Understanding plant microbiomes requires a G x E framework

Jun-Hee Jung[1]*, Frank Reis[1]*, Christina L. Richards[1,2] and Oliver Bossdorf[1]

[1] Plant Evolutionary Ecology, Institute of Evolution and Ecology, Auf der Morgenstelle 5, University of Tübingen, 72076 Tübingen, Germany

[2] Department of Integrative Biology, University of South Florida, Tampa, FL 33620, USA

* indicates equal contributions

Keywords: experimental design, genotype-by-environment, intraspecific variation, plant microbiota

Plant microbiomes have become one of the hottest topics in plant biology. Driven by the increased availability of metagenome sequencing methods, analyses of plant-associated microbiomes have been skyrocketing during the last decade. They have generally taken one of two main perspectives: (1) a focus on the microbiome itself, where researchers describe microbiome diversity and attempt to understand its drivers (Fig. 1A), or (2) a focus on the consequences of microbiomes, where researchers analyse effects of microbiomes on plants (Fig. 1B). Below, we briefly discuss these two perspectives, and we argue that for both a genotype-by-environment (G x E) framework will be key for achieving a deeper and more general understanding of plant microbiomes.

Two perspectives in plant microbiome research

Studies with a microbiome focus typically describe the diversity and composition of the root or leaf microbiomes of plants, and they often test influences of plant characteristics or environmental conditions on the microbiomes (Fig. 1A). For instance, previous studies show that microbiome composition varies within and among plant species, with significant influences of plant genotype (Agler et al., 2016; Wagner et al., 2016; Bowen et al., 2017; Bergelson et al., 2019) and phylogeny (Fitzpatrick et al., 2018), and that plant tissue but also plant age and developmental stage (Chaparro et al., 2014; Wagner et al., 2016) influence plant microbiomes. In addition to plant characteristics, environmental conditions also play a role in microbiome development. For instance, soil conditions and root exudates strongly influence root microbiomes (Fitzpatrick et al., 2018; Hu et al., 2018; Sasse et al., 2018), and leaf microbiomes vary predictably among different habitats (Agler et al., 2016; Wagner et al., 2016).

Microbiome studies with a plant focus, in contrast, are interested in how the microbiome of a plant affects its growth or environmental tolerances. They test - through experiments or association patterns - how plant performance is influenced by the presence or composition of microbiota, sometimes also taking plant or environmental characteristics into account (Fig. 1B). Studies with individual microbe taxa have demonstrated that these can promote growth and stress tolerance of plants (Rodriguez et al., 2008; Lugtenberg and Kamilova, 2009) and influence pathogen and herbivore resistance (Pieterse et al., 2014; Hu et al., 2018). More recent studies with complex microbiomes have confirmed these effects: inoculation with diverse microbiota altered plant growth and physiology (Fitzpatrick et al., 2019; Belimov et al., 2020), phenology (Panke-Buisse et al., 2015) and pathogen resistance (Berendsen et al., 2018; Berg and Koskella, 2018), sometimes in a genotype- or environment-dependent fashion (Berg and Koskella, 2018; Belimov et al., 2020).

In summary, recent research has demonstrated the ubiquity and importance of plant microbiomes, but it has also shown that microbiomes are complex, and influenced by a range of plant and environmental factors. Another challenge is that drivers of microbiome variation often interact. For instance, Wagner et al. (2016) carried out a multi-site field experiment with different genotypes of *Boechera stricta* and found that genotype- and age-effects on bacterial microbiomes were often site-specific. In an experiment with natural ecotypes of *Arabidopsis thaliana*, Fitzpatrick et al. (2019) found that the effects of a natural soil microbiome on plant fitness depended on the plant genotype but also the ecological conditions under which they were tested.

In spite of the many and often interacting drivers of microbiome diversity and microbiome effects, the vast majority of previous studies focused on only one or few drivers, and there have been very few solid multifactorial studies to date that allowed to test for interactions between different factors. As a result, the generality of many previous results remains uncertain, and we are still far from understanding natural plant microbiomes. A significant step forward could therefore be to embrace an important conceptual framework from evolutionary ecology: that of genotype-by-environment interactions.

