FLOWER POWER? THE PLANT MICROBIOME AND HUMAN HEALTH ISSUES

GABRIELE BERG, BIRGIT WASSERMANN, EVELINE ADAM,
ALEXANDER MAHNERT, HENRY MÜLLER and TOMISLAV CERNAVA

Institute of Environmental Biotechnology, Graz University of Technology,
Graz, Austria

SUMMARY

The microbiome is crucial for plant growth and health. Recent studies reveal an unexpected microbial diversity and abundance associated with plants. Plant genotype, soil type, climate, geography as well as pest and pathogens were identified as main drivers of the plant microbiota. The plant-associated microbial diversity seems to be impressive but their dimension is not yet understood. During evolution, microbiomes have secretly co-evolved with their host plants. On the other side, within millennia of domestication, crops underwent traceably many different adaptive trends, allowing rapid speciation and divergence that lead to phenotypic and genotypic distinction to their wild ancestors. Together, domestication and intensive agricultural management shifted the crop microbiota. This resulted in diversity loss, which has consequences for human and *one* health issues. The plant microbiome, which is on one hand specifically adapted to the diverse microhabitats of plants – from the seed to the flower – is on the other hand connected to the whole biosphere. First insights reveal inter-connected microbiomes of plants, e.g. with the built environment, food and humans. These connections need to be better understand for sustainable agriculture as well as plant, human and environmental health and functioning.

INTRODUCTION

Plants and their associated microbes have been interacting with each other for a long time, forming assemblages of species that is referred to as a holobiont (*Vandenkoornhuyse* et al., 2016). The plant-associated microbiota has the ability to contribute multiple aspects to the functioning of the plant holobiont, such as (i) germination and growth support, (ii) supply of nutrients and minerals, (iii) resistance against biotic stress factors (pathogen defense), (iv) resistance against abiotic factors, and (v) production of bioactive metabolites involved in multifold interactions (*Berg* et al., 2017). Plants harbour distinct habitat-specific microbial signatures, which are shaped by a long list of abiotic (soil type, climate, geography) and biotic factors (plant genotype, pest and pathogens). *Whipps* et al., (1988) where the first who implemented the microbiome concept for plant-associated microbial communities. They defined "*the microbiome as a characteristic microbial community occupying a reasonably well-defined habitat which has distinct physio-chemical properties. The term thus not only refers to the microorganisms involved but also encompasses their theatre of activity*".

The rhizosphere, which was introduced for the below-ground, root-associated part of the plant by Lorenz Hiltner already in 1901, is one of the best-studied microbiomes at all (*Phillipot* et al., 2013). The phyllosphere microbiome colonize all above-ground organs, which are exposed to the air and permanently changing abiotic factors such as ultraviolet (UV) radiation, temperature and water, and a general low nutrient availability (*Remus-Emsermann* and *Schlechter*, 2018). The phyllosphere can be further subdivided into the caulosphere (stems), phylloplane (leaves), anthosphere (flowers), and carposphere (fruits). Ectophytic microbial communities acting in the interface with soil and air, while endophytic communities represent an intimate core of the plant microbiota and connecting the different plant microhabitats and development stages are of special importance for health issues (*Hardoim* et al., 2015). A reservoir for plant's endophytes is the rhizosphere,

which represents the below-ground interface with the highly diverse soil microbiota (*Berg* et al., 2005). The flower and seed microbiome are both understudied microhabitats of the plant. The spermosphere is the zone surrounding seeds where interactions between the soil, microbial communities and germinating seeds take place (*Schiltz* et al., 2012). This microenvironment links the above and below-ground microbiome of plants.

Despite more than 100 years of research and deep knowledge about single plant microbiomes, less is known about transmission and interactions of the plant microbiomes. Therefore, this review will summarize our work about microbiome internal and external interactions – within the plants themselves and with its environment in context with the state of current knowledge. Moreover, these interactions will be discussed in view of plant and human health issues.

INTERNAL RELATIONSHIPS: THE MICROBIOME DURING PLANT'S LIFE CYCLE

The seed microbiome and germination

Plant's life cycles are connected with sexual reproduction and characterized by two distinct stages in their life cycle: the gametophyte stage (haploid) and the sporophyte stage (diploid). The haploid gametophyte produces the male and female gametes by mitosis in distinct multicellular structures, after fusion of the diploid zygote develops into the sporophyte. After reaching maturity, the diploid sporophyte produces spores by meiosis, which in turn divide by mitosis to produce the haploid gametophyte. The new gametophyte produces gametes, and the cycle continues. Interestingly, the life cycle of higher

plants is dominated by the sporophyte stage, with the gametophyte borne on the sporophyte while in mosses, the haploid gametophyte is more developed than the sporophyte. For a long time, it was assumed that plants recruit the majority of microorganisms from surrounding soil. Recently it was discovered that all plants transmit a core microbiome from one generation to the other, which is strongly specific for each plant genotype (*Berg* and *Raaijmakers,* 2018).

