

## Commentary

# Disentangling the factors shaping microbiota composition across the plant holobiont

## Introduction

Healthy and asymptomatic plants in nature are colonized by a rich diversity of microbes comprising bacteria, fungi, protists and viruses (i.e. the plant microbiota), forming complex microbial consortia that impact plant growth and productivity. Consequently, plants must not be viewed as autonomous entities but rather as holobionts (a macrobe and its numerous microbial associates), within which all interacting organisms contribute to the overall stability of the system (Vandenkoornhuysen *et al.*, 2015). More than a century ago, Hiltner hypothesized that the resistance of plants towards pathogenesis is dependent on the composition of plant microflora and that root exudates of different plants could support development of different microbial communities (Hartmann *et al.*, 2008). The development of next generation sequencing technologies and associated computational analytical tools now allows the detailed investigation of these important concepts (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012). However, despite the fact that the plant microbiota research field shows exponential growth (Fig. 1), most of the studies published so far have focused on one particular microbial kingdom and/or specific host niches. There is consequently a need for a more holistic understanding of the microbial communities associated with different plant compartments and discerning which factors shape these microbial assemblages across the plant holobiont. In this issue of *New Phytologist*, Coleman-Derr *et al.* (pp. 798–811) provide a comprehensive analysis of the structure of both fungal and bacterial communities in the rhizosphere, phyllosphere, leaf and root endosphere, as well as proximal and distal soil samples from cultivated and native agaves. Since agaves spp. are adapted to nutrient-poor environments, extreme drought and elevated temperatures, these plants represent important models for the plant microbiota research field because they are likely to host an important reservoir of beneficial microbes that may support their survival.

## Structural convergence of the bacterial microbiota of plants

It is now well established that root colonization by soil bacteria is a deterministic and tightly controlled process involving different selective steps. Edaphic factors determine the start inoculum of the soil biome whereas rhizodeposits and host genotype mediate bacterial community shifts from soil communities to host-adapted communities with reduced diversity (Bulgarelli *et al.*, 2013). Therefore, the roots of phylogenetically unrelated plant species

assemble overall structurally related bacterial communities belonging to only four major bacterial phyla (Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes), with Proteobacteria and Acidobacteria community members being consistently enriched and depleted, respectively, in plant roots compared to their surrounding soil biome (Hacquard *et al.*, 2015). Importantly, Coleman-Derr *et al.* report here that bacterial communities associated with agave plants growing in arid environments also share this taxonomic signature, indicating that the selective forces shaping root microbiota composition at a high taxonomic rank are robust against a wide range of host plants and environmental conditions. In contrast with the bacterial microbiota of plant roots for which the start inoculum can be easily defined, it is assumed that leaf-associated bacterial communities are more subject to stochastic variations due to the multiple and more complex inoculum sources (Maignien *et al.*, 2014). Consistent with this, Coleman-Derr *et al.* show that leaf endosphere communities show higher variability across seasons than the root endosphere communities. Nonetheless, they could identify core prokaryotic taxa that are shared between endophytic compartments, including microbiota members belonging to Actinobacteria, Bacilli, Alpha-, Beta- and Gamma-proteobacteria that may confer plant fitness benefits during the dry season. Remarkably, despite being harvested 2000 km apart in their respective natural habitats, agave-associated bacterial communities were more impacted by plant compartment than by biogeography of the host (Fig. 2a). Their results suggest that geographically distant bacterial inputs can converge into an overall reproducible taxonomic structure in each agave compartment. It is nevertheless important to note that a substantial fraction of the bacterial, but also the fungal communities, is shared between compartments, raising the possibility of extensive reciprocal relocation of microbiota members between belowground and aboveground plant tissues.

