

Supplementary Information

Enhancement of Beneficial Microbiomes in Plant-Soil Continuums through Organic Fertilization: Insights into the Composition and Multifunctionality

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Table S1 Beneficial bacteria chosen in this study.

Beneficial Bacteria	Phylum	Functions	Reference
Rhizobium	Proteobacteria	Nitrogen cycle	(Lugtenberg and Kamilova, 2009)
Pseudomonas	Proteobacteria	Phosphate Solubilization; Pathogen suppression; Salinity resistance	(Lugtenberg and Kamilova, 2009; Richardson and Simpson, 2011; Schmitz et al., 2022)
Bacillus	Firmicutes	Phosphate Solubilization; Nitrogen cycle; Pathogen suppression; Salinity resistance	(Kang et al., 2022; Lugtenberg and Kamilova, 2009; Schmitz et al., 2022; Shao et al., 2021)
Nocardioides	Actinobacteria	Nitrogen cycle	(Kuang et al., 2022)
Devosia	Proteobacteria	Nitrogen cycle	(Rivas et al., 2002)
Micromonospora	Actinobacteria	Nitrogen cycle	(Trujillo et al., 2015)
Cellulomonas	Actinobacteria	Nitrogen cycle	(Sonkurt and ÇİĞ, 2019)
Phenylobacterium	Proteobacteria	Nitrogen cycle	(Mehmood et al., 2022)
Streptomyces	Actinobacteria	Nitrogen cycle; Pathogen suppression; Salinity resistance	(Chen et al., 2020; Dahal et al., 2017; Schmitz et al., 2022)
Conexibacter	Actinobacteria	Nitrogen cycle	(Pedrinho et al., 2020)
Asticcacaulis	Proteobacteria	Carbon cycle	(Wilhelm et al., 2019)
Acidovorax	Proteobacteria	ACC deaminase secretion	(Cavite et al., 2020)
Flavobacterium	Bacteroidetes	Pathogen suppression	(Carrion et al., 2019; Kwak et al., 2018)
Sphingomonas	Proteobacteria	Pathogen suppression	(Deng et al., 2022)
Luteimonas	Proteobacteria	Pathogen suppression	(Deng et al., 2022)
Lysobacter	Proteobacteria	Pathogen suppression	(Deng et al., 2022)
Pseudoxanthomonas	Proteobacteria	Pathogen suppression	(Deng et al., 2022)
Stenotrophomonas	Proteobacteria	Pathogen suppression	(Berendsen et al., 2018; Deng et al., 2022; Liu et al., 2021)
Microbacterium	Actinobacteria	Pathogen suppression	(Berendsen et al., 2018)
Paenibacillus	Firmicutes	Pathogen suppression; Hormone secretion	(Bziuk et al., 2021; Mendes et al., 2018)
Erwinia	Proteobacteria	Phosphate Solubilization; Pathogen suppression; Hormone secretion; Siderophore production	(Bziuk et al., 2021; Lugtenberg and Kamilova, 2009; Rodríguez et al., 2006)
Curtobacterium	Actinobacteria	Hormone secretion; Siderophore production	(Bziuk et al., 2021)
Massilia	Proteobacteria	Salinity resistance	(Schmitz et al., 2022)
Enterobacter	Proteobacteria	Salinity resistance	(Schmitz et al., 2022)
Ensifer	Proteobacteria	Salinity resistance	(Schmitz et al., 2022)
Variovorax	Proteobacteria	Hormone manipulation	(Qi et al., 2022)