Mosses, which were the first land plants and which in the past formed the main vegetation, have a long time of co-evolution with their associated microbiobta. This resulted in a highly

specific and specialized microbiome. For example, *Sphagnum* species have, independently of their origin (Norway, Russia, Germany, Austria) a highly similar microbiome (*Opelt* et al., 2007; *Bragina* et al., 2013, 2014). To explain this similarity, the gametophyte as well as the sporophyte was studied. Inside of the sporophyte, a well-defined microbial community was identified, which colonized the gametophyte presenting the moss plant (*Bragina* et al., 2012). Detailed studies showed that specific microorganisms are essential for their germination procedure (*Hornschuh* and *Kutschera,* 2001). Seeds of plants from natural ecosystems are less studied than crop seeds. They have to feature high adaptations in dispersal, persistence and germinative ability under diverse environmental conditions (*Fenner* and *Thompson*, 2005). Seeds and seedlings are exposed to a range of hazards like drought, resource limitation, herbivores and eukaryotic or prokaryotic pathogens (*Bever* et al., 2015). We studied seeds of native alpine plant species growing together for centuries under the same environmental conditions in an Alpine meadow (Hochschwab, Austria). They showed highly unique microbiome signatures and an exceptionally small core microbiome (*Wassermann* et al., 2019). The seeds harboured a unique composition of bacteria, arachea and fungi in abundances with approximately 10^{10} gene copy numbers per gram seed. The plant genotype was clearly identified as the main driver, while different life styles had less, and seed morphology no significant impact.

There exist a lot of knowledge on seed-borne pathogens as well as international surveillance system (ISTA, International Seed Testing Association, www.seedtest.org). In the last decade, crop seeds have been studied as source transmitting a plant-specific core microbiota with neutral or beneficial plant-interaction (*Johnston-Monje* et al., 2011, 2016; *Adam* et al., 2016; *Glassner* et al., 2018; *Gloria* et al., 2018). All of these studies reveal also an unexpectedly high diversity and abundance of the seed-associated microbiota (*Berg* and *Raijmakers*, 2018). They showed up to 20,000 microbial species and up to two billion of bacterial cells in one seed. In general, the seed microbiota consists of bacteria, archaea and fungi. The presented studies focus also on the main drivers of the seed microbiota. Some recent studies on seed-associated microbiomes describe microbial compositions to vary between different geographical sites (*Klaedtke* et al., 2016), and soil types and microbiomes (*Hardoim* et al., 2012; *Liu* et al., 2013). In addition, chemical and microbial seed treatments shaped the microbiota (*Mormile*, 2016; *Rezki* et al., 2016). However, a plant genotype and even cultivar specificity of the seed microbiome has been described frequently (*Barett* et al., 2015; *Adam* et al., 2016; *Rybakova* et al., 2017; *Wassermann*, et al., 2019). Seed endophytes can even be highly conserved across generations of a plant species (*Links* et al., 2014). Besides the horizontal transfer of microbiota from diverse environmental sources, thus, vertical transfer of microbiota to the next generation via seeds plays a key role in adjusting the seed microbiome (*Truyens* et al., 2014).

Assembly and stability of the microbiome in mature plants
Colonization of emerging seedling is controlled by the plant through different strategies, such as the specific profile of root exudates and its immune system (*Doornbos* et al., 2012; *Truyens* et al., 2014; *Sánchez-Cañizares* et al., 2017). Microorganism's from seeds and soil, both colonize the plant
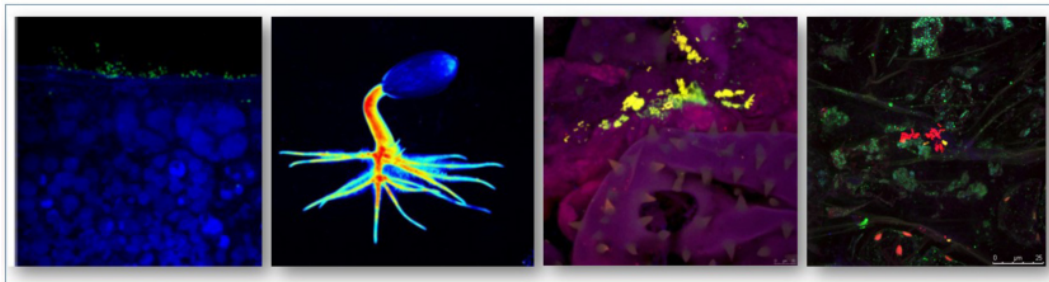
Figure 1: Vizualization of bacterial communities in pumkin microenvironments during its life cycle (seed, seedling, pistal, and petals) by fluorescence *in situ* hybridisation (FISH) in combination with confocal laser scanning microscopy (CLSM) studies

