

# Chapter 9

## Plant Microbiota: Diversity, Transmission and Function



Matthieu Barret, Marc Buée, Christophe Mougel, and Corinne Vacher

### 9.1 Introduction

Plants host a wide array of viruses and microorganisms (eubacteria, archaea, filamentous fungi, oomycetes, protists and nematodes) inside or on the surface of their various organ tissues (Leach et al. 2017). These microorganisms form the plant microbiota. The interactions between a plant and its microbiota can impact the plant's development and its adaptation to abiotic and biotic constraints. Understanding the biological and ecological processes involved in microbiota assembly and dynamics during plant development is vital to implementing strategies to control which microorganisms develop.

### 9.2 Microbial Diversity According to Habitats

The bacterial and fungal taxa present in the plant microbiota have been estimated in many annual and perennial plant species (Muller et al. 2016). These studies revealed the presence of four major bacterial phyla: Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. In lower taxonomic levels, such as family or genus, differences

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M. Barret (✉)

IRHS. Univ Angers, Institut Agro, INRAE, SFR QUASAV, Angers, France  
e-mail: [matthieu.barret@inrae.fr](mailto:matthieu.barret@inrae.fr)

M. Buée

IAM. INRAE, Univ Lorraine, Champenoux, France

C. Mougel

IGEPP. INRAE, Institut Agro, Univ Rennes, Le Rheu, France

C. Vacher

BIOGECO. INRAE, Univ Bordeaux, Pessac, France

in microbial communities have been observed depending on the plant organ, species and environment. For example, differences were observed for fungal communities that are dependent on tree leaves or roots (e.g. Counce et al. 2014). Such differences have also been observed between rhizosphere- and phyllosphere-associated bacterial communities (e.g. Wagner et al. 2016). While some bacterial taxa are preferentially associated with the aboveground or belowground plant components, others colonize both habitats indiscriminately and can therefore be considered generalists (Bai et al. 2015).

Historically, the soil has been considered the major source of plant microorganisms; as such, the rhizosphere has been the most studied plant compartment due to its important relationship with the surrounding soil. Plant root exudation of carbon-based compounds will induce the development of large bacterial and fungal populations ( $10^8$ – $10^{10}$  bacteria and  $10^5$ – $10^6$  fungi per gram of rhizosphere) with a high level of taxonomic diversity (Egamberdieva et al. 2008; Bulgarelli et al. 2013). Some of these microorganism populations will interact more closely with the plant when they are on the surface (epiphytes) or inside the roots (endophytes). Root endophytes colonize healthy plant roots, but do not form complex structures or specialized interfaces with the host to exchange nutrients, unlike what is observed in mycorrhizal fungi (Brundrett 2006). Endophytes are widely found in plant roots, but knowledge about their diversity and ecological functions is lacking. Since nearly 90% of plant species are colonized by mycorrhizal fungi, endophytes commonly coexist in the roots with mycorrhizal fungi as well as with other microbial groups, potentially improving plant fitness (Porrás-Alfaro and Bayman 2011). Dark septate endophytes (DSE) – highly melanized fungi – have been observed in roots colonized by arbuscular and ectomycorrhizal mycorrhizal fungi (Bonito et al. 2016). Under controlled conditions, dark septate endophytes can increase root biomass.

The aerial parts of plants are also colonized by numerous microorganisms. The phyllosphere is thought to be home to around  $10^6$ – $10^7$  bacteria per square centimetre of leaf area (Vorholt 2012). Epiphytic microorganisms living on the leaf surface come from several inoculum sources such as aerosols, precipitation, insects, surrounding vegetation and the soil (Vacher et al. 2016). Unlike in the rhizosphere, nutrient resources on the leaf surface are limited. For example, some bacteria associated with the genus *Methylobacterium* can use the methane or methanol produced by the leaves as a main source of carbon (Vorholt 2012). Additionally, the phyllosphere microbial populations are also subjected to intense UV radiation and develop pigment systems for protection (Ibid.). While the influence of the leaf epiphytic microbiota in the host physiology needs to be clarified, its role in protecting plants against leaf diseases and the mechanisms involved are increasingly understood (Hacquard et al. 2017).

