Impact of plant domestication on spermosphere and rhizosphere microbiome composition

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Chapter 5

The wild side of plant microbiomes

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Abstract

Plants rely on their microbiome for a number of life-support functions including nutrient acquisition and protection against (a)biotic stress factors. For crop plants, however, the process of domestication may have adversely impacted the composition and functions of the associated microbiota, thereby undermining their beneficial effects on plant growth and health. Here, we conducted a meta-analysis to resolve if and how plant domestication affected the composition of the root-associated microbiome. For different plant species, we observed significant and consistent differences in the abundance of Bacteroidetes, Actinobacteria and Proteobacteria. Potential causes and consequences of these microbiome shifts following plant domestication are discussed.

Keywords: rhizosphere and root microbiome, wild relatives, modern cultivars, Bacteroidetes

Introduction

In the search for new strategies to engineer "healthy microbiomes" of plants and humans, considerable attention is given to coevolutionary signatures of host-microbe interactions and mechanisms involved in microbiome assembly and activity (Mueller and Sachs, 2015; Schnorr *et al*., 2016; Crittenden and Schnorr, 2017). For example, comparative analyses of the human microbiome revealed a higher abundance of Bacteroidetes in the gut of hunter-gatherer populations of rural communities in non-industrialized regions than in the gut of Westernized populations, a distinct divergence that appears to be associated with differences in the content of starch, fiber and plant polysaccharides in the food (Schnorr *et al*, 2014; Gomez *et al*., 2016). Similarly, shifts in gut microbiome composition in captive mammals as compared to their wild counterparts have been associated with a loss of dietary fiber and a potential increase in protein consumption (Clayton *et al*., 2016; McKenzie *et al*, 2017). Interestingly, one of the most relevant changes in the gut microbiome of mammals in captivity is an increase in the relative abundance of the genus *Bacteroides* and a decrease of the genus *Prevotella*, both from the Bacteroidetes phylum, a pattern that has also been observed in Westernized humans (McKenzie *et al*, 2017). For plants, several studies have suggested that domestication altered the composition of the root microbiome with an adverse effect on the association with symbiotic nitrogen-fixing rhizobia and mycorrhizal fungi (Pérez-Jaramillo *et al*., 2016). For instance, Kiers *et al*., (2007) showed that older soybean cultivars had a higher yield difference ratio, i.e. the ability of soybean cultivars to reach their full symbiotic potential in the presence of a mix of rhizobial strains with different symbiotic effectiveness, as compared to newer soybean cultivars. Similarly, it has been shown that wild ancestors and primitive landraces of wheat, breadfruit and maize can benefit more from mycorrhizal symbiosis than modern cultivars (Hetrick *et al*., 1992, 1995; Sangabriel-Conde *et al*., 2012; Xing *et al*., 2012). To date, however, the impact of plant domestication on the vast majority of other root-

associated microorganisms is not well understood. In a recent study, we revealed that the rhizosphere microbiome of wild relatives of common bean (*Phaseolus vulgaris*) harbored a higher abundance of Bacteroidetes, while the root microbiome of modern bean accessions was dominated by bacterial families belonging to the Actinobacteria and Proteobacteria (Pérez-Jaramillo *et al*., 20170). Also studies on other plants species, including Arabidopsis (Schlaeppi *et al*., 2014), sugar beet (Zachow *et al*., 2014), barley (Bulgarelli *et al*., 2015) and lettuce (Cardinale *et al*., 2015), suggested that domestication led to compositional changes in the root microbiome. To investigate if these effects of domestication cause similar shifts in microbiome composition for multiple plant species, we set out a meta-analysis of the root microbiome of various crop plants and their wild relatives. The specific objectives of this computational 'walk on the wild side' were to: i) determine differences and patterns in root microbiome composition between wild relatives and their domesticated counterparts, and ii) identify the relative abundance of specific taxa within the Bacteroidetes phylum for crop plants and their wild relatives.