rhizosphere (*Adam* et al., 2016). From the rhizosphere, only a certain subset of microorganisms is able to invade the endosphere (*Berg* et al., 2005; *Hardoim* et al., 2010). Again, plant secondary metabolites and its immune system are the crucial factors for the selection procedure (*Huang* et al., 2019). In early stages of plant's life cycle, the microbiome is characterized by high diversity and fluctuation. Mature plants are characterized by a very stable microbiome, while in the senescent bacterial phyla which are associated with degradation, the microbiome is more abundant (*Smalla* et al., 2001). Microbiome stability and equilibriums are essential for plant functioning during the whole life cycle of a plant (*Berg* et al., 2017).

The flower microbiome
Interestingly, flowering is a plant stage, which is characterized by a highly specific microbiome, which was highly visible in microbial fingerprints of different plant species (*Smalla* et al., 2001). The flower is an underexplored microenvironment. Here, we present some data about pumpkin flowers (*Lukesch*, 2011): petals, pistils and epigynous ovaries of flowers at different maturation stages as well as fruits were analyzed. All parts of the flowers were colonized with bacterial communities at a similar level (2.7×10^6 to 1.4×10^7 16S rRNA gene copies ng^{-1} DNA).

Generally, flower parts at withered maturation stage showed higher abundances than at flourish or youngest stage. Functional analysis showed that a high proportion of the bacterial community had antagonistic traits to a broad-spectrum of phytopathogens; they belong to the classes Alphaproteobacteria and Gammaproteobacteria and the phylum Firmicutes. Deep sequencing of Gammaproteobacteria-specific amplicons revealed highest relative abundances of Pseudomonadaceae and Enterobacteriaceae. The analyses at species level showed a predominance of *Pseudomonas viridiflava*, one of the key pathogens of Styrian oil pumpkin, in pistils and petals. Our results were confirmed by fluorescence *in situ* hybridisation (FISH) in combination with confocal laser scanning microscopy (CLSM) studies. Using the FISH-CLSM approach, the colonization of pistils and petals by diverse bacterial communities could be detected (Figure 1). The highest bacterial density was found on pollen grains attached to pistils, which gives rise to the assumption that pollen act as a vector for bacteria between pumpkin plants. This study suggests the oil pumpkin flower as an interesting niche for plant-associated bacteria, which should be further considered as reservoir for biocontrol agents. It is also in contrast to the general opinion that the mother

56

plant is highly suggested to be responsible for the recruitment of the seed microbiota (*Nelson*, 2018). Recent results by *Manirajan* et al. (2018) confirmed a high microbial diversity influenced by plant species and pollination type associated with pollen. The knowledge about the anthosphere microbiome was even successfully used for manipulation by introducing beneficial bacteria at flowering into progeny seeds *(Mitter* et al., 2018).

Domestication and biodiversity loss
Cultivation of crop plants started 13,000 years ago and today´s divergence of domesticated plants to their wild ancestors emerged as a consequence of selecting wild plants that were gathered and cultivated by hunter-gatherers in early domestication periods. Domestication have impacted plant microbiome assembly and functions via habitat expansion and via changes in crop management practices, root exudation, root architecture, and plant litter quality (*Pérez-Jaramillo* et al., 2016). The authors proposed a "back to the roots" framework that comprises the exploration of the microbiome of indigenous plants and their native habitats for the identification of plant and microbial traits with the ultimate goal to reinstate beneficial associations that may have been undermined during plant domestication. Seeds transmit the footprint of domestication (*Berg* and *Raaijmakers*, 2018), and especially their altered morphology over time is therefore frequently studied by archeobotanic and genomic research.

Hence, threats of plant extinction, driven by modern human culture, affect the whole genomic entirety of the holobiont. Studying the impact of domestication on crop seed microbiomes, the seed microbiomes of plant from natural ecosystems are especially of interest, as solely undisturbed environments are appropriate to explain indigenous plant-microbe-interactions.

