Impact of plant domestication on spermosphere and rhizosphere microbiome composition

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Chapter 1

General introduction and thesis outline

A current paradigm in plant science is that plant fitness partly relies on the association with the root- and shoot-associated microbiota (Vandenkoornhuyse *et al*., 2015; Martin *et al*., 2017; Wallenstein, 2017). Across the different stages of plant development, an intricate chemical communication occurs between plants and microbes, resulting in the selection, enrichment and activation of specific microbial groups (Badri *et al*., 2009; Aleklett and Hart, 2013; Lebeis *et al*., 2015). The plant's influence on the soil microbiota starts immediately after seed imbibition, when organic and inorganic compounds are released by the germinating seed in its direct surroundings, a compartment referred to as the spermosphere (Lemanceau *et al*., 2017; Nelson, 2018) After radicle protrusion and root development, plants exert a strong influence on the surrounding soil promoting an overall change in its physical, chemical and biological nature (Badri and Vivanco, 2009; Jones *et al*., 2009). This root-soil interface, referred to as the rhizosphere, is a dynamic, complex and multifaceted environment that is critical for plant growth and health (McNear Jr., 2013). The rhizosphere is home to a diverse group of (micro)organisms, which includes viruses, bacteria, archaea, protozoa, organotrophic and mycorrhizal fungi (Buée *et al*., 2009; Thompson *et al*., 2017). The taxonomically and functionally diverse microbial communities inhabiting and interacting in the rhizosphere are referred to as the rhizosphere microbiome (Boon *et al*., 2013). The complex interplay in the rhizosphere microbiome can result in neutral, beneficial or deleterious effects on plant growth. For instance, inhibition of soil-borne pathogens by specific beneficial members of the rhizosphere microbiome is of pivotal importance for plant health and productivity (Raaijmakers *et al*., 2009). Beneficial effects provided to the plant by specific members of the rhizosphere microbiome can also be attributed to nutrient acquisition, hormone-mediated plant growth promotion, induced systemic resistance and tolerance to abiotic stresses (Mendes *et al*., 2013).

Soil and plant influence rhizosphere microbiome assembly

Soil physical-chemical characteristics and plant genotypic traits are the main drivers of microbiome assembly in the rhizosphere (Garbeva *et al*., 2004; Berg and Smalla, 2009; Delgado-Baquerizo *et al*., 2016). As soil is an enormous reservoir of microbial diversity and serves as the inoculum for rhizosphere microbiome assembly, abiotic characteristics of the soil environment, such as pH, organic carbon content, moisture availability, texture and structure, shape the composition of the rhizosphere microbiome (Fierer, 2017). Also the plant itself shapes the rhizosphere microbiome via the release of root exudates, volatile compounds, mucilage, and border cells into the surrounding soil (Jones *et al*., 2009). Plant species modulate rhizosphere assembly in a genotype-dependent way (Ofek *et al*., 2014; Ofek-Lalzar *et al*., 2014). Interestingly, phylogenetically closely related plant species often present more similar rhizosphere microbiome compositions. For instance, in a study with maize inbred lines, teosinte (*Zea mays* ssp. *parviglumis*), sorghum and wheat, Bouffaud *et al*. (2014) showed that the composition of the microbiome was more divergent with increasing phylogenetic distances between the plant species tested. The intensity of this plant genotype effect can be stronger for specific plant species on the same soil type as was shown by Turner *et al*. (2013b) by RNA-based metatranscriptomics of the rhizosphere microbiomes of pea, oat and wheat. How plant species and cultivars of a given species affect the rhizosphere microbiome composition and activities is still largely unknown. Nevertheless, there are several studies that give some first insight into the genetic and chemical mechanisms underlying rhizosphere microbiome assembly. For instance, it was found in maize that two plant-genes (*su1*, *sh2*), involved in different carbon storage patterns affecting root exudate composition, promoted different microbial communities in the rhizosphere (Aira *et al*., 2010). Differential recruitment of beneficial microorganisms was also shown for wheat, where the root colonization by specific antifungal *Pseudomonas* species was highly cultivar dependent (Mazzola *et al*., 2004;

Kwak *et al.*, 2012). Another very good example of host genotype-dependent recruitment was shown for eleven *Arabidopsis thaliana* accessions, each with a distinct rhizosphere community composition, probably as a result of genotype-specific patterns of root exudation (Micallef *et al.*, 2009b).