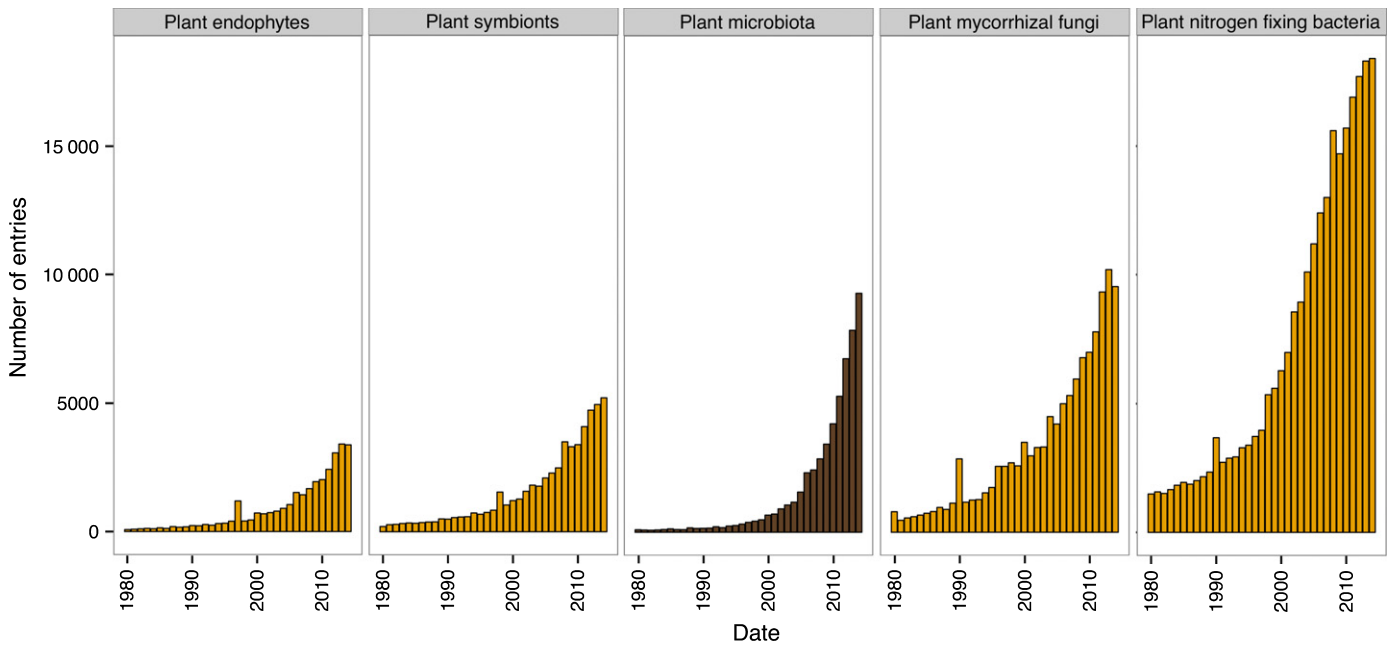
---

*‘Remarkably, despite being harvested 2000 km apart in their respective natural habitats, agave-associated bacterial communities were more impacted by plant compartment than by biogeography of the host.’*

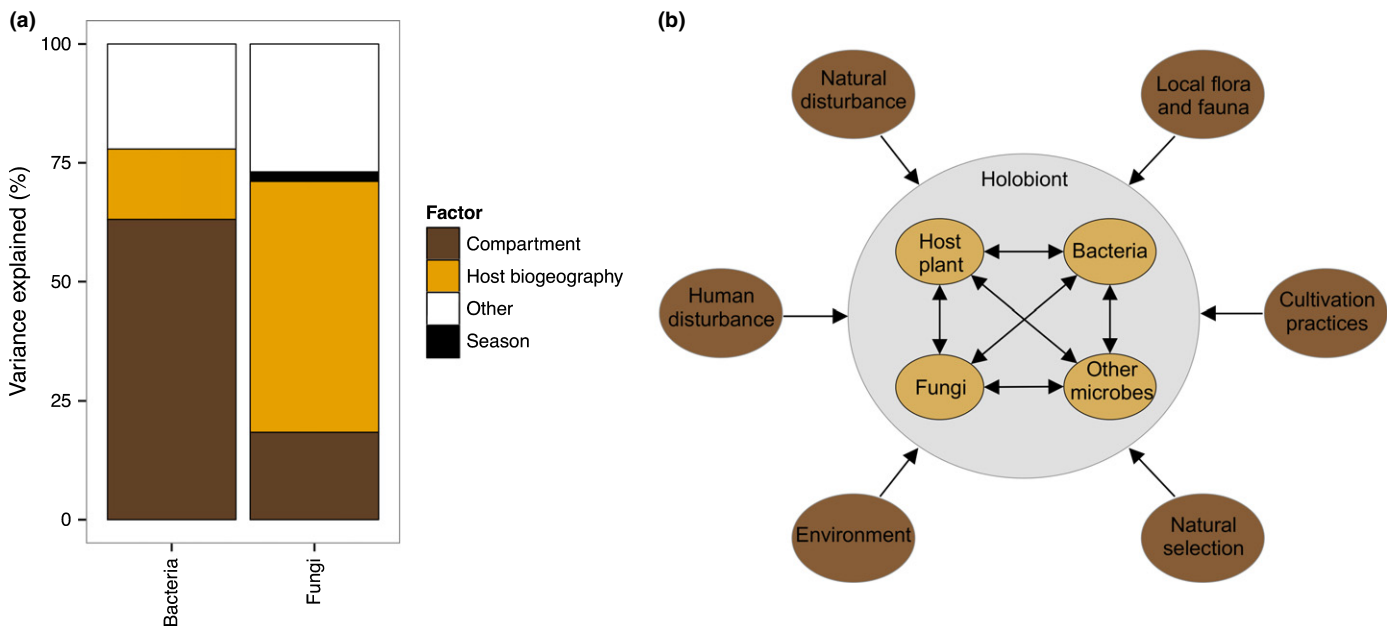
---

## Distinct factors shape fungal and bacterial assemblages on plants

Even though less attention has been given to the fungal microbiota of plants, fungal communities appear to be hyper-diverse in both



**Fig. 1** Exponential increase of the plant microbiota research field. The total number of entries corresponding to the different keywords was evaluated for the time period 1980–2014 using Google Scholar.