In conclusion, the plant microbiome is definitely inter-connect during plant's life cycle. Together, domestication and intensive agricultural management shifted the crop microbiota. This resulted in diversity loss, which has consequences for human and *one* health issues. More research is needed to discover these internal relationships, and use that knowledge for nature conservation and sustainable agriculture.

EXTERNAL RELATIONSHIPS:
THE PLANT MICROBIOME AND HUMAN HEALTH ISSUES

The plant - indoor microbiome connection
The built indoor microbiome has importance for human health because buildings are complex ecosystems that house not only people, but also trillions of microorganisms interacting with each other (*Kembel* et al., 2012). Residents leave their microbial fingerprint mainly from their skin (*Grice* and *Segre*, 2011) but less is known about the transfer from plants. Our hypothesis that indoor plants contribute substantially to the microbial abundance and diversity in the built environment was developed after studying the surface microbiome in intensive care units (ICU) of the Graz university hospital (*Oberauner* et al., 2012). Interestingly, the beneficial part of the ICU microbiome was similar to the phyllosphere microbiome (*Berg* et al., 2014a). We explained that by window ventilation, and in fact we found evidence for a transfer of pollen und their associated microbiome. The

importance of window ventilation for hospitals was already described by *Kembel* et al. (2012), who showed that the phylogenetic diversity of airborne bacterial communities was lower indoors than outdoors, and mechanically ventilated rooms contained less diverse microbial communities than did window-ventilated rooms. In a next step, we experimentally confirmed a proof of principle by analyzing the microbiome of the spider plant *Chlorophytum comosum* in relation to their surrounding environment *(Mahnert* et al., 2014). The abundance of Archaea, Bacteria, and Eukaryota (fungi) increased on surrounding floor and wall surfaces within six months of plant isolation in a cleaned indoor environment, whereas the microbial abundance on plant leaves and indoor air remained stable. We observed a microbiome shift: the bacterial diversity on surfaces increased significantly but fungal diversity decreased. The majority of cells were intact at the time of samplings and thus most probably alive including diverse Archaea as yet unknown phyllosphere inhabitants. The next question we had was to which extent plant-specific diversity contribute to the indoor diversity. To understand the microbiota of indoor phyllospheres and its driving factors in built environments, we used an experiment design under controlled conditions by analyzing 14 phylogenetically diverse plant species grown in the greenhouses of the Botanical Garden in Graz (Austria) demonstrating different climate zones (*Ortega* et al., 2016; *Mahnert* et al., 2018). Statistical analysis showed a significantly higher correlation of community composition - for bacteria as well as for fungi - to plant genotype in comparison to the ambient climatic variables. Finally, we could show that man-made shifts of the microbiome as well as the resistome can be influenced and compensated by the plant microbiome (*Mahnert* et al., 2019).

The food - gut microbiome connection
Recently, the importance of the plant microbiota for human health was evidenced (*David* et al., 2014). The plant-associated microbial diversity can be transferred to the gut microbiome because fruits and vegetables are the major component of a healthy diet (*Berg* et al., 2014b). One prominent example, which was already studied, are Brassicaceae (*Lebeis*, 2015). All family members are characterized by glucosinolates (GLSs) that are part of the effective defense mechanisms of the plant (*Lüthy* and *Matile*, 1984). Moreover, *Brassica* species are known for a bacteria-dominated composition of the microbiome and harbour no mycorrhiza. The hydrolysis of GLSs into highly active breakdown products, mostly isothiocyanates (ITC) and nitriles, is caused by myrosinase activity. Those volatile breakdown products are utilized in biofumigation processes, where *Brassica* residues are incorporated into soil as they provide suppressive or control effect against nematodes and soil-borne fungal pathogens like *Verticillium longisporum (Witzel* et al., 2013). Interestingly, GLSs are also involved in human health issues; the GLS metabolism has become increasingly important over the past decade due to the exploration of anti-cancer activity of ITCs (*Halkier* and *Gershenzon*, 2006). Since humans consume their vegetables often cooked, the GLS-metabolizing ability of bacteria (*Tani* et al., 1974) has recently aroused scientific interest. Some authors consider the addition of myrosinase-active bacteria to a *Brassica* rich diet to supplement inactivated plant myrosinases (*Mullaney* et al., 2013). While the majority of bacterial strains known to exhibit myrosinase activity are ubiquitous inhabitants of