Plant exudates and microbiome assembly

Seed and root exudates comprise low-molecular weight compounds, i.e. sugars, amino acids, organic acids, phenolics and several secondary metabolites, but also high-molecular weight compounds like proteins and mucilage (Nelson, 2004; Bais *et al.*, 2006; Carvalhais *et al.*, 2011; Kawasaki *et al.*, 2016; Sasse *et al.*, 2018). Other compounds commonly found in natural blends of plant exudates are flavonoids, strigolactones and salicylic acid (Cesco *et al.*, 2010; Lebeis *et al.*, 2015; Zwanenburg *et al.*, 2016). Plant genotype, soil type and abiotic factors largely determine the type and quantity of exudates released by seeds and roots (Lareen *et al.*, 2016; Mönchgesang *et al.*, 2016; Iannucci *et al.*, 2017; Lemanceau *et al.*, 2017). Through active and passive mechanisms of exudation, plants impact soil physical-chemical conditions (Rohrbacher and Marc St-Arnaud, 2016; Wang *et al.*, 2017) and the composition and activities of spermosphere and rhizosphere microbiomes (Pérez-Jaramillo *et al.*, 2016; Nelson, 2018). Depending on the type and amount of exudates released, the plant may select and enrich a subset of microbes from the diverse microbial populations inhabiting the soil, or may also act as modulators of soil biogeochemical processes (Hassan and Mathesius, 2012; Coskun *et al.*, 2017). Symbiotic associations with mycorrhizal fungi and rhizobia are initially triggered by plant exudates, and followed by an intricate chemical communication (Chagas *et al.*, 2018). For instance, under N and P limitations, plants release strigolactones into the rhizosphere, which induce several responses in mycorrhizal fungi such as spore germination, hyphal growth and hyphal branching, triggering the symbiotic association with the plant (Lanfranco *et al.*, 2017; Waters *et al.*, 2017). Similarly, in the nodulation process of legumes, the plant root secretes specific flavonoids which are recognized by bacterial Nod factors. This initial step triggers the formation of intracellular structures in the plant that allows compatible rhizobia to enter the inner root tissues, form the nodule and start the N-fixation process (Liu and Murray, 2016). However, not only symbiotic rhizobia inhabit legume nodules. In the model legume *Lotus japonicus,* it was shown that next to Rhizobiales also other bacterial taxa such as Burkholderiales, Flavobacteriales, Pseudomonadales, and Actinobacteridae are found associated with the nodules (Zgadzaj *et al.*, 2016). In the same study, it was shown that an impaired symbiosis ability of the host plant can have a large impact on the bacterial community of the root and rhizosphere compartments (Zgadzaj *et al.*, 2016). Also plant hormones released to the soil environment can impact on the root microbiome assembly. Specifically, it has been shown that wild type Arabidopsis levels of salicylic acid (SA) promoted a particular set of families in the root compartment while limiting colonization of several other bacterial taxa as compared to Arabidopsis mutants deficient in SA biosynthesis (Lebeis *et al.*, 2015). Phenolic acids can also impact the composition of the endophytic microbiome. For instance, cinnamoyl-CoA reductase (*CCR*)-down-regulated poplar trees, which simultaneously display reduced lignin levels and accumulation of extractable phenolic compounds in the xylem, showed an increase in bacterial cell counts as compared to wild type poplar trees. This finding suggested that the capacity to degrade phenolic compounds, in particular pherulic acid, was enhanced in the endosphere of *CCR*-down-regulated poplar trees, directly affecting the endophytic bacterial community (Beckers *et al.*, 2016).

