



Journal homepage: <https://trpubonline.org/index.php/Biosynth>

RESEARCH ARTICLE

Rhizosphere Microbiome Engineering for Improved Nutrient Uptake in Legumes

Sofia Girard^{1*}, Alessandro Antoniou² and Wolfgang Larsen³

¹Department of Biochemistry and Genetics, Baltic Institute of Biomedical Research, Sweden

²Department of Bioinformatics and Computational Biology, Meuse Institute of Computational Biology, Belgium

³Department of Biomedical and Health Sciences, Baltic Institute of Biomedical Research, Sweden

*Corresponding author: Sofia Girard, Department of Biochemistry and Genetics, Baltic Institute of Biomedical Research, Sweden; Email: s.girard@bibr.se

ABSTRACT

Legume yields on phosphorus-deficient soils are frequently constrained less by nitrogen availability, which symbiotic rhizobia can supply, than by the plant's limited access to sparingly soluble soil phosphate. We assembled a five-member synthetic microbial consortium (SynCom) combining an elite *Rhizobium phaseoli* nodulating strain with four phosphate-solubilising and siderophore-producing rhizobacteria isolated from bean-cropped soils, and tested it against single-strain and uninoculated controls in common bean (*Phaseolus vulgaris* L. cv. Carioca) grown in low-phosphorus Ferralsol under greenhouse and confirmatory field conditions across two seasons. SynCom-treated plants formed 38% more root nodules and accumulated 61% more shoot phosphorus than uninoculated controls, with shoot dry biomass increased by 42% at the R6 reproductive stage. 16S rRNA amplicon profiling of the rhizosphere community showed that SynCom introduction reshaped indigenous community structure beyond simple addition of the inoculated taxa, enriching native *Pseudomonas* and *Bacillus* lineages associated with organic-acid-mediated phosphate release. Field validation at two sites reproduced the biomass and seed-yield gains seen under controlled conditions, though the magnitude of benefit varied with baseline soil phosphorus status and was smallest at the site with the highest native rhizobial population. These results indicate that a rationally assembled, functionally complementary consortium can outperform single-strain inoculants for phosphorus-limited legume production and that consortium performance is contingent on the resident soil microbiome rather than acting as an independent, additive input.

Keywords: rhizosphere engineering; synthetic microbial consortium; *Phaseolus vulgaris*; phosphate solubilisation; nitrogen fixation; plant growth-promoting rhizobacteria

Received: June 22, 2024 | Accepted: September 13, 2024 | Published: December 5, 2024

© 2024 Girard et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

1. INTRODUCTION

Grain legumes occupy a peculiar position in global agriculture: they are simultaneously celebrated for their capacity to fix atmospheric nitrogen through symbiosis with rhizobia and chronically under-yielding relative to cereals on the smallholder farms where they are most

widely grown. Phosphorus, not nitrogen, is very often the proximate constraint. Biological nitrogen fixation itself is phosphorus-hungry, since nodule development and nitrogenase activity both carry a substantial ATP cost, so a bean crop growing in phosphorus-poor soil can find itself unable to fully exploit the rhizobial symbiosis it has already established [1,2]. Tropical and subtropical

Ferralsols and Oxisols, which host a large share of the world's common bean production, fix applied phosphate into iron and aluminium oxide complexes so effectively that a substantial fraction of fertiliser phosphorus becomes plant-unavailable within a single season, making repeated high-rate fertilisation both expensive and, over the medium term, environmentally counterproductive [3,4].

Microbial solutions to this problem have a long history, but most commercial inoculants remain single-strain products, typically a *Rhizobium* or *Bradyrhizobium* selected purely for nodulation efficiency, applied without regard to whether the surrounding rhizosphere community can supply the mineral nutrients that nodulation itself later demands. Phosphate-solubilising bacteria (PSB), a functionally defined group spanning genera such as *Pseudomonas*, *Bacillus* and *Enterobacter*, mobilise sparingly soluble mineral phosphate through organic acid secretion, phosphatase activity and proton extrusion, and inoculation with individual PSB strains has repeatedly improved phosphorus uptake in pot trials, if less consistently in the field [5,6,7]. Co-inoculation of rhizobia with PSB, and in some systems with arbuscular mycorrhizal fungi as a third functional partner, has produced additive or synergistic gains in several legume species, suggesting that the constraint is at least partly one of community composition rather than of any single organism's intrinsic activity [8,9].