Methods

Processing of the sequences

Per sample fastq files of 16S metagenome amplicon sequences were kindly provided by the authors of the different studies (Schlaeppi *et al*., 2014; Zachow *et al*., 2014; Bulgarelli *et al*., 2015; Cardinale *et al*., 2015; Leff *et al*., 2017; Pérez-Jaramillo *et al*., 2017). The reads were quality filtered for single end reads with sickle (Joshi *et al*., 2011), and bases below phred score 36 and shorter than 100bp were trimmed. Only high quality filtered reads were mapped to full length 16S sequences from the Silva 119 release (Quast *et al*., 2013) using the usearch global algorithm implemented in VSEARCH version 1.9.6 (Rognes *et al*., 2016). The alignment results were directly converted to BIOM format using biom version 2.1.5 (McDonald *et al*., 2012). Consensus/majority taxonomy was

added as metadata to the biom file. Finally, all BIOM files of each dataset were merged using Qiime version 1.9.1 (Caporaso *et al*. 2010). The Silva 119 reference phylogenetic tree provided by Qiime (clustered at 97%) was filtered using the Qiime command *filter_tree.py* to keep Bacteroidetes taxa which were present only in wild plants. Subsequently, we built phylogenetic trees using the Phyloseq package in R, and for graphic purposes only branches with a relative abundance higher than 0.1% from the total amount of reads were kept.

Description of plant and soils used in the studies

In the study by Pérez-Jaramillo *et al*., (2017), the wild and modern common bean accessions (*Phaseolus vulgaris*) were cultivated in the same soil in a pot trial under the same climatic conditions followed by characterization of the rhizosphere microbiome composition. For Cardinale *et al*. (2015), it is described in the main text that the experiments with *Lactuca serriola*, wild relative of lettuce, and four subspecies of *L. sativa* were done in the field in an experimental farm in Austria, followed by characterization of the rhizosphere microbiome composition. Nevertheless, the description of soil characteristics is not provided and therefore it is not possible to describe growth conditions for the plants neither. In the study by Zachow *et al*. (2014), wild beet plants were collected in the drift line at the Mediterranean Sea coast in Slovenia. From the same region, soil from the coastal drift line was collected and used under unspecified greenhouse conditions in order to grow domesticated beet in the same soil than wild beet. For the study by Schlaeppi *et al*. (2014), several field experiments and greenhouse experiments were done and four different types of soil were used. The root microbiome composition was characterized for *Arabidopsis thaliana*, *Cardamine hirsuta, A. halleri* and *A. lyrata*, while the rhizosphere microbiome composition was characterized for *A. thaliana* and *C. hirsuta*. The latter is an *Arabidopsis* relative species which diverged

~35 Mya and is phylogenetically the most distant species. Finally, in the study by Bulgarelli *et al*. (2015), the microbiome composition of root and rhizosphere compartments of wild barley (*Hordeum vulgare* spp. *spontaneum*), a landrace and a modern variety of barley (*H. vulgare* spp. *vulgare*) were characterized. For this, two pot trials were performed with the three plant accessions in soils that were collected in the same location in two different years. All the information about plant accessions, soil type and experimental conditions are described in Tables S1 and S2.

Statistical analysis

In order to compare the different datasets we rarefied the OTU table up to 500 reads, which was the sequencing depth that allows us to work with most of the data sets available. All the data sets were included except for the data of Leff *et al*. (2017), for which sequencing depth, after processing with the method described above, did not reach the threshold implemented. For Alpha diversity metrics, the command *alpha_diversity.py* in Qiime was applied and the output files were retrieved and plotted in R using the package ggplot2 (v.2.0.0) (Wickham, 2009). As we did not observe significant differences in alpha diversity indexes between wild and domesticated accessions of the same plant species, the data was merged per plant species in order to illustrate exclusively differences between compartments (root/rhizosphere). For beta-diversity calculations, a Bray–Curtis dissimilarity matrix was calculated and used it to build Principal Coordinate Analyses and Permutational multivariate analyses of variance (Adonis function) were performed to evaluate the significance of the variables tested, both retrieved from Phyloseq (v.1.10) (McMurdie and Holmes, 2013) and Vegan (v.2.4-4) (Oksanen *et al*., 2017). For the OTU level analysis, the function calculateEffectiveSamples from the metagenomeSeq R package (v.1.12) (Paulson *et al*., 2017) was applied to the filtered OTU table and features with less than the average number of effective samples in all features