Adopting a G x E framework

Genotype-by-environment (G x E) interactions are statistical interactions between the effects of genotypes and environment on phenotypes, i.e. when phenotypic differences among genotypes depend on the environment in which they are tested, or *vice versa* phenotypic responses to environment depend on the genotype. The G x E concept has long been central to plant evolutionary ecology (Sultan, 2000), and a large body of research has often found strong G x E interactions in many plant species - to the extent that genotype effects may be strong in some but completely absent in other environments, and phenotypic responses to environment are sometimes opposite for different genotypes. Similar results in animal research confirmed that G x E interactions are the rule in natural populations, and that one therefore needs to be cautious with generalisation from single-factor studies.

The classic experimental approach to testing G x E interactions is a common garden experiment where multiple genotypes are replicated across different environments in a multi-factorial design, so that the generality of both genotype and environment effects, as well as their interactions, can be statistically tested. The results of such experiments are often visualized through reaction norm plots that show genotype-specific responses to environment, or other relevant interactions (Fig. 1C,D). We can easily apply these concepts and experimental approaches to a multi-factorial study of plant microbiomes, both for the microbiome and the plant perspective.

Microbiome-focused studies with a G x E character will essentially treat the microbiome as an 'extended phenotype' of the plant that is subject to the same complex influences as other plant phenotypes. Such studies will e.g. test the influence of plant genotype on plant microbiomes under different environmental conditions, or *vice versa* environmental effects on plant microbiomes across multiple plant genotypes (Fig. 1C). For instance, field experiments can transplant multiple plant genotypes into different habitats and test the interactive effects of genotype and habitat on spontaneous microbiome development (Wagner et al., 2016). Lab experiments can inoculate different plant genotypes with identical microbial communities and follow their dynamics under different growth conditions. With a generous interpretation of the G x E concept, these studies may also include plant factors other than genotype, such as plant tissue, plant age, or even plant species. More complex studies may include several environmental factors and/or additional microbial drivers of microbiome composition (Fig. 1A).

Plant-focused studies with a G x E framework generally test microbiome effects on plant performance or stress tolerance not only for one narrow type of experimental set-up but across a range of different environments and/or multiple plant genotypes (Fig. 1B). For instance, lab experiments can study the beneficial or pathogenic effects of different microbial inoculates under several, controlled levels of resource availability or abiotic stress (Fitzpatrick et al., 2019). For more realistic tests, field experiments can plant seedlings inoculated with different microbial communities into a range of natural habitats. Depending on one's perspective and strategy of data analysis, these approaches will examine how microbiome effects on plants are modulated by environmental influences or – an equally important perspective – how plant responses to the environment (phenotypic plasticity; environmental tolerances) are modulated by microbes (Fig.

1D). Finally, the ‘environment’ component in such experiments may also include additional biotic factors, such as competitors (Fitzpatrick et al., 2019), herbivores or other (background) microbiota, which will allow for testing microbe-microbe interactions or other complex biotic interactions.

Conclusions

The study of plant microbiomes is an important research frontier in current plant biology, with many open questions, particularly from an ecological-evolutionary (Koskella et al., 2017; Fitzpatrick et al., 2020) and agricultural perspective (Toju et al., 2018). Irrespective of whether their focus is on the plant or the microbiome, plant microbiome studies are challenged by the complexity of their subject. Plant microbiome studies that are too simple may therefore overlook important interactions between different factors, and they run the risk of overestimating or overgeneralizing their results. A more thorough understanding of plant microbiomes will require not only working with a broader range of plant genotypes and non-model species, but also to take a G x E perspective and explicitly test the generality of plant-microbiome interactions across multiple, and interacting, drivers.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (DFG; BO 3241/10-1 and BO 3241/11-1) as part of the priority programme 2125 Deconstruction and Reconstruction of the Plant Microbiota “DECRyPT”.

Author contributions

All authors jointly developed the ideas in this essay and contributed to writing the manuscript.

Literature Cited

Agler, M. T., J. Ruhe, S. Kroll, C. Morhenn, S.-T. Kim, D. Weigel, and E. M. Kemen. 2016. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biology* 14: e1002352.

Belimov, A. A., A. I. Shaposhnikov, D. S. Syrova, A. A. Kichko, P. V. Guro, O. S. Yuzikhin, T. S. Azarova, et al. 2020. The role of symbiotic microorganisms, nutrient uptake and rhizosphere bacterial community in response of pea (Pisum sativum L.) genotypes to elevated Al concentrations in soil. *Plants* 9.

Berendsen, R. L., G. Vismans, K. Yu, Y. Song, R. de Jonge, W. P. Burgman, M. Burmølle, et al. 2018. Disease-induced assemblage of a plant-beneficial bacterial consortium. *The ISME Journal* 12: 1496–1507.