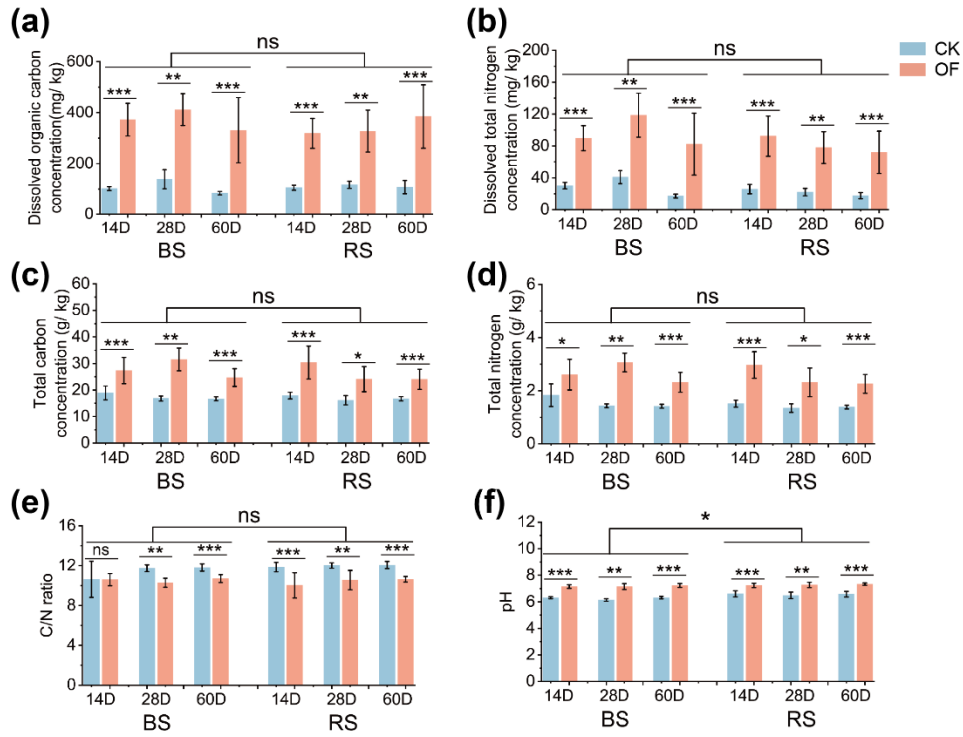


Fig. S1 Changes of dissolved organic carbon (a), dissolved total nitrogen (b), total carbon (c), total nitrogen (d), C/N ratio (e), pH (f) in bulk soil (BS) and rhizosphere soil (RS) across different fertilization treatments and growth time. P values are indicated by *, e.g., * represents $P < 0.05$, ** represents $P < 0.01$, and *** represents $P < 0.001$.

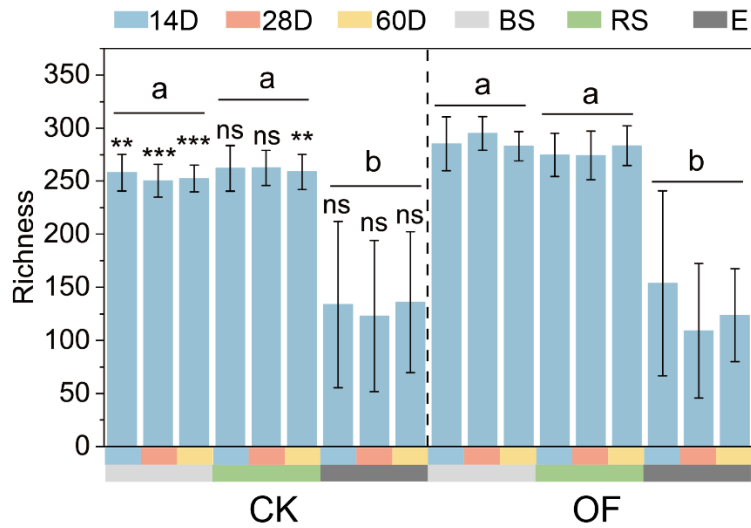


Fig. S2 The richness of bacterial communities in bulk soil (BS), rhizosphere soil (RS) and endosphere (E) with different fertilization treatments and growth time. The richness differences between CK groups and OF groups are tested by t-test. ** represents $P < 0.01$ and *** represents $P < 0.001$. The richness differences across the three compartments are tested by one-way ANOVA. Different letters above the bars indicate significant differences at $P < 0.05$.

Table S2 The effects of plant growth time on bacterial richness in bulk soil (BS), rhizosphere soil (RS) and endosphere (E) based on one-way ANOVA.