were removed. For the analysis at OTU level, we used normalized tables applying a cumulative-sum scaling normalization. Then, a Zero-Inflated Gaussian Distribution Mixture Model was applied using the fitZig function from metagenomeSeq. With the coefficients from the model, we applied moderated t-tests between accessions using the makeContrasts and eBayes commands retrieved from the R package Limma (v.3.22.7) (Ritchie *et al*., 2015). Obtained P-values were adjusted using the Benjamini–Hochberg correction method. Differences in the abundance of taxa between accessions were considered significant when adjusted P-values were lower than 0.05 at OTU level. Treemap (v.3.7.3) was used to visualize the significantly abundant OTU's, the taxonomy, the adjusted *P*-value and per mil relative abundance in bubble graphs, in which the size of the bubbles indicates the relative abundance per hundred of the raw read counts.

Results and discussion

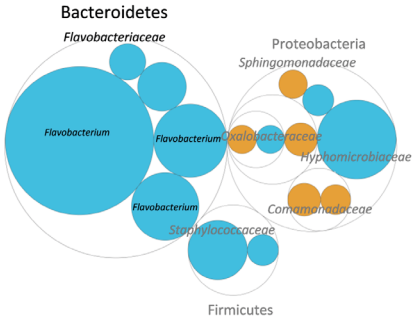
In this study, we retrieved the raw 16S rDNA sequences from 6 independent common garden experiments with a total of 9 plant species and adopted the same computational pipeline to assess the root/rhizosphere bacterial community composition (Table S1, Table S2). Regarding the analysis of the Arabidopsis root microbiome by Schlaeppi *et al.* (2014), our comparison was made based on divergence time estimates with *Cardamine hirsuta* considered as the 'ancient/wild' species and members of the genus *Arabidopsis* as the 'modern/evolved' counterpart.

First, we observed marked differences in the diversity of bacterial communities associated with roots of the different plant species, which were largely explained by the study (29.1%, PERMANOVA, $P<0.001$) (Fig. S1) and the microhabitat sampled, i.e. root or rhizosphere (Fig. S2). These results reinforce the preponderant role of soil type in the assembly of the root microbiome (Peiffer *et al*., 2013). Also, the higher diversity in the

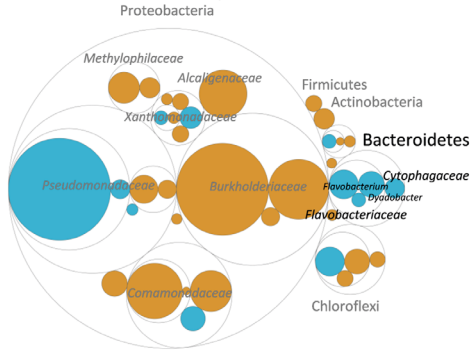
rhizosphere as compared to the endosphere (Fig. S2) is in accordance with previous reports (Edwards *et al*., 2015). Subsequent pairwise comparisons showed that, for each plant species, the Bacteroidetes were consistently enriched in the root or rhizosphere of wild relatives and a comparable difference was observed between *Cardamine hirsuta* and *Arabidopsis halleri* (moderated t-tests; $P<0.05$, BH corrected) (Fig. 1a). For the ancestor of sugar beet, *Beta vulgaris* ssp. *maritima*, we also observed a higher prevalence of Bacteroidetes taxa as compared to modern sugar beet, although this difference could not be analyzed statistically as the replicate samples in that study (Zachow *et al*., 2014) were pooled. Next to the Bacteroidetes, we observed a higher relative abundance of some other bacterial families on roots of wild relatives of the different plant species. In common bean, Planctomycetes, Verrucomicrobia and Acidobacteria together with some Proteobacteria families were also more abundant on roots of the wild accession. For wild barley, a few Proteobacteria families were enriched as well as two Firmicutes families. For wild lettuce and *Cardamine hirsuta,* also several Proteobacteria families were enriched. Overall, Proteobacteria and Actinobacteria were consistently enriched on roots of the modern counterpart, while Bacteroidetes was found almost exclusively enriched on roots of the wild relatives irrespective of the plant species and study. The phylum Bacteroidetes has also been found as a prevalent and abundant member in the rhizosphere of several other wild plant species (Aleklett *et al*., 2015; Shi *et al*., 2015).