Bergelson, J., J. Mittelstrass, and M. W. Horton. 2019. Characterizing both bacteria and fungi improves understanding of the Arabidopsis root microbiome. *Scientific Reports* 9: 24.

Berg, M., and B. Koskella. 2018. Nutrient- and dose-dependent microbiome-mediated protection against a plant pathogen. *Current biology: CB* 28: 2487–2492.e3.

Bowen, J. L., P. J. Kearns, J. E. K. Byrnes, S. Wigginton, W. J. Allen, M. Greenwood, K. Tran, et al. 2017. Lineage overwhelms environmental conditions in determining rhizosphere bacterial community structure in a cosmopolitan invasive plant. *Nature Communications* 8: 433.

Chaparro, J. M., D. V. Badri, and J. M. Vivanco. 2014. Rhizosphere microbiome assemblage is affected by plant development. *The ISME Journal* 8: 790–803.

Fitzpatrick, C. R., J. Copeland, P. W. Wang, D. S. Guttman, P. M. Kotanen, and M. T. J. Johnson. 2018. Assembly and ecological function of the root microbiome across angiosperm plant species. *Proceedings of the National Academy of Sciences of the United States of America* 115: E1157–E1165.

Fitzpatrick, C. R., Z. Mustafa, and J. Viliunas. 2019. Soil microbes alter plant fitness under competition and drought. *Journal of Evolutionary Biology* 32: 438–450.

Fitzpatrick, C. R., I. Salas-González, J. M. Conway, O. M. Finkel, S. Gilbert, D. Russ, P. J. P. L. Teixeira, and J. L. Dangl. 2020. The plant microbiome: from ecology to reductionism and beyond. *Annual Review of Microbiology* 74: 81–100.

Hu, L., C. A. M. Robert, S. Cadot, X. Zhang, M. Ye, B. Li, D. Manzo, et al. 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications* 9: 2738.

Koskella, B., L. J. Hall, and C. J. E. Metcalf. 2017. The microbiome beyond the horizon of ecological and evolutionary theory. *Nature Ecology & Evolution* 1: 1606–1615.

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

Panke-Buisse, K., A. C. Poole, J. K. Goodrich, R. E. Ley, and J. Kao-Kniffin. 2015. Selection on soil microbiomes reveals reproducible impacts on plant function. *The ISME journal* 9: 980–989.

Pieterse, C. M. J., C. Zamioudis, R. L. Berendsen, D. M. Weller, S. C. M. Van Wees, and P. A. H. M. Bakker. 2014. Induced systemic resistance by beneficial microbes. *Annual review of phytopathology* 52: 347–375.

Rodriguez, R. J., J. Henson, E. Van Volkenburgh, M. Hoy, L. Wright, F. Beckwith, Y.-O. Kim, and R. S. Redman. 2008. Stress tolerance in plants via habitat-adapted symbiosis. *The ISME Journal* 2: 404–416.

Sasse, J., E. Martinoia, and T. Northen. 2018. Feed your friends: do plant exudates shape the root microbiome? *Trends in Plant Science* 23: 25–41.

Sultan, S. E. 2000. Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science* 5: 537–542.

Toju, H., K. G. Peay, M. Yamamichi, K. Narisawa, K. Hiruma, K. Naito, S. Fukuda, et al. 2018. Core microbiomes for sustainable agroecosystems. *Nature Plants* 4: 247–257.

Wagner, M. R., D. S. Lundberg, T. G. Del Rio, S. G. Tringe, J. L. Dangl, and T. Mitchell-Olds. 2016. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nature Communications* 7: 12151.

Figure 1. Two main perspectives in plant microbiome research, and reaction norm plots illustrating possible G x E interactions tested in each. With a microbiome perspective, researchers usually study community-level characteristics of the microbiome, and test effects of plant genotype or other plant characteristics (P1/P2) and environmental conditions (E1/E2). With a plant perspective, the dependent variables are measures of plant performance, and experiments test influences of microbiomes (M1/M2), environmental conditions, and their interactions. The dashed lines in the upper graphs indicate indirect effects where some drivers of plant or microbiome variation alter plant or microbiome responses to others. Note that while the bottom graphs display only categorical variables, the G x E framework can be equally applied to continuous explanatory variables.