58
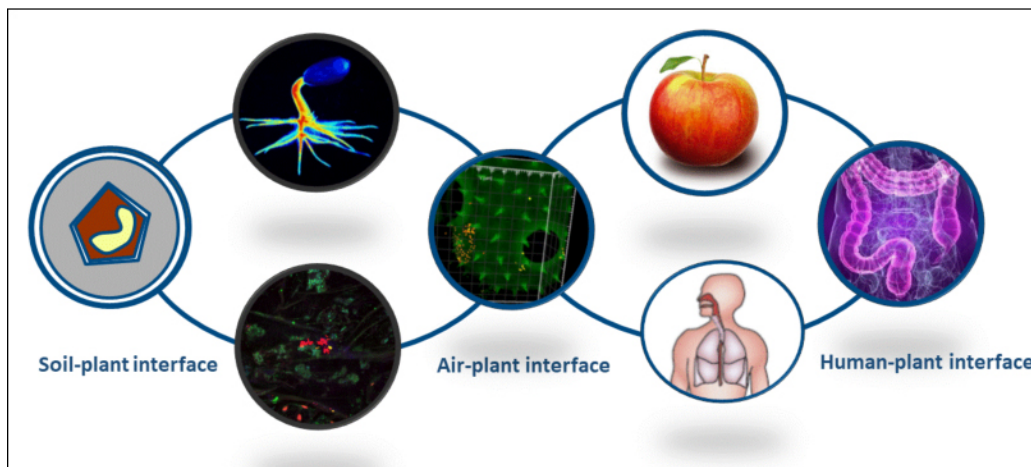
Figure. 2: Inter-connected microbiomes and their interfaces.

the human intestinal tract (*Mullaney* et al., 2013), still little is known about myrosinase-active bacteria colonizing edible plants tissues. We showed that due to their GLS content *Brassica* harbour a very specific microbiota containing also myrosinase-active bacteria (*Wassermann* et al., 2017).

Why is that plant-human microbiome connection so important? The loss of microbial diversity in the gut is associated with acute outbreaks as well as with chronic disease, e.g. allergies, obesity, mental diseases (*Turnbaugh* et al., 2006). *Hanski* et al. (2012) found first answers about the global question why biodiversity is so important. They showed a correlation between allergies in children and surrounding microbial biodiversity. Increasing chronic diseases in children were explained by the "missing microbe theory", which was published by *Blaser* (2014). In 2017, this was further developed into the "theory of disappearing microbiota and the epidemics of chronic diseases", which postulate that losses of particular bacterial species of our ancestral microbiota have altered the context in which immunological, metabolic and cognitive development occur in early life, which results in increased disease. Structural and especially functional microbial diversity is already established as a key factor in preventing human diseases, and is suggested as biomarker for plant health as well (*Berg* et al., 2017). However, despite many indications, this is not well understood and especially mechanistic studies understanding microbial diversity are missing. To our opinion, to study the plant - human microbiome connection offers an enormous potential to solve human health problems in future.

The microbiome of soil and plants plays a crucial role in plant and ecosystem health (*Berg* et al., 2017; *Laforest-Lapointe* et al., 2017). However, overlapping compositions, and interconnected microbiomes of human, animal and plant in connection with health should be considered, and used to expand the version of "One health" that includes environmental health and its relation to human cultures and habits (*Flandroy* et al., 2018). The interlinked microbiomes are shown in Figure 2, but the links and transmission routes have to be studied much more in detail.

CONCLUSIONS

Microbial biodiversity associated with plants is important for plant health; the balance between the microbiota and the host is crucial during the whole life cycle. Diversity loss associated with plants cause plant diseases, outbreaks of human pathogens of plants-origin as well as human health problems. Knowledge on the plant microbiota and their inter-connection can provide solutions to face health problems, e.g. to fight against multi-resistant pathogens and outbreaks. However, it can also provide solutions for crop production under climate change conditions. Plant microbiome engineering and biotechnology open novel options to develop microbials, which fulfil important functions for the plant host, e.g. nutrient, mineral and vitamin supply, and protection against biotic and abiotic stresses. Altogether, the plant microbiome will be the key to the next green revolution (Science Breakthroughs by 2030; http://nas-sites.org).

LITERATURE

Adam, E., Bernhart, M., Müller, H., Winkler, J., and Berg, G.: The *Cucurbita pepo* seed microbiome: Genotype-specific composition and implications for breeding. Plant Soil 422, 35-49 (2016).

Barret, M., Briand, M., Bonneau, S., Préveaux, A., Valière, S., Bouchez, O., and Jacques, M.A.: Emergence shapes the structure of the seed microbiota. Appl. Environ. Microbiol. 81, 1257-1266 (2015).