a

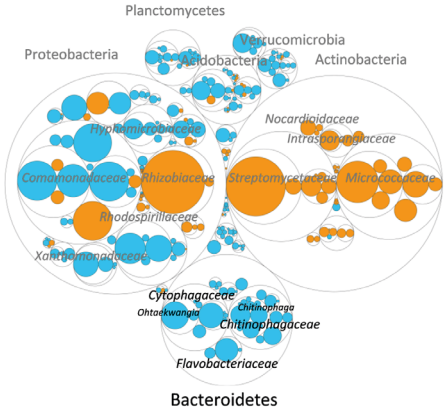
i) *Hordeum vulgare* ssp. *spontaneum* vs *Hordeum vulgare* ssp. *vulgare*



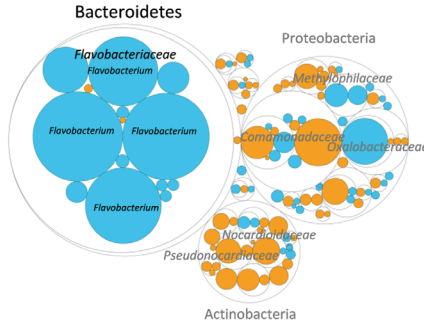
ii) *Lactuca serriola* vs *Lactuca sativa* ssp. *capitata*



iii) *Phaseolus vulgaris* (G22304-wild) vs *Phaseolus vulgaris* (G5773-modern)