Plant exudates may be also released in order to trigger microbiome-dependent defensive responses. Chapelle *et al.* (2016), found an overrepresentation of ppGpp metabolism, which is a mechanism associated with stress perception and stress response regulation in bacteria, in the rhizosphere microbiome of sugar beet grown in a soil suppressive to the fungal root pathogen *Rhizoctonia solani*. They hypothesized that this defensive response may have been triggered by plant-derived compounds following initial root infection by *R. solani* or directly by organic acids produced by the pathogenic fungus prior to or during infection, such as oxalic and phenylacetic acid (Chapelle *et al*., 2016). Besides soluble compounds, plants can also release volatile organic compounds (VOCs) to communicate with bacterial communities. In a recent study by Schulz-Bohm *et al.* (2018), it was shown that roots of *Carex arenaria* infected with the fungal pathogen *Fusarium culmorum* emitted a blend of VOCs that attracted bacteria with antifungal properties. In conclusion, plants can release several types of compounds into the soil environment to directly impact the composition of the microbiome assembly. Furthermore, these and other studies indicated that plants under attack alter their exudation profiles to recruit specific members of the microbiome with the ability of antagonize subsequent pathogen attacks. For instance, infection of Arabidopsis leaves with *Pseudomonas syringae* pv *tomato* induced an increased secretion of malic acid from the roots, promoting the colonization and biofilm formation by the beneficial rhizobacterium strain *Bacillus subtilis* FB17 (Rudrappa *et al*., 2008). Similarly, under foliar pathogen infection, Arabidopsis plants activated a salicylic acid dependent defensive response in order to recruit specific bacterial taxa in the rhizosphere which provided systemic protection against the pathogen (Berendsen *et al*., 2018). This ‘cry for help’ was already well-described aboveground as an indirect defense mechanism of plants in response to insect herbivory (Dicke, 2009) but is less well studied belowground. One of the first conclusive studies on the “crying for help” hypothesis belowground was shown for maize. Upon attack by insect larvae, a volatile sesquiterpene (E)-β-caryophyllene was emitted via the roots attracting an entomopathogenic nematode (Rasmann *et al*., 2005). It is highly likely that the same principle holds for plant-pathogen interactions in the rhizosphere environment, where specific members of the microbiome may act as an indirect plant defense mechanism (Cook *et al*., 1995).

The search for beneficial rhizosphere traits

Because the importance of the microbiome for plant growth and health has been largely underestimated, there is a renewed interest in identifying plant and microbial traits involved in the beneficial relationships between microbes and plants (Busby *et al*., 2017; Kroll *et al*., 2017; Wallenstein, 2017). One of the first attempts to identify plant traits and genes involved in beneficial interactions with soil microorganisms, other than rhizobia and arbuscular mycorrhizal fungi (AMF), was in a study by Smith *et al*. (1997) with six tomato lines and the biocontrol strain *Bacillus cereus* UW85. In this study, the authors found significant differences between the tomato lines for resistance to the pathogen *Pythium torulosum* and for support of root colonization and disease suppression by the biocontrol strain UW85. Subsequent analysis of recombinant inbred line (RIL) populations, derived from a cross between a cultivated tomato and a wild tomato relative, showed that three quantitative trait loci (QTL) were associated with disease suppression by *B. cereus* UW85, and three other QTL were associated with the growth of *B. cereus* on the seed (Smith *et al*., 1999). More recently, genetic loci have been identified for their involvement in the ability of Arabidopsis accessions to respond to the activity of plant growth-promoting bacteria (Wintermans *et al*., 2016). Furthermore, Mendes *et al*. (2018) showed that resistance breeding in common bean against the root pathogen *Fusarium oxysporum* also had unintentionally co-selected for plant traits that support rhizosphere microbes with potential antagonistic activities to this fungal root pathogen. On the other side, some genes and traits related to bacterial adaptation to plants have been also recently characterized. Briefly, gene categories related to carbohydrate metabolism and transport were enriched in the genomes of several plant- and root- associated bacteria, while mobile genetic elements were found underrepresented in plant-associated taxa (Levy *et al.*, 2018). However, several conceptual and experimental efforts are yet to be made to enable the use of these traits in rhizosphere-based plant breeding programs (Bakker *et al.*, 2012). These efforts include the identification of the specific plants traits involved and complementing the still limited knowledge of the microbial diversity responsible for the beneficial effects on plant growth and health. Meta-'omics approaches have opened new possibilities to also identify those microbial groups and their functional genes in the rhizosphere involved in biocontrol or in plant growth promotion that were invisible due to poor culturability (Ramírez *et al.*, 2017; Thompson *et al.*, 2017). These technologies also allow us to unravel changes in the abundance, evenness and richness as well as in their functions, and provide a first step in the selection of prominent plant genotypes that are better equipped in shaping a beneficial rhizosphere microbiome.