Berg, G., Krechel, A., Ditz, M., Sikora, R.A., Ulrich, A., and Hallmann J.: Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiol. Ecol. 51, 215-29 (2005).

Berg, G., Mahnert, A., and Moissl-Eichinger, C.: Beneficial effects of plant-associated microbes on indoor microbiomes and human health? Front. Microbiol. 5, 15 (2014a).

Berg, G., Erlacher, A., Smalla, K., and Krause, R.: Vegetable microbiomes: Is there a connection among opportunistic infections, human health and our 'gut feeling'? Microb. Biotechnol. 7, 487-95 (2014b).

Berg, G., Rybakova, D., Grube, M., and Köberl, M.: The plant microbiome explored: Implications for experimental botany. J. Exp. Bot. 67, 995-1002 (2016).

Berg, G., Köberl, M., Rybakova, D., Müller, H., Grosch, R., and Smalla, K.: Plant microbial diversity is suggested as the key to future biocontrol and health trends. FEMS Microbiol. Ecol. 93, fix050 (2017).

Berg, G. and Raaijmakers, J.M.: Saving seed microbiomes. ISME J. 12, 1167-1170 (2018).

Bever, J.D., Mangan, S.A., and Alexander, H.M.: Maintenance of plant species diversity by pathogens. Annu. Rev. Ecol. Evol. Syst. 46, 305-325 (2015).

Blaser, M.J: Missing microbes. How the overuse of antibiotics is fueling our modern plagues. Henry Holt and Company, New York (2014).

Blaser, M.J: The theory of disappearing

microbiota and the epidemics of chronic diseases. Nat. Rev, Immunol. 17, 461-463 (2017).

Bragina, A., Berg, C., Cardinale, M., Shcherbakov, A., Chebotar, V., and Berg, G.: *Sphagnum* mosses harbour highly specific bacterial diversity during their whole lifecycle. ISME J. 6, 802-813 (2012).

Bragina, A., Berg, C., Müller, H., Moser, D., and Berg, G.: Insights into functional bacterial diversity and its effects on Alpine bog ecosystem functioning. Sci. Rep. 3, 1955 (2013).

Bragina, A., Oberauner-Wappis, L., Zachow, C., Halwachs, B., Thallinger, G.G., Müller, H., and Berg, G.: The *Sphagnum* microbiome supports bog ecosystem functioning under extreme conditions. Mol. Ecol. 23, 4498-4510 (2014).

David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., Biddinger, S.B., Dutton, R.J., and Turnbaugh, P.J.: Diet rapidly and reproducibly alters the human gut microbiome. Nature 505, 559-563 (2014).

Doornbos, R.F., van Loon, L.C., and Bakker, P.A.H.M.: Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. Agron. Sustain. Dev. 32, 227-243 (2012).

Fenner, M. and Thompson, K.: The ecology of seeds. Cambridge University Press, Cambridge, New York (2005).

Grice, E.A. and Segre, J.A: The skin microbiome. Nat. Rev. Microbiol. 9, 244-253 (2011).

Flandroy, L., Poutahidis, T., Berg, G., Clarke, G., Dao, M.C., Decaestecker, E., Furman, E., Haahtela, T., Massart, S., Plovier, H., Sanz, Y., and Rook, G.: The impact of human activities and lifestyles on the interlinked microbiota and health of humans and of ecosystems. Sci. Total Environ. 627, 1018-1038 (2018).

Glassner, H., Zchori-Fein, E., Yaron, S., Sessitsch, A., Sauer, U., and Compant, S.: Bacterial niches inside seeds of *Cucumis melo* L. Plant Soil 422, 101-113 (2018).

Gloria, T.C., Bonneau, S., Bouchez, O., Genthon, C., Briand, M., Jacques, M.A., and Barret, M.: Functional microbial features driving community assembly during seed germination and emergence. Front. Plant Sci. 9, 902 (2018).

Hanski, I., von Hertzen, L., Fyhrquist, N., Koskinen, K., Torppa, K., Laatikainen, T., Karisola, P., Auvinen, P., Paulin, L., Mäkelä, M.J., Vartiainen, E., Kosunen, T.U., Alenius, H., and Haahtela, T.: Environmental biodiversity, human microbiota, and allergy are interrelated. Proc. Natl. Acad. Sci. USA 109, 8334-8339 (2012).

Halkier, B.A. and Gershenzon, J.: Biology and biochemistry of glucosinolates. Annu. Rev. Plant Biol. 57, 303-333 (2006).

Hardoim, P.R., van Overbeek, L.S., Berg, G., Pirttilä, A.M., Compant, S., Campisano, A., Döring, M., and Sessitsch, A.: The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol. Mol. Biol. Rev. 79, 293-320 (2015).