		Time	
CK	BS	14D	ns
		28D	
		60D	
	RS	14D	ns
		28D	
		60D	
	E	14D	ns
		28D	
		60D	
OF	BS	14D	ns
		28D	
		60D	
	RS	14D	ns
		28D	
		60D	
	E	14D	ns
		28D	
		60D	

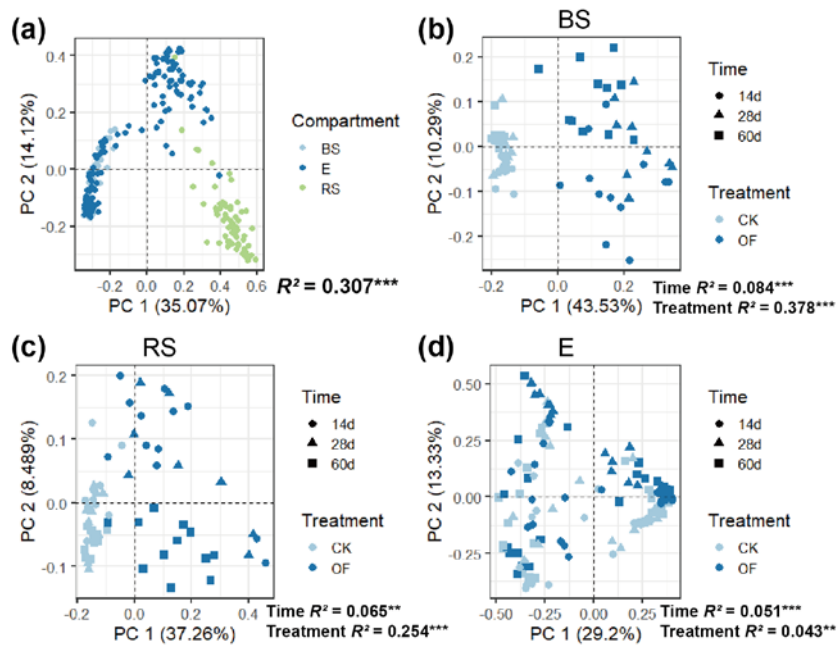


Fig. S3 Principal component analysis (PCA) indicating the dissimilarity of bacterial communities in different compartments (a) and the effects of growth time and fertilization treatments on the dissimilarity of bacterial communities in bulk soil (BS) (b), rhizosphere soil (RS) (c) and endosphere (E) (d). P values are indicated by *, e.g., ** represents $P < 0.01$ and *** represents $P < 0.001$.

Table S3 The effects of fertilization treatments and growth time on bacterial communities in different compartments based on Adonis.

	Fertilization		Time		Fertilization × Time	
	R^2	P	R^2	P	R^2	P
Bulk soil	0.378	0.001	0.084	0.001	0.041	0.014
Rhizosphere soil	0.254	0.001	0.065	0.004	0.038	0.050
Endosphere	0.043	0.002	0.051	0.001	0.031	0.009

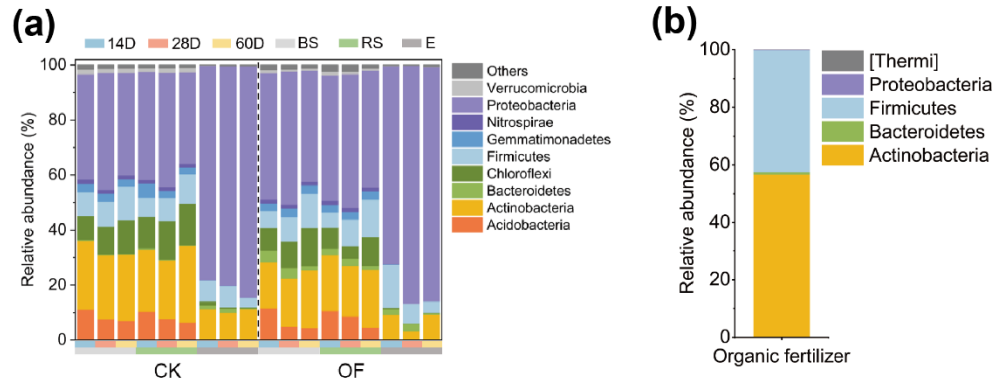


Fig. S4 Changes of bacterial community composition in bulk soil (BS), rhizosphere soil (RS) and endosphere (E) across different fertilization treatments and growth time at phylum level (a). The composition of bacterial communities in the organic fertilizer at a phylum level (b).