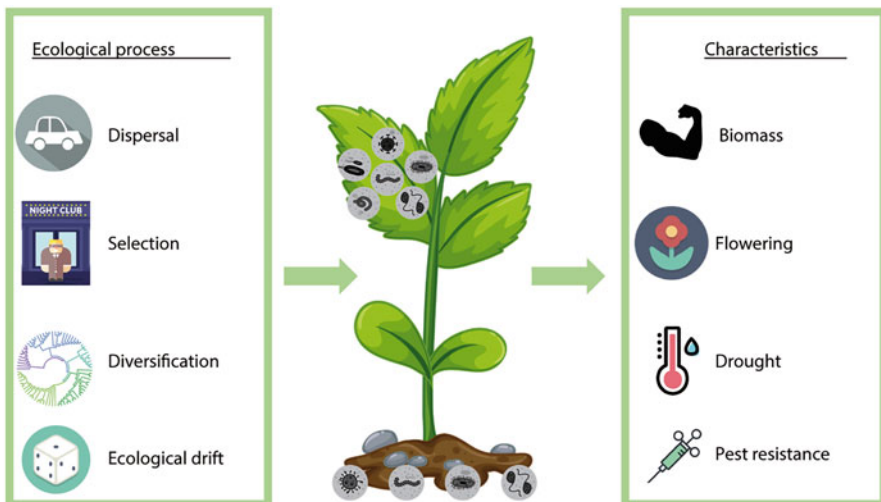
Initial work on the plant microbiota taxa mainly focused on the phyllosphere and rhizosphere microbial communities during the vegetative phase of plant growth. More recently, other habitats associated with the reproductive organs of plants that reproduce sexually, such as flowers (anthosphere), fruits (carposphere) and dry or germinated seeds (in this latter case, the spermosphere), have also been analysed (Nelson 2018; Shade et al. 2017). While these habitats generally have less microbial

diversity than the phyllosphere and rhizosphere, the main bacterial and fungal phyla are found within these compartments. Thus, it is likely that part of the plant microbiota is inherited via vertical transmission. While the percentage of taxa transmitted vertically is undoubtedly smaller than that transmitted horizontally by the local habitat, the seed microbiota is the primary inoculum source for the plant microbiota and can thus contribute to the early stages of its development, especially during germination (Shade et al. 2017).

### 9.3 Microbiota Assembly and Transmission Processes

The ecological processes involved in microbial community assembly can be grouped into four distinct categories (Fig. 9.1): (i) selection, (ii) diversification, (iii) dispersal and (iv) ecological drift (Nemergut et al. 2013).

Selection is generally considered to be the main ecological process that modulates microbial community assembly and is therefore the most studied process. It refers to changes in microbiota composition caused by a difference in fitness between microorganisms. The fittest microorganisms on a given host plant and in a given environment are those that are favoured by selection and increase proportionally in the microbiota. Differences in fitness may result from interactions between the host and its microorganisms or be related to environmental parameters. For example, soil type, farming practices and climate are essential components of the rhizosphere microbiota composition (Philippot et al. 2013). Other studies have shown a significant effect of the host genotype in both the rhizosphere microbiota (Walters et al. 2018) and phyllosphere composition (Horton et al. 2014). This impact of the host



**Fig. 9.1** Assembly of the plant’s microbiota and impact on host fitness

genotype on the selection of certain microbial taxa can be explained in part by the quality and quantity of carbon-based compounds exuded by the roots or by the plant's immune response (Hacquard et al. 2017).

New genetic variations by mutation, homologous recombination or horizontal gene transfer is similar to evolutionary diversification. Depending on the selective pressures exerted by the host and the environment on the microbial assemblage, this diversification can lead to the emergence of new species. This process could have a major impact on microbial community assembly because, unlike with macroorganisms, microorganisms have a short generation time and can therefore evolve rapidly. However, the impact of diversification within microbial communities remains difficult to assess. Evolution experiments, consisting of repeated successive inoculations of microorganisms on plants over several generations, have nevertheless made it possible to identify adaptive mutations in certain plant-associated bacteria (Guidot et al. 2014).