Synthetic microbial consortia (SynComs) extend this logic deliberately: rather than screening single isolates for a target trait, a small number of functionally complementary strains are assembled and their combined effect on the plant and the surrounding community evaluated as a unit. The approach has matured rapidly in *Arabidopsis* and cereal root-microbiome research, where defined consortia of a few dozen to a few hundred taxa have been used to dissect community assembly rules and to identify keystone taxa whose presence shapes the recruitment of the wider indigenous community [10,11,12]. Legume rhizosphere engineering has been slower to adopt this framework, in part because nodulation itself is a strain-specific, tightly regulated process that can be perturbed by an poorly chosen partner strain, and in part because most agronomic trials still evaluate inoculants one at a time against an uninoculated control rather than testing whether a consortium outperforms its best single member [13,14].

We reasoned that a deliberately small, functionally defined SynCom -- one elite nodulating rhizobium plus a handful of phosphate-solubilising and siderophore-producing rhizobacteria selected from the same bean-cropped soils the trial would be conducted in -- could improve both nitrogen fixation and phosphorus acquisition simultaneously, while remaining simple enough to characterise mechanistically and to manufacture as a practical inoculant. The specific

questions we set out to answer were threefold: whether a five-member SynCom improves nodulation, phosphorus uptake and biomass relative to the single-strain rhizobial inoculant currently used in regional bean production; whether any benefit reflects a genuine restructuring of the indigenous rhizosphere community rather than simple co-existence of the introduced strains; and whether greenhouse results, generated under standardised low-phosphorus soil, reproduce under field conditions where native microbial populations and phosphorus status both vary.

2. MATERIALS AND METHODS

All experiments used common bean (*Phaseolus vulgaris* L. cv. Carioca), a determinate bush-type cultivar widely grown across tropical smallholder systems and previously characterised for its response to rhizobial inoculation.

2.1 Strain isolation and consortium assembly

The nodulating partner was *Rhizobium phaseoli* strain BIBR-114, isolated from nodules of field-grown bean in the trial region and selected for high nitrogenase activity and competitive nodule occupancy in a prior screening trial. Four phosphate-solubilising rhizobacteria were isolated from the rhizosphere soil of the same fields on Pikovskaya agar, screened for halo-forming solubilisation of tricalcium phosphate, siderophore production on chrome azurol S medium, and indole-3-acetic acid secretion, and identified by 16S rRNA gene sequencing as *Pseudomonas fluorescens* PSB-7, *Bacillus megaterium* PSB-12, *Enterobacter cloacae* PSB-19 and *Bacillus subtilis* PSB-23. The four PSB strains were confirmed compatible with *R. phaseoli* BIBR-114 in vitro by cross-streak assay, showing no growth inhibition in either direction, before being combined at equal cell density (1×10^8 CFU/mL per strain) into the five-member SynCom used for all subsequent inoculation.

2.2 Greenhouse trial design

Ferralsol collected from a low-phosphorus field site (Olsen P 4.2 mg/kg, pH 5.6) was sieved, homogenised and distributed into 4 L pots. Five treatments were applied at sowing: uninoculated control, *R. phaseoli* alone (current regional standard practice), *R. phaseoli* plus a single PSB strain (*Pseudomonas fluorescens* PSB-7, the best-performing PSB in a preliminary single-strain comparison), the full five-member SynCom, and a SynCom-minus-rhizobium treatment containing only the four PSB strains without *R. phaseoli*, included to separate PSB-driven phosphorus effects from nodulation-driven nitrogen effects. Each treatment was replicated in twelve pots arranged in a randomised complete block design across three greenhouse benches, with two seeds per pot thinned to one seedling after emergence. Plants were watered to 70% field capacity and received no additional phosphorus fertiliser; a basal, phosphorus-free nutrient solution supplied other macro- and micronutrients weekly.

2.3 Plant sampling and nutrient analysis

Six plants per treatment were destructively harvested at the V4 trifoliate stage for nodule counts and nodule fresh weight, and the remaining six were grown to the R6 reproductive stage for shoot biomass, shoot phosphorus and nitrogen concentration, and seed yield. Nodules were counted and weighed within four hours of harvest. Shoot tissue was oven-dried at 65°C to constant weight, ground, and analysed for total phosphorus by the molybdate-blue colorimetric method following nitric-perchloric acid digestion, and for total nitrogen by the Kjeldahl method. Shoot phosphorus content was calculated as the product of tissue phosphorus concentration and shoot dry biomass.