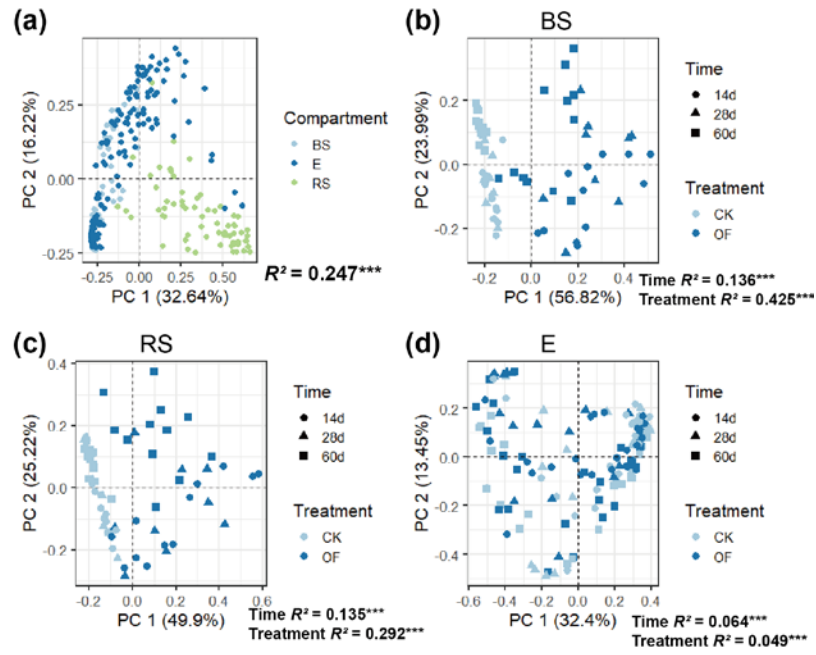


Fig. S5 Principal component analysis (PCA) indicating the dissimilarity of beneficial bacterial communities in different compartments (a) and the effects of growth time and fertilization treatments on the dissimilarity of beneficial bacterial communities in bulk soil (BS) (b), rhizosphere soil (RS) (c) and endosphere (E) (d). *** represents $P < 0.001$.

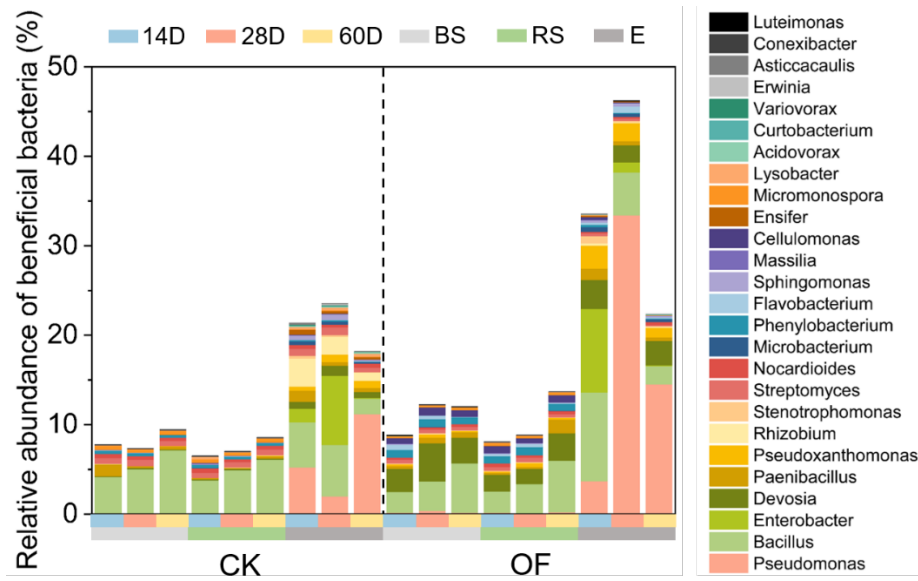


Fig. S6 The composition of beneficial bacteria in bulk soil (BS), rhizosphere soil (RS) and endosphere (E) with different fertilization treatments and growth time.

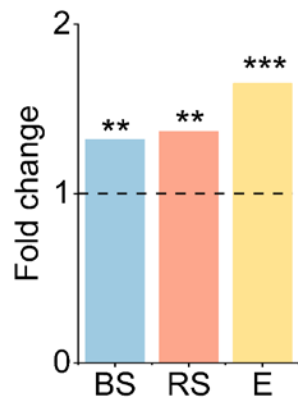


Fig. S7 The fold changes in the relative abundance of beneficial bacteria in the three compartments treated with organic fertilizers compared to that treated without organic fertilizers. Samples of different growth time are included. t-test is used to test differences. ** represents $P < 0.01$ and *** represents $P < 0.001$.