Plant domestication and microbiome assembly

Over the past decades, plant breeders have exploited genes from wild relatives of modern crop species to improve plant growth and health (Hajjar and Hodgkin, 2007). Similarly, entomologists explore native habitats and wild ancestors of crop plants to identify natural enemies of insect pests (Chen, 2016). In plant microbiome research, however, relatively few efforts have been made to study the identity and diversity of beneficial microbial communities present in the native habitats of ancestors of modern agricultural crop species. Smith *et al.* (1999) postulated that plant genotypic traits involved in beneficial plant-microbe interactions may have been lost in the process of plant breeding, primarily as a consequence of the targeted selection of a few specific traits related with higher yields or other profitable plant traits. In fact, it is known that loss of genetic diversity is a common feature of domesticated crops as compared to their wild relatives (Bitocchi *et al.*, 2013). This reduction in genetic diversity could have undermined the abilities of modern plant cultivars to interact with and benefit from the microbiome. Genes involved in active root exudation and/or root development could have been inadvertently lost in domesticated plant accessions. Therefore, I postulate that wild relatives of crop plants, which are more genetically diverse, represent a valuable source of genotypic and phenotypic traits associated with the recruitment and assembly of the microbiome. Furthermore, domestication also led to environmental changes associated with agricultural practices which in turn made biotic interactions with belowground diversity presumably less relevant to sustain plant growth and health (Wissuwa *et al.*, 2009). Consequently, I postulate that native habitats may harbor a soil microbial diversity that co-evolved with wild relatives of crop plants and that agricultural management and habitat expansion of domesticated varieties caused a decoupling between plants and their microbiome. This could have led to the loss of microbial diversity, the 'missing plant microbes', which we hypothesized were formerly a functionally important component of the microbiome of wild plants in their native habitats. This concept has been explored in human microbiome research, where antibiotic overuse and modern lifestyle also are proposed to have caused a loss of several components of the microbiota (Blaser, 2017). In this context, I postulate that modern crops grown in soils under agricultural management have lost specific members of the rhizosphere microbiome typically found on roots of their wild relatives growing in the native soils in the centres of origin.

Common bean as a 'model' in plant microbiome and domestication research

Common bean (*Phaseolus vulgaris* L.) is currently the most important legume crop, grown worldwide in a broad range of environmental conditions. It is of high importance in developing countries because it is a good source of protein, carbohydrate and micronutrients. Furthermore, common bean is the basis of subsistence economy for low income farmers especially in Latin America and Africa, currently the largest common bean producing regions (Broughton *et al*., 2003; Akibode and Maredia, 2011; CGIAR, 2013). The common bean rhizosphere microbiome has been studied primarily because of the symbiotic associations with nitrogen-fixing bacteria of the genus *Rhizobium*. Briefly, five *Rhizobium* species have been recognized as microsymbionts of *P. vulgaris* (Amarger, 2001), and the species *R. etli* as the most commonly associated with beans in the Americas (Aguilar *et al*., 2004). The regulation of this symbiotic association is dependent on compounds produced by the plant (flavonoids) for the induction of nodulation genes in the bacterial species, which in turn secrete nodulation factors essential for nodule formation (Schultze and Kondorosi, 1998). Apart from this well-studied plant-microbe association, the relation between rhizobia and several nonsymbiotic bacteria in the rhizosphere of common bean has been assessed, searching for an increment in nodule formation mediated by bacterial inoculants (Burdman *et al*., 1996; Petersen *et al*., 1996). To our knowledge, the differences between wild relatives, landraces and cultivars of common bean in the composition and functions of the microbial communities in the rhizosphere have not been established, nor the relative influence of plant genotype and soil type.