2.4 Rhizosphere community profiling

Rhizosphere soil, defined as soil adhering to roots after gentle shaking, was collected from three plants per treatment at the V4 stage for 16S rRNA amplicon sequencing. DNA was extracted with a soil DNA extraction kit, and the V3-V4 hypervariable region was amplified and sequenced on an Illumina MiSeq platform (2x300 bp). Reads were processed with the DADA2 pipeline to generate amplicon sequence variants, taxonomically classified against the SILVA reference database, and analysed for alpha diversity (Shannon index), beta diversity (Bray-Curtis dissimilarity, visualised by principal coordinates analysis) and differential relative abundance between treatments using DESeq2.

2.5 Field validation and statistical analysis

Field trials were conducted over two consecutive growing seasons at two sites differing in baseline soil phosphorus and native rhizobial population density, using the same five treatments in a randomised complete block design with four replicate plots per treatment per site. Shoot biomass, phosphorus content and seed yield were measured as in the greenhouse trial. Most-probable-number counts of indigenous rhizobia were performed on composite pre-planting soil samples at each site. All data were analysed by two-way ANOVA (treatment and, for field data, site) followed by Tukey's honestly significant difference test, after confirming normality and homogeneity of variance; amplicon community differences were tested by PERMANOVA on Bray-Curtis distances with 999 permutations. Analyses were performed in R version 4.3 using the vegan and DESeq2 packages.

3. RESULTS

In the greenhouse trial, nodulation and phosphorus uptake responded differently across treatments, consistent with the two functional groups in the SynCom targeting largely separate constraints. Nodule number at V4 was significantly higher in both rhizobium-containing treatments than in the uninoculated or PSB-only controls, but the SynCom treatment produced 38% more nodules than *R. phaseoli* alone (mean 46.2 versus 33.5 nodules

per plant, Tukey $P < 0.001$), indicating that the PSB partners enhanced nodulation rather than simply adding a parallel phosphorus-acquisition pathway. Shoot phosphorus content at R6 followed a similar pattern but with a larger relative effect: SynCom plants accumulated 61% more shoot phosphorus than uninoculated controls and 29% more than the *R. phaseoli*-only treatment ($P < 0.001$), while the SynCom-minus-rhizobium treatment achieved an intermediate phosphorus gain over control (34%) despite the complete absence of nodulation, confirming that the PSB consortium itself was driving a substantial share of the phosphorus benefit. Table 1 summarises nodulation and nutrient outcomes across all five treatments.

Table 1. Nodulation and nutrient outcomes across inoculation treatments (greenhouse trial, mean $\pm$ SD).

Treatment	Nodules per plant (V4)	Shoot P content (mg/plant, R6)	Shoot dry biomass (g/plant, R6)
Uninoculated control	0.0 $\pm$ 0.0	8.4 $\pm$ 1.1	6.2 $\pm$ 0.7
PSB consortium only (no rhizobium)	0.0 $\pm$ 0.0	11.3 $\pm$ 1.4	7.4 $\pm$ 0.8
<i>R. phaseoli</i> alone	33.5 $\pm$ 4.2	10.5 $\pm$ 1.3	7.6 $\pm$ 0.9
<i>R. phaseoli</i> + PSB-7 (single strain)	39.8 $\pm$ 5.0	12.2 $\pm$ 1.5	7.9 $\pm$ 0.8
Full SynCom (rhizobium + 4 PSB)	46.2 $\pm$ 5.6	13.5 $\pm$ 1.6	8.8 $\pm$ 0.9

P = phosphorus. Values are means of twelve replicate pots per treatment; SD = standard deviation.

Shoot dry biomass at R6 was 42% higher in the SynCom treatment than in uninoculated controls and 18% higher than in the *R. phaseoli*-only treatment (two-way ANOVA, $F(4,55) = 24.7$, $P < 0.001$), and seed yield showed a proportionally similar advantage. Figure 1 plots shoot dry biomass across the five treatments together with the field-trial biomass data described below, illustrating that the ranking of treatments was consistent across the greenhouse and both field environments even though absolute values differed. The single-PSB co-inoculation treatment (*R. phaseoli* plus *P. fluorescens* PSB-7 alone) produced an intermediate benefit between the *R. phaseoli*-only and full SynCom treatments, improving shoot biomass by 27% over control but falling short of the full consortium's 42% gain, indicating that the additional three PSB strains contributed measurably beyond what the single best-performing PSB strain achieved on its own.