iv) *Cardamine hirsuta* vs *Arabidopsis halleri*



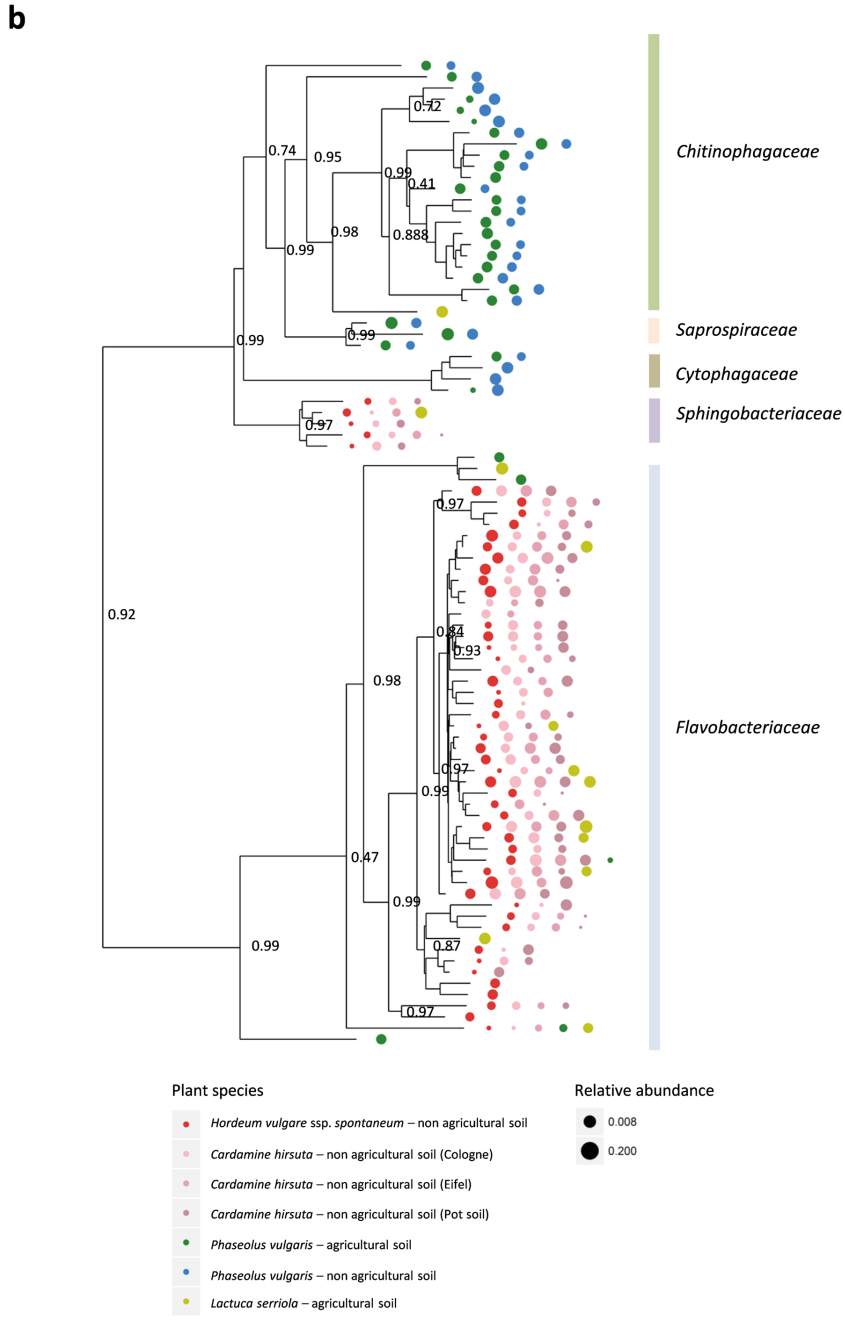


Fig. 1. Enrichment and taxonomic diversity of bacterial taxa in wild and domesticated plant species. a) Differential abundance of bacterial OTUs between wild plant accessions and their domesticated counterparts. Presented here are selected pairwise comparisons between **i)** wild barley (*Hordeum vulgare* ssp. *spontaneum*) and modern barley (*Hordeum vulgare* ssp. *vulgare*); **ii)** wild lettuce (*Lactuca serriola*)

and cultivated lettuce (*Lactuca sativa* ssp. *capitata*); **iii)** wild and modern accessions of common bean (*Phaseolus vulgaris*), and **iv)** *Cardamine hirsuta* and *Arabidopsis halleri.* Each comparison was made using a zero-inflated Gaussian distribution mixture model followed by moderated t-test and a Bayesian approach. Only OTUs significantly enriched in one of the two accessions are shown (FDR<0.05). The largest circles represent Phylum level and the inner circles represent Class and Family level. The color of the circles represents the OTUs enriched in the rhizosphere/roots of wild relatives (cyan) or of modern crop plants (orange), with the assigned genus in italics. The size of the circle is the mean read relative abundance of the differentially abundant OTU. b) Phylogenetic tree of bacterial members of the Bacteroidetes phylum associated with different wild plant species. The Bacteroidetes taxa were selected from microbiome data of wild plant species to construct the phylogenetic tree. The size of the circles corresponds to the relative abundance for each Bacteroidetes taxa. Only data with a relative abundance higher than 0.1% is depicted in the tree. Each abundance data is the average of at least 3 samples per plant species and site.

Our analysis further revealed that the extent of the Bacteroidetes enrichment on roots of wild plant relatives exhibits plant species-specific signatures. For example, approximately 50% of the bacterial species differentially enriched on roots of wild barley belonged to the Bacteroidetes, while for *Cardamine hirsuta,* wild lettuce and wild common bean the Bacteroidetes represented 33.3%, 24.5% and 18.9%, respectively, of the root-associated bacterial community. Subsequent phylogenetic analysis of the Bacteroidetes that were more abundant (>0.1%) on wild relatives showed two main clusters: one composed mainly of members of the *Chitinophagaceae* family and the other of members of the *Flavobacteriaceae* family (Fig. 1b). The family *Flavobacteriaceae* was represented by a high diversity in *Cardamine hirsuta* and wild barley, whereas *Chitinophagaceae* and *Cytophagaceae* families were predominant in the root microbiome of wild relatives of common bean (Fig. 1b). Collectively, these results indicate that plant domestication resulted in a similar overall taxonomic shift in the prokaryotic root microbiome with a reduced abundance of the Bacteroidetes phylum on modern accessions and a concomitant increase of members of the Actinobacteria and Proteobacteria (Fig. 2). At higher taxonomic levels, we observed that the plant species-specific effects observed on Bacteroidetes families may be probably due to differences in the physicochemical

characteristics of the diverse soils used in these independent studies, such as divergent pH values and the organic carbon content (Table S1).