Wild common bean (*Phaseolus vulgaris*) originated in central Mexico (Bitocchi *et al*., 2012; Desiderio *et al*., 2013) and from there spread throughout Central and South America (Gepts, 1998). This wide distribution led to the establishment of two different genetic pools: Mesoamerican and Andean (Gepts and Bliss, 1985). Additional to these genetic pools, a third intermediate genetic pool can be distinguished composed of wild common bean accessions from Ecuador and northern Peru with a combination of both Mesoamerican and Andean alleles (Debouck *et al*., 1993) (Fig. 1). The Mesoamerican and Andean wild common bean populations were the basis for two independent domestication processes, which presumably occurred in the Oaxaca Valley in Mexico for

Mesoamerica and eastern Bolivia or northern Argentina for the Andes (Beebe *et al*., 2001; Bitocchi *et al*., 2013). After domestication, common bean started a journey across the American continent with human groups as its main dispersion force and successfully adapted to several dissimilar environments and human preferences, leading to several morphological and physiological changes (Gepts and Debouck, 1991). Simultaneously, domesticated common bean races experienced a reduction in genetic diversity as compared to its wild counterparts, a fact that encouraged plant breeders to look into wild common bean genotypes for useful traits that could be used for the improvement of domesticated races. The broadening of the cultivable gene pool through the use of wild relatives or through the recombination of Mesoamerican and Andean gene pools has been postulated as a response to abiotic and biotic stresses, and also as a strategy to improve the yield of commercial bean varieties (Gepts, 1998; Singh, 2001).

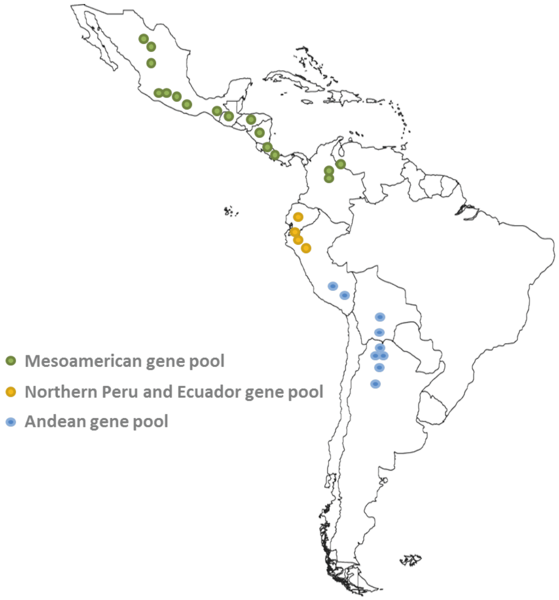

Fig. 1. Distribution of wild common bean (*Phaseolus vulgaris*) in Central and South America. Accessions of common bean have been collected from Mexico to Argentina and three genetic pools can be recognized based on phenotypic and molecular data: Mesoamerican, Andean and an intermediate gene pool

in northern Peru and Ecuador. Adapted from "Origin and evolution of common Bean: Past events and recent trends", Gepts, (1998).

Wild beans have coevolved with several pests and diseases, symbiotic organisms such as rhizobia and mycorrhizal fungi, and at the same time acquired adaptations to several abiotic conditions (Toro *et al*., 1990). Recently, it was proposed that wild relatives could be reservoirs of genes associated with drought tolerance (Cortés *et al*., 2012). An example of successful wild bean gene mining was the transfer of genes associated with high grain yield from wild beans to cultivars of domesticated common bean (Blair *et al*., 2006). In another study, it was observed that when a wild common bean was used as one of the parents, more plants with a higher yield were found in the segregating populations compared to the domesticated parent (Herrera-Flores and Acosta-Gallegos, 2008). However, in a recent survey in which 19 important crops were evaluated with regard to the contributions of wild relatives to their overall crop performance, it was noticed that common bean is amongst those species, together with soybean, pigeon pea, sorghum, lentil, and cowpea, that have not incorporated wild genes into the cultivated varieties, whilst for the remaining 13 crops over 100 traits have been acquired from over 60 wild species (Hajjar and Hodgkin, 2007). The lack of wild traits in modern common bean varieties indicates that from the plant breeding perspective more efforts are yet to be made in order to identify and integrate beneficial traits from wild common bean genotypes into modern cultivars. As mentioned above, these traits could be used to address problems associated with biotic and abiotic stress factors and to improve plant productivity, but also the potential of a still unknown genetic diversity could be used to overcome the future production constraints in the scenery of climate change (Porch *et al*., 2013). Thousands of accessions of modern common bean cultivars, landraces and wild relatives are held in the Program of Genetic Resources from the International Centre for Tropical Agriculture (CIAT) to perform basic and applied research (Toro, 1990; CIAT-PRG, 2018). With the aforementioned plant material we can determine the inherent rhizosphere microbial diversity of cultivated varieties, landraces and wild relatives, allowing comparisons which can be used to assess whether there is a core microbiome of the numerous common bean accessions. By contrast, it can be also determined whether wild relatives and landraces, which possess a more diverse genetic background, can promote different rhizosphere microbial communities as compared to those present in domesticated modern varieties. Finally, the relative importance of the plant genotype in shaping the rhizosphere microbial diversity can be unraveled with common bean as a model plant and if/how microbiome diversity impacts the plant's overall performance.