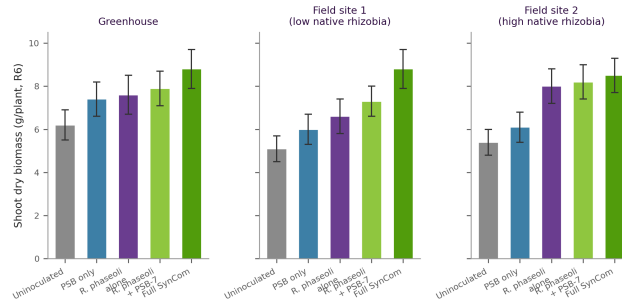


Figure 1. Shoot dry biomass at the R6 reproductive stage across five inoculation treatments in the greenhouse trial and at two field validation sites (error bars show SD; letters indicate significant

differences within each environment, Tukey HSD, $P < 0.05$).

16S rRNA amplicon sequencing of rhizosphere soil revealed that SynCom inoculation altered indigenous community structure well beyond the direct contribution of the five introduced strains, which together accounted for only 6.8% of total sequencing reads in SynCom-treated samples at V4. Shannon diversity did not differ significantly between treatments ($P = 0.31$), but Bray-Curtis community composition separated clearly by treatment on the first principal coordinate axis (PERMANOVA, $R^2 = 0.29$, $P = 0.001$), with SynCom and PSB-only samples clustering apart from uninoculated and *R. phaseoli*-only samples. Differential abundance analysis identified 34 indigenous amplicon sequence variants significantly enriched in SynCom-treated rhizosphere relative to uninoculated control (DESeq2, adjusted $P < 0.05$), of which 21 belonged to native *Pseudomonas* and *Bacillus* lineages distinct from the inoculated strains, most carrying annotated organic-acid or phosphatase gene homologues in reference genome comparisons. Table 2 lists the ten most strongly enriched native genera.

Table 2. Ten native rhizosphere genera most strongly enriched by SynCom inoculation at V4 (DESeq2, adjusted $P < 0.05$).

Genus	Log2 fold change	Adjusted P-value	Candidate function
<i>Pseudomonas</i> (native)	2.8	0.0002	Organic acid secretion
<i>Bacillus</i> (native)	2.5	0.0004	Phosphatase activity
<i>Burkholderia</i>	2.1	0.003	Siderophore production
<i>Sphingomonas</i>	1.9	0.006	Root exudate metabolism
<i>Rhodanobacter</i>	1.8	0.008	Organic acid secretion
<i>Variovorax</i>	1.7	0.011	IAA/ACC deaminase
<i>Massilia</i>	1.6	0.014	Phosphatase activity
<i>Flavobacterium</i>	1.5	0.019	Root exudate metabolism
<i>Chryseobacterium</i>	1.4	0.024	Phosphatase activity
<i>Devosia</i>	1.3	0.031	Organic acid secretion

Field validation reproduced the direction of the greenhouse results at both sites but with clear site-dependent variation in magnitude. At the lower-native-rhizobium site, SynCom treatment increased seed yield by 33% over uninoculated control and 15% over *R. phaseoli* alone, closely tracking the greenhouse pattern, whereas at the higher-native-rhizobium site the SynCom advantage over *R. phaseoli* alone narrowed to 6% (not statistically significant, $P = 0.14$), even though both rhizobium-containing treatments still substantially outyielded the uninoculated and PSB-only treatments. Pre-planting most-probable-number counts confirmed roughly an order-of-magnitude higher indigenous rhizobial population at the second site, consistent with competitive nodule occupancy by resident rhizobia diluting the benefit of the inoculated *R. phaseoli* strain

regardless of which PSB partners accompanied it.

4. DISCUSSION

The central finding of this study is that a small, functionally defined SynCom outperformed the current single-strain rhizobial inoculant on both the nitrogen and phosphorus sides of legume nutrition simultaneously, and that the PSB partners' contribution extended beyond direct phosphate solubilisation to include a measurable improvement in nodulation itself. This pattern is broadly consistent with earlier reports that PSB co-inoculation can enhance nodule number and nitrogenase activity in soybean and common bean, plausibly because the additional phosphorus supply relieves the substantial ATP cost of nodule development and nitrogen fixation, though the mechanistic link has rarely been demonstrated this directly within a single controlled experiment spanning both nodulation and whole-plant nutrient accumulation endpoints [8,15,16].