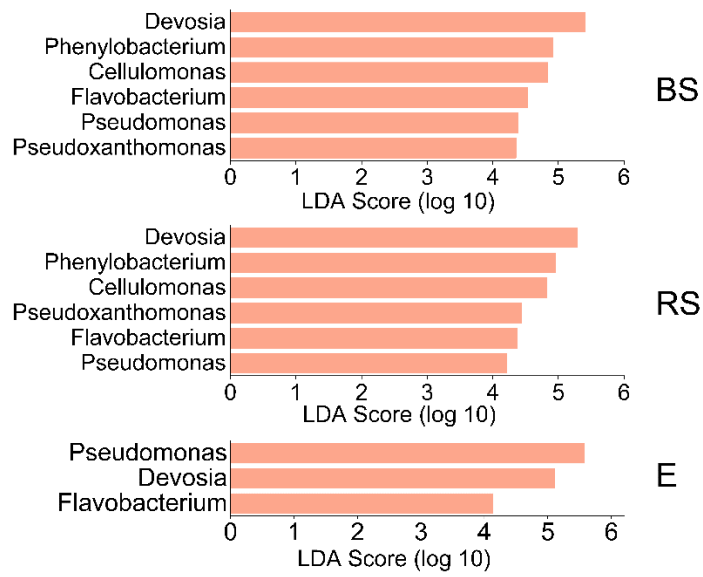


Fig. S8 LEfSe (Linear discriminant analysis Effect Size) showing beneficial bacteria with significant differences between CK groups and OF groups. Samples of different growth time are included.