Through the combination of evolutionary diversification and selection, each microbial species acquires various traits that help it adapt to a particular habitat and occupy a unique ecological niche. In contrast to the ecological niche theory, the neutral theory of biodiversity postulates that all members of a microbial community, whatever their traits, have the same fitness in the habitat being considered. According to this theory, the distribution of community members is mainly due to dispersal and ecological drift. Microbial dispersal refers to the movement of microorganisms from an original habitat to a new habitat. Unlike for macroorganisms, the dispersal capacity of microorganisms has not been widely studied. This lack of data on microorganisms can be explained by the simple fact that it is difficult to quantify their dispersal due to their size, rapid generation time and high number of individuals. Thus, in microorganisms, spatial distribution is used to estimate dispersal. For example, differences in the abundance of microbial taxa in the phyllosphere can be partly explained by dispersal (Maignien et al. 2014). Similarly, the fungal taxa of the seed microbiota also appears to be impacted by dispersal (Rezki et al. 2018). Microbial dispersal is a predominantly passive process, mediated by phenomena such as spores being carried by wind or air. The influence of propagule dispersal has been studied more comprehensively in mycorrhizal fungi macromycetes, which form spore-bearing fruiting bodies called sporophores. In these microorganisms, the number of spores produced per species is one of the key factors involved in the composition of plant-associated communities (Peay et al. 2012).

The last of the four ecological processes mentioned, ecological drift, refers to stochastic, i.e. random, changes in relative species abundance. Ecological drift can even lead some organisms within a community to become extinct over time (Nemergut et al. 2013). This process plays a major role in community assembly when selection is weak and species richness, i.e. the number of species, is low. Microbial taxa with low relative abundance are more sensitive to the stochastic effects of ecological drift, which can drive them to extinction. It seems that the composition of the bacterial communities of seeds (Rezki et al. 2018) and the phyllosphere (Maignien et al. 2014) are partly influenced by ecological drift.

## 9.4 Impact of the Plant Microbiota on Host Fitness

Groups of plant-associated microorganisms can modify many of a host's phenotypic traits by stimulating the plant's growth or influencing its adaptation to abiotic and biotic constraints.

It is accepted that plant growth is strongly related to the biotic characteristics of the soil in which they grow. For example, differences in the biomass of the aerial parts of *Arabidopsis thaliana* have been associated with changes in the composition of soil bacterial communities (Sugiyama et al. 2013). However, it is difficult to separate the effects due to microbial activity from those related to the soil's physicochemical properties in this phenotypic modification. Another trait impacted by plant-microorganism interactions concerns the transport of minerals in the soil. One strategy that plants use to increase their capacity to absorb minerals (e.g. nitrogen or phosphate) is to establish beneficial relationships with soil microorganisms such as nitrogen-fixing bacteria in the Rhizobiales order or mycorrhizal fungi. These bipartite plant-microorganism symbiotic interactions modulate and can be modulated by other members of the plant microbiota (Zgadzaj et al. 2016). Thus, microbial-interactions have a direct impact on mineral accumulation in plant tissue.

Soil microbial communities can also affect plant flower development. For example, differences in the soil microbiota can lead to delayed flowering in *A. thaliana* (Panke-Buisse et al. 2015). Although the microbial taxa and molecular determinants involved in modulating flowering are still unknown, the use of microbial inoculum to modulate the flowering time offers interesting possibilities, such as to prevent flowering too early.

Fluctuations in environmental conditions will impact a plant's physiology, which can in turn induce changes in its microbiota. For example, a drought episode can cause a water deficit in plants. In response to this abiotic stress, a plant will modulate its root architecture as well as the quantity and quality of the exudates produced. These changes in plant physiology will affect the root-associated microbiota (Naylor and Coleman-Derr 2018). During repeated drought episodes, changes in rhizosphere microbiota can improve the host plant's ability to withstand water stress. In a study conducted on *Brassica rapa*, the plants showed greater drought resistance when they were grown in previously dry soils (Lau and Lennon 2012). When these plants were grown in dry soils over several generations, they had a higher bacterial abundance and diversity around their roots compared to plants cultivated in irrigated soils, suggesting these microorganisms had an influence on their water stress tolerance.