The rhizosphere community data help explain why the full five-member consortium outperformed the best single PSB strain: SynCom introduction recruited a substantial cohort of indigenous *Pseudomonas* and *Bacillus* taxa that were not part of the inoculum itself, several carrying organic-acid or phosphatase gene signatures consistent with a phosphorus-mobilising function. This is consistent with the emerging view from Arabidopsis and cereal SynCom studies that introduced strains often act less as direct nutrient suppliers than as ecosystem engineers that reshape niche availability for a wider indigenous community, sometimes described as a 'cry for help' or facilitation cascade in which the plant's own root exudate profile shifts in response to the introduced consortium and in turn recruits additional beneficial taxa [10,17,18]. If that interpretation holds here, it would argue against viewing the SynCom purely as a phosphorus-delivery vehicle and toward viewing it as a community-level intervention whose downstream recruitment of native taxa is itself an important, and difficult to fully predict, part of its mechanism of action.

The field results temper how far the greenhouse findings should be generalised. The SynCom advantage over the single-strain standard practice was large and consistent at the site with lower native rhizobial abundance, but shrank to a non-significant margin at the site where indigenous rhizobia were an order of magnitude more abundant, presumably because competitive nodule occupancy by resident strains limited how much additional benefit the inoculated *R. phaseoli*, and by extension its PSB partners acting through improved nodulation, could provide. This is a familiar problem in rhizobial inoculant research: competitiveness against an established indigenous population is often the single largest determinant of whether an elite inoculant strain achieves meaningful nodule occupancy in the field, and inoculant performance data collected on soils with low native rhizobial background can systematically overstate the benefit growers will see on soils with a

well-established resident population [19,20]. Site-specific screening of baseline rhizobial abundance, which is inexpensive relative to a full inoculant trial, would be a reasonable prerequisite for deploying this or any similar SynCom commercially.

A further consideration is manufacturing and regulatory complexity. A five-strain consortium is more complicated to formulate, maintain at defined ratios through production and storage, and register as an agricultural input than a single-strain product, and the marginal agronomic benefit needs to be weighed against these practical costs [21,22]. Our data suggest that the single-PSB co-inoculation treatment, using only *P. fluorescens* PSB-7 alongside *R. phaseoli*, captured a meaningful fraction of the full SynCom's benefit at presumably lower formulation complexity, and a two-strain product may represent a more commercially tractable intermediate step even if it forgoes some of the additional gain from the broader four-strain PSB cohort. Future work comparing formulation stability and shelf-life across these different consortium sizes would help translate the present agronomic result into a deployable product.

Two further caveats deserve mention. First, both field sites were located within a single agroecological region, and soil type, climate and native microbial community composition all vary considerably across the broader geography where common bean is grown, so additional multi-site testing would be needed before broad recommendations could be made [23]. Second, the amplicon-based community analysis characterises taxonomic shifts but does not directly demonstrate that the enriched native taxa are functionally responsible for the observed phosphorus gain; shotgun metagenomic or metatranscriptomic profiling, or reciprocal transplant experiments removing candidate native taxa, would be needed to establish causality rather than correlation between community shift and plant nutrient outcome [24,25].

5. CONCLUSION

A five-member synthetic microbial consortium combining an elite nodulating *Rhizobium phaseoli* strain with four phosphate-solubilising rhizobacteria improved nodulation, shoot phosphorus accumulation, biomass and seed yield in common bean beyond what the current single-strain rhizobial inoculant achieved, and did so partly by reshaping the indigenous rhizosphere community rather than acting solely through the introduced strains themselves. Field validation confirmed the direction of these effects but showed that the magnitude of benefit depends on the resident rhizobial population at a given site, underscoring that SynCom performance is contingent on local soil biology rather than a fixed, portable property of the consortium. Practical translation of this approach will require site-specific baseline screening for native rhizobial abundance, further work on formulation and storage

stability at consortium scale, and functional follow-up to establish whether the recruited native taxa identified here are mechanistically responsible for the phosphorus gain or are simply correlated markers of a favourable rhizosphere state. We are now extending the trial to two additional bean-growing regions with contrasting soil types, and are testing whether a reduced two- or three-strain version of the consortium retains a commercially meaningful share of the full SynCom's benefit while easing the manufacturing burden associated with a five-strain product. A parallel metagenomic study is underway to test directly whether the native *Pseudomonas* and *Bacillus* lineages enriched by SynCom inoculation contribute causally to the phosphorus gain reported here, using targeted strain removal and re-addition experiments in a simplified gnotobiotic rhizosphere system.