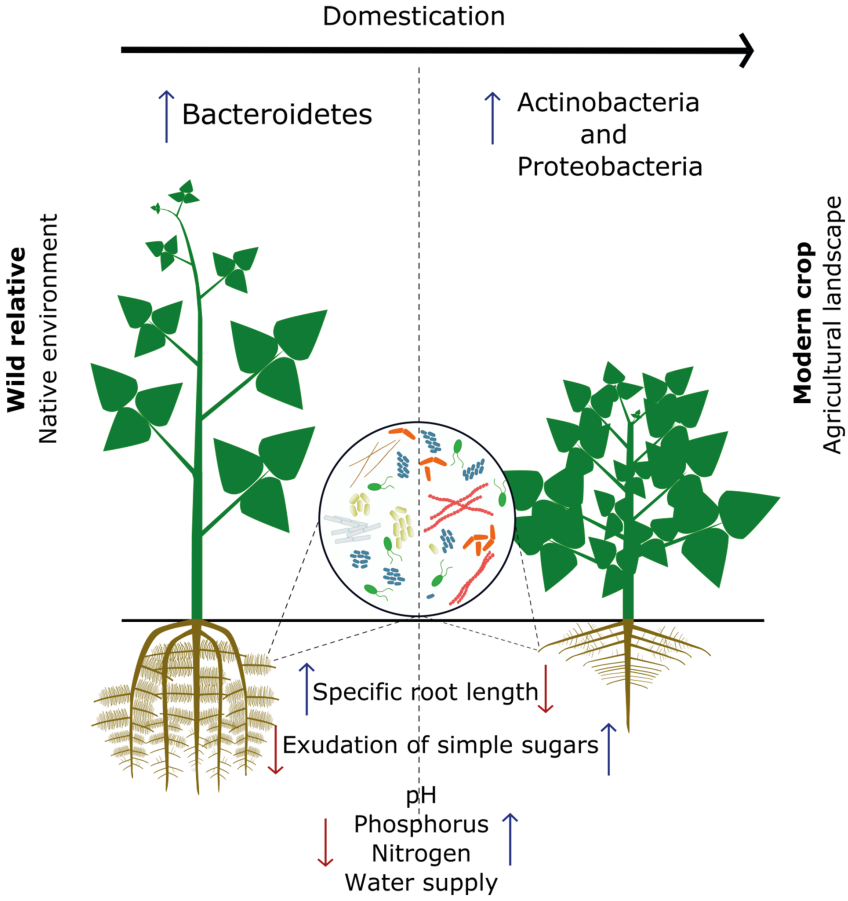


Fig. 2. Impact of domestication on soil management, plant phenotype, plant physiology and rhizobacterial diversity. In this hypothetical schematic representation, the root morphology of the wild relative substantially differs from that of the modern counterpart. Readily available macronutrients and water associated with agricultural management led to shallower roots in modern crop cultivars as compared to roots of wild relatives, which are rooting deeper with conspicuous lateral roots. Domesticated crop plants presumably also exude more 'simple' sugars than their wild relatives. The impact of the domestication process on rhizobacterial community composition is reflected in a decrease in Bacteroidetes abundance on modern crop plants, while the abundances of the Actinobacteria and Proteobacteria are increased.