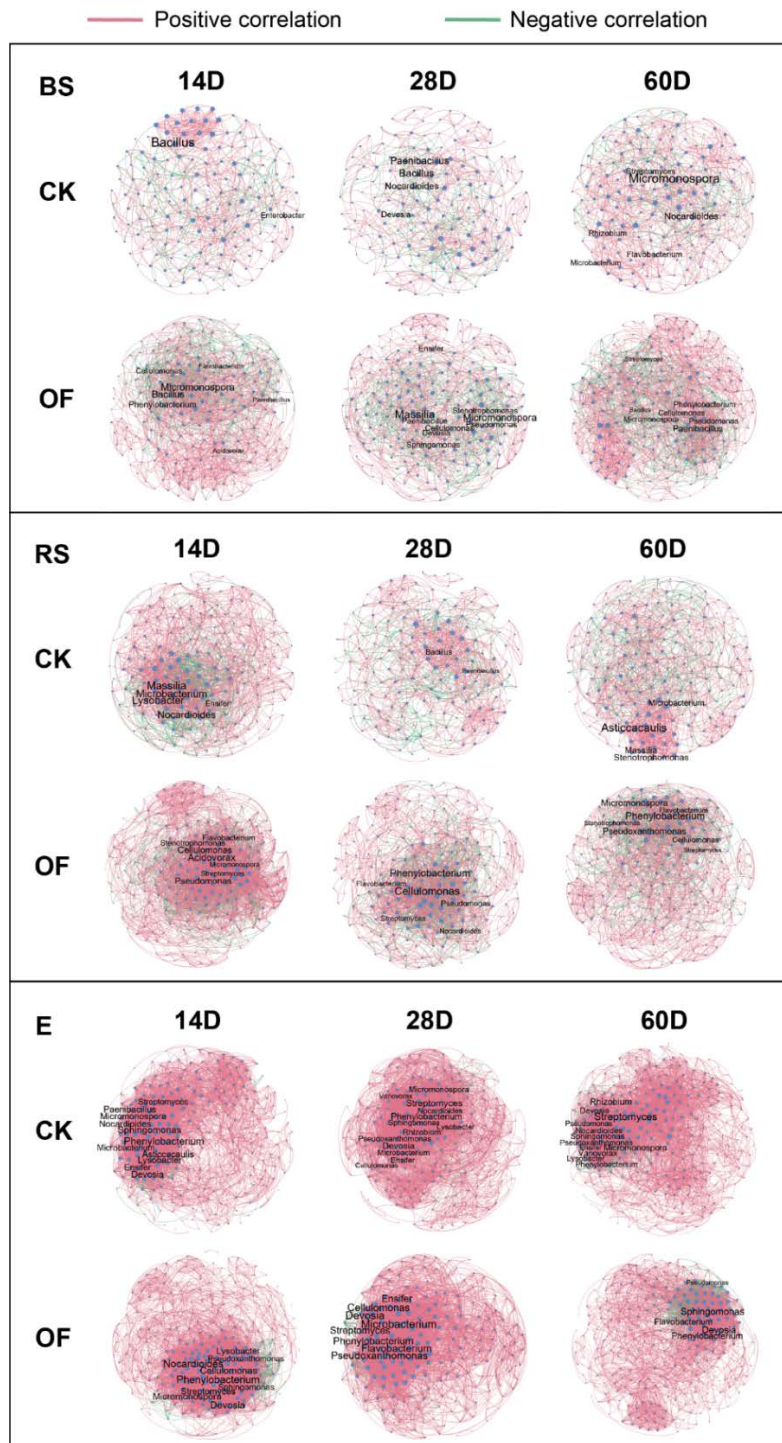


Fig. S9 The networks showing effects of organic fertilizations and growth time on co-occurrence patterns of bacteria taxa in bulk soil (BS), rhizosphere soil (RS) and endosphere (E) at a genus level. Beneficial bacteria with high degree are marked in networks.

Reference

- Berendsen, R.L., Vismans, G., Yu, K., Song, Y., de Jonge, R., Burgman, W.P., Burmolle, M., Herschend, J., Bakker, P., Pieterse, C.M.J., 2018. Disease-induced assemblage of a plant-beneficial bacterial consortium. *ISME Journal* 12, 1496-1507.
- Bziuk, N., Maccario, L., Straube, B., Wehner, G., Sorensen, S.J., Schikora, A., Smalla, K., 2021. The treasure inside barley seeds: microbial diversity and plant beneficial bacteria. *Environmental Microbiome* 16, 20.
- Carrion, V.J., Perez-Jaramillo, J., Cordovez, V., Tracanna, V., de Hollander, M., Ruiz-Buck, D., Mendes, L.W., van Ijcken, W.F.J., Gomez-Exposito, R., Elsayed, S.S., Mohanraju, P., Arifah, A., van der Oost, J., Paulson, J.N., Mendes, R., van Wezel, G.P., Medema, M.H., Raaijmakers, J.M., 2019. Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. *Science* 366, 606-612.
- Cavite, H.J.M., Mactal, A.G., Evangelista, E.V., Cruz, J.A., 2020. Growth and yield response of upland rice to application of plant growth-promoting rhizobacteria. *Journal of Plant Growth Regulation* 40, 494-508.
- Chen, D., Wang, X., Zhang, W., Zhou, Z., Ding, C., Liao, Y., Li, X., 2020. Persistent organic fertilization reinforces soil-borne disease suppressiveness of rhizosphere bacterial community. *Plant and Soil* 452, 313-328.
- Dahal, B., NandaKafle, G., Perkins, L., Brozel, V.S., 2017. Diversity of free-living nitrogen fixing *Streptomyces* in soils of the badlands of South Dakota. *Microbiological Research* 195, 31-39.
- Deng, X.H., Zhang, N., Li, Y.C., Zhu, C.Z., Qu, B.Y., Liu, H.J., Li, R., Bai, Y., Shen,

Q.R., Salles, J.F., 2022. Bio-organic soil amendment promotes the suppression of *Ralstonia solanacearum* by inducing changes in the functionality and composition of rhizosphere bacterial communities. *New Phytologist* 235, 1558-1574.

Kang, A., Zhang, N., Xun, W., Dong, X., Xiao, M., Liu, Z., Xu, Z., Feng, H., Zou, J., Shen, Q., Zhang, R., 2022. Nitrogen fertilization modulates beneficial rhizosphere interactions through signaling effect of nitric oxide. *Plant Physiology* 188, 1129-1140.

Kuang, B., Xiao, R., Hu, Y., Wang, Y., Zhang, L., Wei, Z., Bai, J., Zhang, K., Acuna, J.J., Jorquera, M.A., Pan, W., 2022. Metagenomics reveals biogeochemical processes carried out by sediment microbial communities in a shallow eutrophic freshwater lake. *Frontiers in Microbiology* 13, 1112669.