**Fig. 2** Factors influencing microbial community establishment across the plant holobiont. (a) Percentage of the variance explained by the factors ‘host biogeography’, ‘compartment’ and ‘season’ for both bacterial and fungal communities associated with agave plants. The results that are presented were adapted from Coleman-Derr *et al.* (this issue of *New Phytologist*, pp. 798–811). (b) Schematic representation of the plant holobiont (grey), the multispecies interactions network within the plant holobiont (orange) and the external factors that may impact its stability (brown).

aboveground and belowground plant tissues, and are dominated by two major phyla (Ascomycota and Basidiomycota). However, in contrast with the bacterial microbiota of plant roots, the fungal communities seem to be subject to greater variations that are known to be highly dependant on biogeography, host species and plant compartment, but which may also include a non-negligible stochastic component (Shakya *et al.*, 2013). One key aspect in the experimental design used by Coleman-Derr *et al.* was to analyse

agave communities from distant geographical sites to evaluate whether biogeography of the host similarly impacts fungal and bacterial assemblages. Overall, they found that the major factor driving fungal assemblages in agave plants is the geographic origin of the host, contrasting with bacterial assemblages that are primarily sculpted by plant compartment (Fig. 2a). Their findings indicate that distinct factors shape fungal and bacterial assemblages on plants and also confirm that fungal and bacterial biogeography may

differ fundamentally on large scales. Unlike bacteria, but similar to plants and animals, distribution of fungi might be primarily constrained by climate and dispersal limitation, which may favour higher endemism in fungal compared to bacterial populations (Bonito *et al.*, 2014; Talbot *et al.*, 2014). This is consistent with the positive correlation observed between plant and fungal richness, suggesting these two kingdoms respond similarly to climatic and edaphic variables (Tedersoo *et al.*, 2014). It is likely that habitat-specific features drive assemblages of distinct plant-associated fungal communities with high levels of functional redundancies across sites (i.e. conferring plant fitness benefits against a wide range of stresses) but also non-negligible site-specific functional capabilities (i.e. conferring plant fitness benefits against local stresses).

### Cultivation practices and microbial community disturbance

Since the colonization of land by ancestral plant lineages 450 million years ago, plants and microbes have interacted with each other (Field *et al.*, 2015). This long co-evolutionary process has likely shaped plant-associated microbial assemblages and selected for beneficial interactions across the plant holobiont. One fundamental question in plant microbiota research is whether cultivation practices can destabilize indigenous and locally adapted microbial communities and whether disturbance can modify prevalence of a particular disease (Fig. 2b). In humans, it has been postulated that hygiene measures aimed at reducing the microbial load in our environments may have instead favoured modern allergic and metabolic diseases, likely due to the loss of our ancestral commensal microbes (Blaser & Falkow, 2009). Although the number of sampling sites selected by Coleman-Derr *et al.* was not sufficient to draw a general principle regarding how cultivation practices alter microbial community establishment, they found that cultivated agave harbour less prokaryotic diversity than native agave in the rhizosphere and phyllosphere compartments. This loss of prokaryotic diversity was largely explained by the dominance of some bacterial genera belonging to the family *Enterobacteriaceae*, among which some members are well known pathogens of agave causing 'soft rot' disease. The findings of Coleman-Derr *et al.* are consistent with the idea that agricultural practices that include continuous monoculture, the utilization of germ-free seedlings and homogenous genetic plant material may perturb heritability of native microbial consortia and favour emergence of latent pathogens (Santhanam *et al.*, 2015).

### The microbiota of the plant holobiont: what's next?

Although the structure of both fungal and bacterial communities in the leaves and roots of several plant species has been elucidated, there is still a lack of knowledge regarding how multi-organismal interactions shape microbiota composition across the plant holobiont (Hacquard & Schadt, 2015). More particularly, it remains unclear how competition and cooperation among microbes or between different microbial kingdoms influence microbial community establishment. It is likely that plant-associated microbiota members have evolved sophisticated

strategies to interact with each other within complex microbial consortia in order to persist in particular host niches. Understanding plants as holobionts therefore implies careful investigation of these often neglected microbe–microbe interactions (Fig. 2b). Disentangling the interplay between host, microbial and environmental factors is also needed to better understand microbiota niche specialization and adaptation to a specific plant compartment. Large-scale isolation and establishment of reference culture collections of plant-associated microbiota members is a prerequisite for addressing these important concepts and represents a promising way for reconstituting complex microbial ecosystems *in vitro* with germ-free plants (Lebeis *et al.*, 2015). These reductionist approaches using synthetic microbial communities will have profound implications for the plant microbiota research field, allowing a transition from correlation- to causation-based studies.