Hornschuh, M., Grotha, R., and Kutschera, U: Moss-associated methylobacteria as phytosymbionts: An experimental study. Naturwissenschaften 93, 480-486 (2006).

Huang, A.C., Jiang, T., Liu, Y.X., Bai, Y.C., Reed, J., Qu, B., Goossens, A., Nützmann, H.W., Bai, Y, and Osbourn, A.: A specialized metabolic network selectively modulates *Arabidopsis* root microbiota. Science 364, eaau6389 (2019).

Johnston-Monje, D. and Raizada, M.N.: Conservation and diversity of seed associated endophytes in *Zea* across boundaries of evolution, ethnography and ecology. PLoS One 6, e20396 (2011).

Johnston-Monje, D., Lundberg, D.S., Lazarovits, G., Reis, V.M., and Raizada, M.N.: Bacterial populations in juvenile maize rhizospheres originate from both seed and soil. Plant Soil, 405, 337-355 (2016).

Kembel, S.W., Jones, E., Kline, J., Northcutt, D., Stenson, J., Womack, A.M., Bohannan, B.J., Brown, G.Z., and Green, J.L.: Architectural design influences the diversity and structure of the built environment

microbiome. ISME J. 6, 1469-1479 (2012).

Klaedtke, S., Jacques, M.A., Raggi, L., Préveaux, A., Bonneau, S., Negri, V., and Barret, M.: Terroir is a key driver of seed-associated microbial assemblages. Environ. Microbiol. 18, 1792-1804 (2016).

Laforest-Lapointe, I., Paquette, A., Messier, C., and Kembel, S.W.: Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. Nature 546, 145-147 (2017).

Lebeis, S.L.: Greater than the sum of their parts: Characterizing plant microbiomes at the community-level. Curr. Opin. Plant Biol. 24, 82-86 (2015).

Links, M.G., Demeke, T., Gräfenhan, T., Hill, J.E., Hemmingsen, S.M., and Dumonceaux, T.J.: Simultaneous profiling of seed-associated bacteria and fungi reveals antagonistic interactions between microorganisms within a shared epiphytic microbiome on Triticum and Brassica seeds. New Phytol. 202, 542-553 (2014).

Liu, Y., Zuo, S., Zou, Y., Wang, J., and Song, W.: Investigation on diversity and population succession dynamics of endophytic bacteria from seeds of maize (Zea mays L., Nongda108) at different growth stages. Ann. Microbiol. 63, 71-79 (2013).

Lüthy, B. and Matile, P.: The mustard oil bomb: Rectified analysis of the subcellular organisation of the myrosinase system. Biochem. Physiol. Pflanzen 179, 5-12 (1984).

Lukesch, B.: Investigations of bacterial communities on the Styrian oil pumpkin with a special focus on female flowers. Master thesis, Graz University of Technology, Graz, Austria (2011).

Mahnert, A., Moissl-Eichinger, C., and Berg, G.: Microbiome interplay: Plants alter microbial abundance and diversity within the built environment. Front. Microbiol. 6, 887 (2015).

Mahnert, A., Ortega, R.A., Berg, C., Grube, M., and Berg, G.: Leaves of indoor ornamentals are biodiversity and functional hotspots for fungi. Front. Microbiol. 9, 2343 (2018).

Mahnert, A., Moissl-Eichinger, C., Zojer, M., Bogumil, D., Mizrahi, I., Rattei, T., Martinez, J.L., and Berg, G.: Man-made microbial resistances in built environments. Nat. Commun. 10, 968 (2019).

Manirajan, B.A., Maisinger, C., Ratering, S., Rusch, V., Schwiertz, A., Cardinale, M., and Schnell S.: Diversity, specificity, co-occurrence and hub taxa of the bacterial-fungal pollen microbiome. FEMS Microbiol. Ecol. 94, fiy112 (2018).

Mitter, B., Pfaffenbichler, N., Flavell, R., Compant, S., Antonielli, L., Petric, A., Berninger, T., Naveed, M., Sheibani-Tezerji, R., von Maltzahn, G., and Sessitsch, A.: A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. Front. Microbiol. 8, 11 (2018).

Mormile, B.W.: Influence of seed microbiome on fitness of Epichloë infected tall Fescue seedlings. Doctoral dissertation, Southern Connecticut State University, New Haven (2016).