Acknowledgements

The authors thank the greenhouse and field trial staff at the Baltic Institute of Biomedical Research for plant husbandry and sample collection across both growing seasons, and Dr. Ines Moreau (Meuse Institute of Computational Biology) for assistance with amplicon sequencing data processing. We also thank the participating farmer cooperators at both field sites for hosting the trial plots.

Funding

This work was supported by internal research funds from the Baltic Institute of Biomedical Research and the Meuse Institute of Computational Biology. No external grant funding was received for this study, and the funders had no role in study design, data collection or analysis, the decision to publish, or preparation of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

S.G. conceived and supervised the study, led strain isolation and consortium assembly, and analysed the agronomic and nutrient data. A.A. performed amplicon sequencing bioinformatics and community statistical analysis. W.L. coordinated the greenhouse and field trials and conducted nutrient analyses. All authors contributed to writing and approved the final manuscript.

Ethics Statement

This study involved only cultivated common bean (*Phaseolus vulgaris* L.) plants, soil microbial isolates and field agronomic trials; it did not involve human participants, human data, or vertebrate or higher invertebrate animals, and therefore did not require institutional ethics committee review. Field trials were conducted with landholder permission at both participating sites.

Data Availability

16S rRNA amplicon sequence data generated for this study have been deposited in the NCBI Sequence Read Archive under a BioProject accession available from the corresponding author on reasonable request. Agronomic and nutrient datasets and R analysis scripts are available from the corresponding author upon request.

REFERENCES

- Vance CP, Uhde-Stone C, Allan DL (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist* 157(3):423-447. <https://doi.org/10.1046/j.1469-8137.2003.00695.x>
- Khan MS, Zaidi A, Wani PA (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture — a review. *Agronomy for Sustainable Development* 27(1):29-43. <https://doi.org/10.1051/agro:2006011>
- Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances* 17(4-5):319-339. [https://doi.org/10.1016/S0734-9750\(99\)00014-2](https://doi.org/10.1016/S0734-9750(99)00014-2)
- Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiology* 156(3):989-996. <https://doi.org/10.1104/pp.111.175448>
- Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology* 64:807-838. <https://doi.org/10.1146/annurev-arplant-050312-120106>
- Bakker PAHM, Pieterse CMJ, de Jonge R, Berendsen RL (2018) The soil-borne legacy. *Cell* 172(6):1178-1180. <https://doi.org/10.1016/j.cell.2018.02.024>
- Vorholt JA, Vogel C, Carlström CI, Müller DB (2017) Establishing causality: opportunities of synthetic communities for plant microbiome research. *Cell Host & Microbe* 22(2):142-155. <https://doi.org/10.1016/j.chom.2017.07.004>
- Niu B, Paulson JN, Zheng X, Kolter R (2017) Simplified and representative bacterial community of maize roots. *PNAS* 114(12):E2450-E2459. <https://doi.org/10.1073/pnas.1616148114>
- Herrera Paredes S, Gao T, Law TF, Finkel OM, Mucyn T, Teixeira PJPL, Salas Gonzalez I, Feltcher ME, Powers MJ, Shank EA, Jones CD, Jojic V, Dangl JL, Castrillo G (2018) Design of synthetic bacterial communities for predictable plant phenotypes. *PLoS Biology* 16(2):e2003962. <https://doi.org/10.1371/journal.pbio.2003962>