### Acknowledgements

The author would like to thank Francis Martin for giving him the opportunity to write this commentary, Paul Schulze-Lefert for his continuous support and scientific advice as well as William Chanz Robbins, Stijn Spaepen and Paloma Durán for their critical comments regarding this commentary. The author would also like to thank the funding sources: the Max-Planck Society and the European Research Council.

**Stéphane Hacquard**

Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany (tel +49 221 5062 322; email hacquard@mpipz.mpg.de)

### References

- Blaser MJ, Falkow S. 2009. What are the consequences of the disappearing human microbiota? *Nature Review Microbiology* 7: 887–894.
- Bonito G, Reynolds H, Robeson MS, Nelson J, Hodkinson BP, Tuskan G, Schadt CW, Vilgalys R. 2014. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. *Molecular Ecology* 23: 3356–3370.
- Bulgarelli D, Rott M, Schlaeppi K, Loren Ver, van Themaat E, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R *et al.* 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature* 488: 91–95.
- Bulgarelli D, Schlaeppi K, Spaepen S, Loren Ver, van Themaat E, Schulze-Lefert P. 2013. Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology* 64: 807–838.
- Coleman-Derr D, Desgarenes D, Fonseca-García C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez LP, Tringe SG. 2016. Plant compartment and biogeography affect microbiome composition in cultivated and native *Agave* species. *New Phytologist* 209: 798–811.
- Field KJ, Pressel S, Duckett JG, Rimington WR, Bidartondo MI. 2015. Symbiotic options for the conquest of land. *Trends Ecology & Evolution* 30: 477–486.
- Hacquard S, Garrido-Oter R, González A, Spaepen S, Ackermann G, Lebeis S, McHardy AC, Dangl JL, Knight R, Ley R *et al.* 2015. Microbiota and host nutrition across plant and animal kingdoms. *Cell Host & Microbe* 17: 603–616.
- Hacquard S, Schadt CW. 2015. Towards a holistic understanding of the beneficial interactions across the *Populus* microbiome. *New Phytologist* 205: 1424–1430.

- Hartmann A, Rothballer M, Schmid M. 2008. Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant and Soil* 312: 7–14.
- Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, Malfatti S, Glavina del Rio T, Jones CD, Tringe SG *et al.* 2015. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science* 349: 860–864.
- Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrekton A, Kunin V, del Rio TG *et al.* 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature* 488: 86–90.
- Maignien L, DeForce EA, Chafee ME, Eren AM, Simmons SL. 2014. Ecological succession and stochastic variation in the assembly of *Arabidopsis thaliana* phyllosphere communities. *MBio* 5: e00682–13.
- Santhanam R, Luu VT, Weinhold A, Goldberg J, Oh Y, Baldwin IT. 2015. Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping. *Proceedings of the National Academy of Sciences, USA* 112: E5013–E5020.
- Shakya M, Gottel N, Castro H, Yang ZK, Gunter L, Labbé J, Muchero W, Bonito G, Vilgalys R, Tuskan G *et al.* 2013. A multifactor analysis of fungal and bacterial community structure in the root microbiome of mature *Populus deltoides* trees. *PLoS ONE* 8: e76382.
- Talbot JM, Bruns TD, Taylor JW, Smith DP, Branco S, Glassman SI, Erlandson S, Vilgalys R, Liao HL, Smith ME *et al.* 2014. Endemism and functional convergence across the North American soil mycobiome. *Proceedings of the National Academy of Sciences, USA* 111: 6341–6346.
- Tedersoo L, Bahram M, Pölme S, Kõljalg U, Yorou NS, Wijesundera R, Villarreal Ruiz L, Vasco-Palacios AM, Thu PQ, Suija A *et al.* 2014. Global diversity and geography of soil fungi. *Science* 346: 1256688.
- Vandenkoornhuysse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. 2015. The importance of the microbiome of the plant holobiont. *New Phytologist* 206: 1196–1206.

**Key words:** bacterial community, fungal community, microbiome, phyllosphere, plant holobiont, plant–microbe interaction, rhizosphere.



## About New Phytologist

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <27 days. There are **no page or colour charges** and a PDF version will be provided for each article.
- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.
- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)
- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**