Mullaney, J.A., Kelly, W.J., McGhie, T.K., Ansell, J., and Heyes, J.A.: Lactic acid bacteria convert glucosinolates to nitriles efficiently yet differently from Enterobacteriaceae. J. Agric. Food Chem. 61, 3039-3046 (2013).

Nelson, E.B.: The seed microbiome: Origins, interactions, and impacts. Plant Soil, 422, 7-34 (2018).

Oberauner, L., Zachow, C., Lackner, S., Högenauer, C., Smolle, K.H., and Berg, G.: The ignored diversity: Complex bacterial communities in intensive care units revealed by 16S pyrosequencing. Sci. Rep. 3, 1413 (2013).

Opelt, K., Berg, C., Schönmann, S., Eberl, L., and Berg. G.: High specificity but contrasting biodiversity of Sphagnum-associated bacterial and plant communities in bog ecosystems independent of the geographical region. ISME J. 1, 502-516 (2007).

Ortega, R.A., Mahnert, A., Berg, C., Müller, H., and Berg, G.: The plant is crucial: Specific composition and function of the phyllosphere microbiome of indoor ornamentals. FEMS Microbiol. Ecol. 92 fiw173

(2016).

Pérez-Jaramillo, J.E., Mendes, R., and Raaijmakers, J.M.: Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol. Biol. 90, 635-644 (2016).

Philippot, L., Raaijmakers, J.M., Lemanceau, P., and van Der Putten, W.H.: Going back to the roots: The microbial ecology of the rhizosphere. Nat. Rev. Microbiol. 11, 789-799 (2013).

Remus-Emsermann, M.N.P. and Schlechter, R.O.: Phyllosphere microbiology: At the interface between microbial individuals and the plant host. New Phytol. 8, 1327-1333 (2018).

Rezki, S., Campion, C., Iacomi-Vasilescu, B., Preveaux, A., Toualbia, Y., Bonneau, S., Briand M, Laurent E, Hunault G, Simoneau P, Jacques MA, and Barret M.: Differences in stability of seed-associated microbial assemblages in response to invasion by phytopathogenic microorganisms. PeerJ. 4, e1923 (2016).

Rybakova, D., Mancinelli, R., Wikström, M., Birch-Jensen, A.S., Postma, J., Ehlers, R.U., and Berg, G.: The structure of the *Brassica napus* seed microbiome is cultivar-dependent and affects the interactions of symbionts and pathogens. Microbiome 5, 104 (2017).

Sánchez-López, A.S., Thijs, S., Beckers, B., González-Chávez, M.C., Weyens, N., Carrillo-González, R., and Vangronsveld, J.: Community structure and diversity of endophytic bacteria in seeds of three consecutive generations of *Crotalaria pumila* growing on metal mine residues. Plant Soil, 422, 51-66 (2018).

Schiltz, S., Gaillard, I., Pawlicki-Jullian, N., Thiombiano, B., Mesnard, F., Gontier, E: A review: What is the spermosphere and how can it be studied? J. Appl. Microbiol. 119, 1467-1481 (2012).

Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H, and Berg, G.: Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: Plant-dependent enrichment and seasonal shifts revealed. Appl. Environ. Microbiol. 67, 4742-4751 (2001).

Truyens, S., Weyens, N., Cuypers, A., and Vangronsveld, J.: Bacterial seed endophytes: Genera, vertical transmission and interaction with plants. Environ. Microbiol. Rep. 7, 40-50 (2014).

Tani, N., Ohtsuru, M., and Hata, T.: Isolation of myrosinase producing microorganism. Agric. Biol. Chem. 38, 1617-1622 (1974).

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., and Gordon, J.I.: An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444, 1027-1031 (2006).

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., and Dufresne, A.: The importance of the microbiome of the plant holobiont. New Phytol. 206, 1196-1206 (2015).

Wassermann, B., Rybakova, D., Müller, C., and Berg, G.: Harnessing the microbiomes of *Brassica* vegetables for health issues. Sci. Rep. 7, 17649 (2017).

Wassermann, B., Cernava, T., Müller, H., Berg, C., and Berg, G.: Seeds of native alpine plants host unique microbial communities embedded in cross-kingdom networks. Microbiome 7, 108 (2019).

Whipps, J.M., Lewis, K., and Cooke, R.C.: Mycoparasitism and plant disease control. In: Biological control systems (Ed.: Burge, N.M.). Manchester University Press, Manchester 161-187 (1988).

Witzel, K., Hanschen, F.S., Schreiner, M., Krumbein, A., Ruppel, S., and Grosch R.: Verticillium suppression is associated with the glucosinolate composition of *Arabidopsis thaliana* leaves. PLoS One 8, e71877 (2013).