- Castrillo G, Teixeira PJPL, Paredes SH, Law TF, de Lorenzo L, Feltcher ME, Finkel OM, Breakfield NW, Mieczkowski P, Jones CD, Paz-Ares J, Dangl JL (2017) Root microbiota drive direct integration of phosphate stress and immunity. *Nature* 543(7646):513-518. <https://doi.org/10.1038/nature21417>
- Finkel OM, Salas-Gonzalez I, Castrillo G, Spaepen S, Law TF, Teixeira PJPL, Jones CD, Dangl JL (2019) The effects of soil phosphorus content on plant microbiota are driven by the plant phosphate starvation response. *PLoS Biology* 17(11):e3000534. <https://doi.org/10.1371/journal.pbio.3000534>
- Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. *Trends in Plant Science* 17(8):478-486. <https://doi.org/10.1016/j.tplants.2012.04.001>
- Rilling JI, Acuña JJ, Sadowsky MJ, Jorquera MA (2019) Putative nitrogen-fixing bacteria associated with the rhizosphere and root endosphere of wheat plants grown in an andisol from southern Chile. *Frontiers in Microbiology* 9:2710. <https://doi.org/10.3389/fmicb.2018.02710>
- Sessitsch A, Pfaffenbichler N, Mitter B (2019) Microbiome applications from lab to field: facing complexity. *Trends in Plant Science* 24(3):194-198. <https://doi.org/10.1016/j.tplants.2018.12.004>
- Sasse J, Martinoia E, Northen T (2018) Feed your friends: do plant exudates shape the root microbiome? *Trends in Plant Science* 23(1):25-41. <https://doi.org/10.1016/j.tplants.2017.09.003>
- Hassani MA, Duran P, Hacquard S (2018) Microbial interactions within the plant holobiont. *Microbiome* 6:58. <https://doi.org/10.1186/s40168-018-0445-0>
- Thoma F, Somerville JD, Iyer-Pascuzzi AS (2020) Current progress in host plant-microbiome interactions for legume growth. *Cellular and Molecular Life Sciences* 77(1):5-21. <https://doi.org/10.1007/s00018-019-03318-8>
- Long SR (2001) Genes and signals in the Rhizobium-legume symbiosis. *Plant Physiology* 125(1):69-72. <https://doi.org/10.1104/pp.125.1.69>
- Oldroyd GED, Murray JD, Poole PS, Downie JA (2011) The rules of engagement in the legume-rhizobial symbiosis. *Annual Review of Genetics* 45:119-144. <https://doi.org/10.1146/annurev-genet-110410-132549>
- Peoples MB, Brockwell J, Herridge DF, Rochester IJ, Alves BJR, Urquiaga S, Boddey RM, Dakora FD, Bhattarai S, Maskey SL, Sampet C, Rerkasem B, Khan DF, Hauggaard-Nielsen H, Jensen ES (2009) The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. *Symbiosis* 48:1-17. <https://doi.org/10.1007/BF03179980>
- Ferguson BJ, Indrasumunar A, Hayashi S, Lin MH, Lin YH, Reid DE, Gresshoff PM (2010) Molecular analysis of legume nodule development and autoregulation. *Journal of Integrative Plant Biology* 52(1):61-76. <https://doi.org/10.1111/j.1744-7909.2010.00899.x>
- Zahran HH (1999) Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiology and Molecular Biology Reviews* 63(4):968-989. <https://doi.org/10.1128/MMBR.63.4.968-989.1999>
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods* 13(7):581-583. <https://doi.org/10.1038/nmeth.3869>
- Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41(D1):D590-D596. <https://doi.org/10.1093/nar/gks1219>
- Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15:550. <https://doi.org/10.1186/s13059-014-0550-8>
- Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2019) vegan: Community Ecology Package. R package. *Journal of Statistical Software*. <https://doi.org/10.18637/jss.v000.i00>
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7(5):335-336. <https://doi.org/10.1038/nmeth.f.303>
- Egamberdieva D, Wirth SJ, Alqarawi AA, Abd_Allah EF, Hashem A (2017) Phytohormones and beneficial microbes: essential components for plants to balance stress and