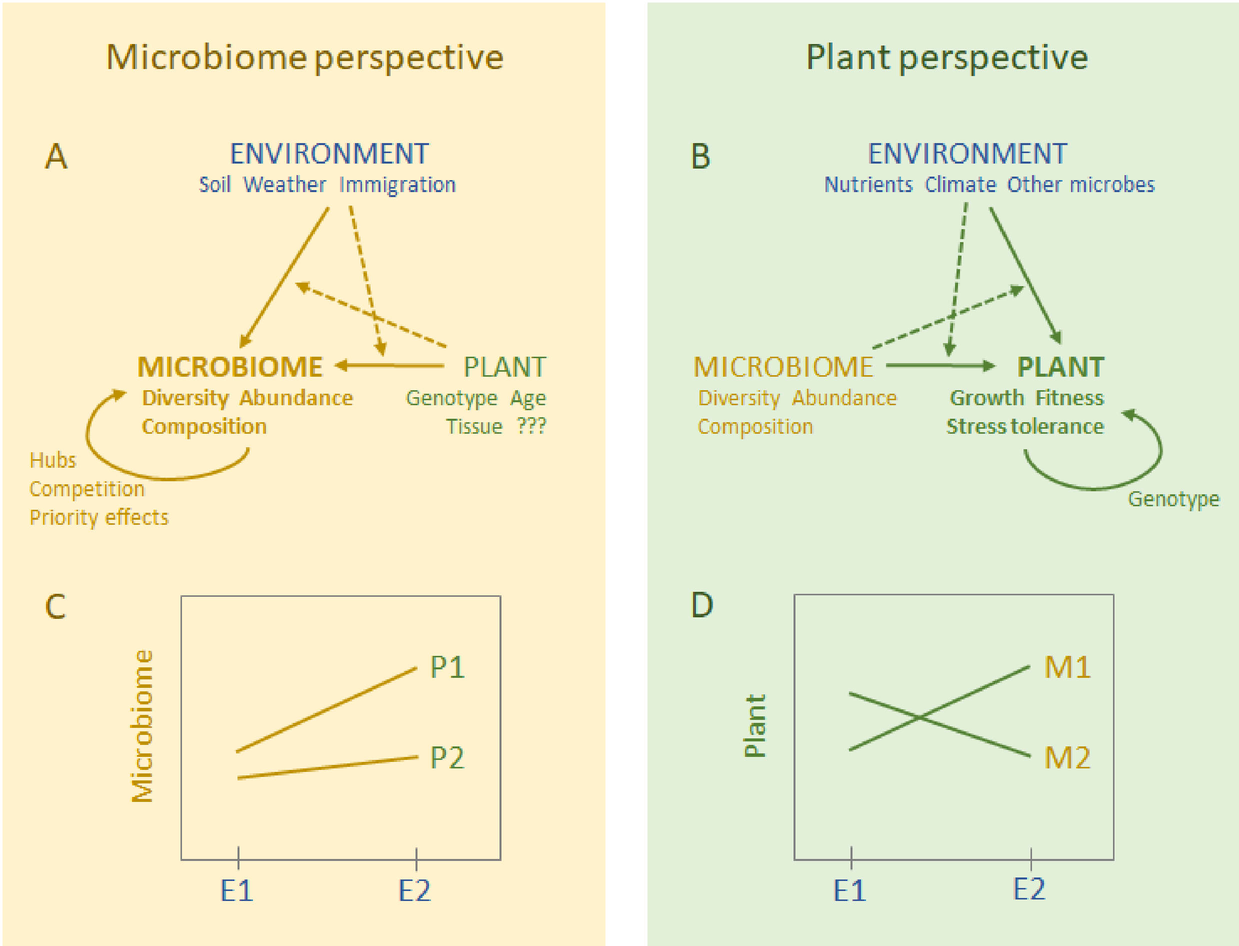

Figure 1. Two main perspectives in plant microbiome research, and reaction norm plots illustrating possible G x E interactions tested in each. With a microbiome perspective, researchers usually study community-level characteristics of the microbiome, and test effects of plant genotype or other plant characteristics (P1/P2) and environmental conditions (E1/E2). With a plant perspective, the dependent variables are measures of plant performance, and experiments test influences of microbiomes (M1/M2), environmental conditions, and their interactions. The dashed lines in the upper graphs indicate indirect effects where some drivers of plant or microbiome variation alter plant or microbiome responses to others. Note that while the bottom graphs display only categorical variables, the G x E framework can be equally applied to continuous explanatory variables.