Thesis Outline

The overall aim of my thesis was to decipher the impact of plant domestication on the spermosphere and rhizosphere microbiome composition of common bean (*Phaseolus vulgaris*). Common bean is currently the most important legume crop, grown worldwide in a broad range of environmental conditions with an outstanding nutritional importance in developing countries. In order to explore the effect of domestication on the plant microbiome, an extensive literature review was undertaken (**Chapter 2).** This chapter also presents the "Back-to-the-Roots" approach, which is a general workflow to investigate the impact of domestication on microbiome composition and functioning and how you can use that knowledge to reinstate beneficial partnerships in modern cultivars by studying the microbiome of wild plants in native soils. **Chapter 3** links genotypic and phenotypic traits of wild and domesticated common bean accessions with their rhizosphere microbiome composition. Genotypic and root morphology analyses of the common bean accessions, followed by a detailed characterization of the rhizosphere microbiome by 16S rDNA amplicon sequencing, allowed a robust statistical analysis to find associations between genotypic and root phenotypic traits of common bean and the abundance of certain rhizobacterial families. **Chapter 4** addresses how the transition of common bean from a native soil to an agricultural context impacts the composition of the rhizosphere microbiome. Soil sampling was performed in Colombia, one of the centres of diversification of common bean. Subsequently, the native and the agricultural soils collected were used to grow different wild and modern common bean accessions in a pot trial under controlled conditions. An integral assessment was undertaken, including diversity measurements, niche-neutral abundance modelling, core microbiome assessment, differential abundance and co-ocurrence network analyses to search for the 'missing plant microbes', i.e. microbes that were lost during domestication of common bean including the transition from a native to an agricultural soil. **Chapter 5** describes how domestication of plant species other than common bean impacted the root-associated microbiome. To this end, we analyzed publicly available metagenomic data from microbiome studies on different modern crop cultivars and their wild relatives. Following the same pipeline in this 'computational walk on the wild side', we determined differences in root microbiome composition between wild relatives and their domesticated counterparts. We specifically identified the relative abundance of specific taxa within the Bacteroidetes phylum.

Different plant developmental stages may recruit and enrich different microbial groups. The spermosphere, which is a short-lived, dynamic developmental stage, can also have an impact on microbiome assembly. **Chapter 6** evaluates through amplicon sequencing the magnitude of the spermosphere effect on the bacterial diversity and abundance to assess if a plant genotype-dependent influence is already discernible in the spermosphere of common bean. Subsequently, collection, characterization and quantification of seed exudates were performed. In a further experiment, one of the most abundant amino acids (i.e. glutamate) released by common bean seeds was used as inoculant of a soil solution, in order to evaluate if a single seed exudate can simulate the compositional shifts observed between the spermosphere microbiomes of wild and modern bean accessions. **Chapter 7** brings together the research findings of this thesis, discusses the implications of the conceptual and experimental work and proposes further directions on how to translate the fundamental knowledge on rhizosphere microbiomes of wild relatives and native habitats to modern agriculture.