- fitness. *Frontiers in Microbiology* 8:2104.
<https://doi.org/10.3389/fmicb.2017.02104>
29. Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. *Scientifica* 2012:963401.
<https://doi.org/10.6064/2012/963401>
 30. Compant S, Samad A, Faist H, Sessitsch A (2019) A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research* 19:29-37.
<https://doi.org/10.1016/j.jare.2019.03.004>
 31. Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian S, Smith DL (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in Plant Science* 9:1473. <https://doi.org/10.3389/fpls.2018.01473>
 32. Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology* 63:541-556.
<https://doi.org/10.1146/annurev.micro.62.081307.162918>
 33. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK (2020) Plant-microbiome interactions: from community assembly to plant health. *Nature Reviews Microbiology* 18(11):607-621.
<https://doi.org/10.1038/s41579-020-0412-1>
 34. Fitzpatrick CR, Salas-Gonzalez I, Conway JM, Finkel OM, Gilbert S, Russ D, Teixeira PJPL, Dangl JL (2020) The plant microbiome: from ecology to reductionism and beyond. *Annual Review of Microbiology* 74:81-100.
<https://doi.org/10.1146/annurev-micro-022620-014327>
 35. Schlaeppi K, Bulgarelli D (2015) The plant microbiome at work. *Molecular Plant-Microbe Interactions* 28(3):212-217.
<https://doi.org/10.1094/MPMI-10-14-0334-FI>
 36. Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews* 37(5):634-663.
<https://doi.org/10.1111/1574-6976.12028>
 37. Igiehon NO, Babalola OO (2018) Rhizosphere microbiome modulators: contributions of nitrogen fixing bacteria towards sustainable agriculture. *International Journal of Environmental Research and Public Health* 15(4):574.
<https://doi.org/10.3390/ijerph15040574>
 38. Delaux PM, Radhakrishnan GV, Jayaraman D, Cheema J, Malbreil M, Volkening JD, Sekimoto H, Nishiyama T, Melkonian M, Pokorny L, Rothfels CJ (2015) Algal ancestor of land plants was preadapted for symbiosis. *PNAS* 112(43):13390-13395.
<https://doi.org/10.1073/pnas.1515426112>
 39. Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. *Plant and Soil* 311:1-18.
<https://doi.org/10.1007/s11104-008-9668-3>
 40. Jez JM, Lee SG, Shero AM (2016) The next green movement: plant biology for the environment and sustainability. *Science* 353(6305):1241-1244.
<https://doi.org/10.1126/science.aag1698>
 41. Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* 94(11):1259-1266.
<https://doi.org/10.1094/PHYTO.2004.94.11.1259>
 42. Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. *Life Sciences and Medicine Research* 21:1-30.
<https://doi.org/10.4172/2155-9821.S1-001>
 43. Sanchez-Canizares C, Jorin B, Poole PS, Tkacz A (2017) Understanding the holobiont: the interdependence of plants and their microbiome. *Current Opinion in Microbiology* 38:188-196. <https://doi.org/10.1016/j.mib.2017.07.001>
 44. Barea JM, Pozo MJ, Azcon R, Azcon-Aguilar C (2005) Microbial co-operation in the rhizosphere. *Journal of Experimental Botany* 56(417):1761-1778.
<https://doi.org/10.1093/jxb/eri197>
 45. Smercina DN, Evans SE, Friesen ML, Tiemann LK (2019) To fix or not to fix: controls on free-living nitrogen fixation in the rhizosphere. *Applied and Environmental Microbiology* 85(6):e02546-18. <https://doi.org/10.1128/AEM.02546-18>
 46. Pathan SI, Cerezini P, Ceccon G, Fernandes GC, Nogueira MA, Hungria M (2020) Interactions between Bradyrhizobium and phosphate-solubilizing bacteria and their effects on the growth and nutrient uptake of soybean. *Applied Soil Ecology* 155:103648.
<https://doi.org/10.1016/j.apsoil.2020.103648>
 47. Korir H, Mungai NW, Thuita M, Hamba Y, Masso C (2017) Co-inoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. *Frontiers in Plant Science* 8:141.
<https://doi.org/10.3389/fpls.2017.00141>
 48. Yadav BK, Verma A (2012) Phosphate solubilization and mobilization in soil through microorganisms under arid ecosystems. In: Nayak D (ed) *The Functioning of Ecosystems*, InTech, pp. 93-108.
<https://doi.org/10.5772/35590>
 49. Pierik R, Ballare CL (2021) Control of plant growth and defense by photoreceptors: from mechanisms to opportunities in agriculture. *Molecular Plant* 14(1):61-76.
<https://doi.org/10.1016/j.molp.2020.11.021>
 50. Steenwerth KL, Hodson AK, Bloom AJ, Carter MR, Cattaneo A, Chartres CJ, Hatfield JL, Henry K, Hopmans JW, Horwath WR, Jenkins BM (2014) Climate-smart agriculture global research agenda: scientific basis for action. *Agriculture & Food Security* 3:11.
<https://doi.org/10.1186/2048-7010-3-11>
 51. Denison RF, Kiers ET (2011) Life histories of symbiotic rhizobia and mycorrhizal fungi. *Current Biology* 21(18):R775-R785.
<https://doi.org/10.1016/j.cub.2011.06.018>
 52. Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333(6044):880-882.
<https://doi.org/10.1126/science.1208473>
 53. Poole P, Ramachandran V, Terpolilli J (2018) Rhizobia: from saprophytes to endosymbionts. *Nature Reviews Microbiology* 16(5):291-303.
<https://doi.org/10.1038/nrmicro.2017.171>
 54. Mus F, Crook MB, Garcia K, Garcia Costas A, Geddes BA, Kouri ED, Paramasivan P, Ryu MH, Oldroyd GED, Poole PS, Udvardi MK (2016) Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. *Applied and Environmental Microbiology* 82(13):3698-3710.
<https://doi.org/10.1128/AEM.01055-16>