Kwak, M.J., Kong, H.G., Choi, K., Kwon, S.K., Song, J.Y., Lee, J., Lee, P.A., Choi, S.Y., Seo, M., Lee, H.J., Jung, E.J., Park, H., Roy, N., Kim, H., Lee, M.M., Rubin, E.M., Lee, S.W., Kim, J.F., 2018. Rhizosphere microbiome structure alters to enable wilt resistance in tomato. *Nature Biotechnology* 36, 1100.

Liu, H., Li, J., Carvalhais, L.C., Percy, C.D., Prakash Verma, J., Schenk, P.M., Singh, B.K., 2021. Evidence for the plant recruitment of beneficial microbes to suppress soil-borne pathogens. *New Phytologist* 229, 2873-2885.

Lugtenberg, B., Kamilova, F., 2009. Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology* 63, 541-556.

Mehmood, M.A., Fu, Y., Zhao, H., Cheng, J., Xie, J., Jiang, D., 2022. Enrichment of bacteria involved in the nitrogen cycle and plant growth promotion in soil by sclerotia of rice sheath blight fungus. *Stress Biology* 2,

Mendes, L.W., Mendes, R., Raaijmakers, J.M., Tsai, S.M., 2018. Breeding for soil-borne pathogen resistance impacts active rhizosphere microbiome of common bean. *ISME Journal* 12, 3038-3042.

Pedrinho, A., Mendes, L.W., Merloti, L.F., Andreote, F.D., Tsai, S.M., 2020. The natural recovery of soil microbial community and nitrogen functions after pasture abandonment in the Amazon region. *FEMS Microbiology Ecology* 96,

Qi, M., Berry, J.C., Velez, K.W., O'Connor, L., Finkel, O.M., Salas-Gonzalez, I., Kuhs, M., Jupe, J., Holcomb, E., Glavina Del Rio, T., Creech, C., Liu, P., Tringe, S.G., Dangl, J.L., Schachtman, D.P., Bart, R.S., 2022. Identification of beneficial and detrimental bacteria impacting sorghum responses to drought using multi-scale and multi-system microbiome comparisons. *ISME Journal* 16, 1957-1969.

Richardson, A.E., Simpson, R.J., 2011. Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiology* 156, 989-996.

Rivas, R., Velazquez, E., Willems, A., Vizcaino, N., Subba-Rao, N.S., Mateos, P.F., Gillis, M., Dazzo, F.B., Martinez-Molina, E., 2002. A new species of *Devosia* that forms a unique nitrogen-fixing root-nodule symbiosis with the aquatic legume *Neptunia natans* (L.f.) druce. *Applied and Environmental Microbiology* 68, 5217-5222.

Rodríguez, H., Fraga, R., Gonzalez, T., Bashan, Y., 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil* 287, 15-21.

Schmitz, L., Yan, Z., Schneijderberg, M., de Roij, M., Pijnenburg, R., Zheng, Q.,

Franken, C., Dechesne, A., Trindade, L.M., van Velzen, R., Bisseling, T., Geurts, R., Cheng, X., 2022. Synthetic bacterial community derived from a desert rhizosphere confers salt stress resilience to tomato in the presence of a soil microbiome. *ISME Journal* 16, 1907-1920.

Shao, J., Miao, Y., Liu, K., Ren, Y., Xu, Z., Zhang, N., Feng, H., Shen, Q., Zhang, R., Xun, W., 2021. Rhizosphere microbiome assembly involves seed-borne bacteria in compensatory phosphate solubilization. *Soil Biology and Biochemistry* 159,

Sonkurt, M., ÇİĞ, F., 2019. The effect of plant growth-promoting bacteria on the development, yield and yield components of bread (*Triticum Aestivum* L.) and durum (*Triticum Durum*) wheats. *Applied Ecology and Environmental Research* 17, 3877-3896.

Trujillo, M.E., Riesco, R., Benito, P., Carro, L., 2015. Endophytic Actinobacteria and the interaction of *Micromonospora* and nitrogen fixing plants. *Frontiers in Microbiology* 6, 1341.

Wilhelm, R.C., Singh, R., Eltis, L.D., Mohn, W.W., 2019. Bacterial contributions to delignification and lignocellulose degradation in forest soils with metagenomic and quantitative stable isotope probing. *ISME Journal* 13, 413-429.