31:959–971. <https://doi.org/10.1016/j.tim.2023.03.018>
- Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol* 11:789–799. <https://doi.org/10.1038/nrmicro3109>
- Porter SS, Sachs JL (2020) Agriculture and the disruption of plant-microbial symbiosis. *Trends Ecol Evol* 35:426–439. <https://doi.org/10.1016/j.tree.2020.01.006>
- Raaijmakers JM, Kiers ET (2022) Rewilding plant microbiomes. *Science* 378:599–600. <https://doi.org/10.1126/science.abn6350>
- Singh BK, Trivedi P, Egidi E, Macdonald CA, Delgado-Baquerizo M (2020) Crop microbiome and sustainable agriculture. *Nat Rev Microbiol* 18:601–602. <https://doi.org/10.1038/s41579-020-00446-y>
- Sun K, Jiang HJ, Pan YT, Lu F, Zhu Q, Ma CY, Zhang AY, Zhou JY, Zhang W, Dai CC (2023) Hyphosphere microorganisms facilitate hyphal spreading and root colonization of plant symbiotic fungus in ammonium-enriched soil. *ISME J* 17:1626–1638. <https://doi.org/10.1038/s41396-023-01476-z>
- Tkacz A, Hortala M, Poole PS (2018) Absolute quantitation of microbiota abundance in environmental samples. *Microbiome* 6:110. <https://doi.org/10.1186/s40168-018-0491-7>
- Valadares RV, Costa MD, Neves JCL, Vieira Netto JAF, Silva IRD, Moro E, Alves MR, Fernandes LA (2020) Rhizosphere microbiological processes and eucalypt nutrition: synthesis and conceptualization. *Sci Total Environ* 746:141305. <https://doi.org/10.1016/j.scitotenv.2020.141305>
- Vandeputte D, Kathagen G, Dhoe K, Vieira-Silva S, Valles-Colomer M, Sabino J, Wang J, Tito RY, De Commer L, Darzi Y, Vermeire S, Falony G, Raes J (2017) Quantitative microbiome profiling links gut community variation to microbial load. *Nature* 551:507–511. <https://doi.org/10.1038/nature24460>
- Wagner MR, Lundberg DS, Del Rio TG, Tringe SG, Dangl JL, Mitchell-Olds T (2016) Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nat Commun* 7:12151. <https://doi.org/10.1038/ncomms12151>
- Wang XL, Wang MX, Xie XG, Guo SY, Zhou Y, Zhang XB, Yu N, Wang ET (2020) An amplification-selection model for quantified rhizosphere microbiota assembly. *Sci Bull (beijing)* 65:983–986. <https://doi.org/10.1016/j.scib.2020.03.005>
- Wang XL, Feng H, Wang YY, Wang MX, Xie XG, Chang HZ, Wang L, Qu JC, Sun K, He W, Wang C, Dai CC, Chu ZH, Tian CF, Yu N, Zhang XB, Liu H, Wang ET (2021) Mycorrhizal symbiosis modulates the rhizosphere microbiota to promote rhizobia-legume symbiosis. *Mol Plant* 14:503–516. <https://doi.org/10.1016/j.molp.2020.12.002>
- Wang XL, Wang MX, Wang LK, Feng H, He X, Chang SH, Wang DP, Wang L, Yang J, An GY, Wang XJ, Kong LR, Geng Z, Wang ET (2022) Whole-plant microbiome profiling reveals a novel geminivirus associated with soybean stay-green disease. *Plant Biotechnol J* 20:2159–2173. <https://doi.org/10.1111/pbi.13896>
- Wang MX, Ge AH, Ma XZ, Wang XL, Xie QJ, Wang LK, Song XW, Jiang MC, Yang WB, Murray JD, Wang YY, Liu H, Cao XF, Wang ET (2024) Dynamic root microbiome sustains soybean productivity under unbalanced fertilization. *Nat Commun* 15:1668. <https://doi.org/10.1038/s41467-024-45925-5>
- Yu P, He XM, Baer M, Beirinckx S, Tian T, Moya YAT, Zhang XC, Deichmann M, Frey FP, Bresgen V, Li CJ, Razavi BS, Schaaf G, von Wirén N, Su Z, Bucher M, Tsuda K, Goormachtig S, Chen XP, Hochholdinger F (2021) Plant flavones enrich rhizosphere Oxalobacteraceae to improve maize performance under nitrogen deprivation. *Nat Plants* 7:481–499. <https://doi.org/10.1038/s41477-021-00897-y>
- Zhang JY, Zhang N, Liu YX, Zhang XN, Hu B, Qin Y, Xu HR, Wang H, Guo XX, Qian JM, Wang W, Zhang PF, Jin T, Chu CC, Bai Y (2018) Root microbiota shift in rice correlates with resident time in the field and developmental stage. *Sci China Life Sci* 61:613–621. <https://doi.org/10.1007/s11427-018-9284-4>