Finally, the expression of certain soil-borne or leaf diseases can be modulated by the plant's microbiota. One of the best documented cases concerns suppressive soils. In these soils, disease development is limited by certain microbial assemblages near roots. Examples of pathogens suppressed by such soils include (i) take-all in wheat caused by the fungus *Gaeumannomyces graminis* var. *tritici*, (ii) black root rot in tobacco caused by *Thielaviopsis basicola*, and (iii) damping off of sugar beet seedlings caused by *Rhizoctonia solani* (Weller et al. 2002). The decrease in symptoms shown in these suppressive soils is due to the gradual selection of certain

rhizobacterial populations. Rhizobacteria can inhibit the growth of the pathogen by producing different antimicrobial compounds such as rhamnolipids, lipopeptides or polyketides (see Chap. 12).

Plants can also be protected against plant pathogens indirectly by stimulating the plant immune system by members of root-associated microbial communities, which is known as induced systemic resistance (ISR). ISR will limit or inhibit the penetration of plant pathogens into the plant tissue via a hypersensitive response that results in rapid cell death at the point of infection, thus reducing disease severity. ISR can reduce various leaf and root diseases caused by a range of bacterial and fungal plant pathogens (Pieterse et al. 2014).

The protective activity of plant-associated microbial communities is not limited to the rhizosphere. Indeed, correlations between resistance to biotic stresses and certain changes in the microbiota composition have been observed in the phyllosphere (Ritpitakphong et al. 2016). Resistance to plant pathogens can be induced by a microbial assemblage or a specific microbial population. For example, bacteria related to *Sphingomonas*, a bacterial genus found abundantly in the phyllosphere of several plant species, can significantly reduce the degree of colonization of the phyllosphere of *Arabidopsis thaliana* by *Pseudomonas syringae* pv. *tomato*. While the exact nature of this protective effect observed in *A. thaliana* is still unknown, several mechanisms have been put forward, such as competition for resources between these bacterial populations or the induction of defence reactions in the plant via salicylic or jasmonic acid-dependent pathways (Innerebner et al. 2011).

## 9.5 Leveraging the Microbiota to Improve Plant Growth and Health

For several decades, assessing the plant microbiota composition according to phenotypic changes in the plant has been one of the first steps in finding associations between certain microbial taxa and traits of interest.

Exploiting the plant microbiota to promote the growth and health of its host is one alternative to using plant protection products. To date, several bacterial and fungal strains are already being sold commercially and used as natural control agents capable of reducing some of the effects of plant diseases (see Chap. 11). This is the case of certain fungal strains of the genus *Trichoderma*, which can inhibit plant pathogens in the soil through their antagonistic activities (Hermosa et al. 2012), or arbuscular mycorrhizal fungi, such as *Rhizophagus irregularis*, which promote plant growth in soils with low fertility (Khasa et al. 2009). Although the activity of these commercial strains is satisfactory under controlled or semi-controlled conditions, their effectiveness in situ may prove disappointing. Such variations in effectiveness can be explained by several factors such as environmental fluctuations or farming practices, which may impact the colonization, survival or activity of the inoculated strain (see Chap. 11). It is also likely that interactions between members of the plant

microbiota and the introduced biological control agents are also involved in the successful colonization of the latter.

The impact of the inoculated or selected microorganism on the ecosystem is a fundamental issue in classical biological control (see Chap. 3). One of the best-known examples is the case of a cultivar of tall fescue (*Festuca arundinacea* Schreb). The cultivar, Kentucky 31, was initially selected for its ability to grow in nutrient-poor soils and tolerance to water stress. An endophytic fungus (*Epichloë coenophiala*, Morgan-Jones & W. Gams), transmitted vertically by the plant's seeds, was responsible for the grass being able to adapt to these abiotic stresses (Schardl et al. 2004). Unfortunately, following the mass commercialization of the Kentucky 31 variety, significant ecological and economic damage was caused by this grass-endophyte partnership. The fungus produces several alkaloids that are toxic to animals, and the livestock that fed on the fescue were plagued by various pathologies (Hoveland 1993). This example highlights both the value of identifying the components of the plant microbiota with a view to managed co-selection, as well as the need to control and better understand the underlying effects of this biotic partnership and anticipate undesirable effects.