Firstly, it is important to emphasize that in our analyses the same computational pipeline was used adopting a rarefaction of the OTU table to the same sequencing depth. However, the approach used in this study cannot address all biases associated with this type of meta-analysis. Differences in soil types, sampling strategies, nucleic acid extraction protocols and sequencing techniques between the different studies may have affected the reach of our meta-analysis and the interpretation of the results. Nevertheless, it is noteworthy that despite all these constraints we found similar and consistent differences between the prokaryotic composition of the root/rhizosphere microbiome of wild and domesticated plant species with a significantly higher abundance of Bacteroidetes on/in roots of wild plant relatives. Why Bacteroidetes are relatively more abundant in the root and rhizosphere compartments of wild relatives of various crop plant species is yet unknown. They are recognized for their ability to degrade complex biopolymers, a trait associated with a diverse set of carbohydrate processing enzymes (Thomas *et al*., 2011; Berlemont *et al*., 2015). Hence, their prevalence in the root compartments of wild plant species may be a phylogenetic signal associated with the presence of complex biopolymers in their root exudates (Fig. 2). Plant root exudates can have a major impact on the structure and functioning of microbial communities in soil environments (Bais *et al*., 2006; Micallef *et al*., 2009a). A recent study on mutants of poplar trees, silenced in the cinnamyl-Co reductase (*CCR*) gene of the monolignol-specific lignin pathways, showed significant effects on the density and composition of culturable rhizosphere and endosphere bacteria, microbiome shifts that were proposed to be mediated, at least in part, by changes in extractable plant phenolic compounds such as ferulic acid (Beckers *et al*., 2016). In this context it is worth noting that one of the most common domestication syndrome traits is related with changes in the type and amount of secondary metabolites, such as the loss of specific compounds that are toxic for humans or livestock or the reduction of flavonoid content in the leaves (Gepts, 2004; Meyer *et al*., 2012; Chacón-Fuentes *et al*., 2017). To

date, however, very little is known about qualitative and quantitative differences between root exudation profiles of crop plants and their wild relatives. For wheat, it has been shown that a modern wheat variety exuded three to five times more 'simple' sugars (mainly fructose, glucose and maltose) than an ancient wheat cultivar under stress conditions, a feature that might be related with a lower capacity of the modern wheat cultivar to control sugar exudation (Shaposhnikov *et al*., 2016). Whether the higher levels of these 'easy-digestible' sugars are also the case for other plant species and may contribute to a competitive advantage and a concomitant higher abundance of Proteobacteria and Actinobacteria on roots of modern crop cultivars remains to be addressed.

Also differences in root architecture between crop plants and their wild relatives may impact root microbiome assembly. More specifically, the prevalence of Bacteroidetes in the rhizosphere of wild bean correlated significantly with a higher specific root length (SRL, i.e. root length per unit of root dry mass) and a lower root density (Pérez-Jaramillo *et al*., 2017). A high SRL has been associated with a higher efficiency of water search and uptake for the plant and is considered a strategy to acquire nutrients in low-fertile soils (Comas *et al*., 2013; Kramer-Walter *et al*., 2016). Along with changes in plant genotype and phenotype, the domestication process also involves changes in the environment and the concomitant need of management practices, such as the use of chemical pesticides and fertilizers, to sustain growth and health of the crop plants (Pérez-Jaramillo *et al*., 2016). Therefore, altered root morphology traits (Fig. 2) as well as changes in plant physiology and root exudation may have contributed to the observed and consistent shifts in the prokaryotic root microbiomes between wild plant relatives and their domesticated counterpart. This hypothesis needs to be validated by experiments where morphological and physiological traits, in particular root architecture and

exudation profiles, of wild relatives of crop plants are assessed in agricultural soils as well as in soils from their centres of origin and diversification.

Whether a higher relative abundance of Bacteroidetes affects plant growth and health as was shown for growth (i.e. obesity) and health of humans (Ley *et al*., 2006; Arrieta *et al*., 2014; Liu *et al*, 2017) is not known to date. Some studies suggested that representatives of this phylum can affect plant growth and health. In particular strains of the genus *Flavobacterium* have been associated with plant growth promotion and disease protection (Kolton *et al*., 2012). For the legume plant *Trifolium pratense*, however, *Flavobacterium* led to impaired shoot growth (Hartman *et al*., 2017). For the genus *Chryseobacterium,* disease protective effects have been described (Yin *et al*., 2013), but effects on plant growth and health by most other Bacteroidetes, including members of the *Chitinophagaceae* and *Cytophagaceae* families detected here, remain to be discovered. Establishing a phenotypically and genomically diverse and well-characterized collection of Bacteroidetes species from multiple wild plant relatives followed by controlled bioassays to test the effects of individual species/strains and consortia on plant growth and health under diverse environmental conditions will shed more light on their functional importance for the growth and survival of wild plant species in their native, environmentally harsh habitats. Understanding the functional importance of these 'missing plant microbes' can be highly instrumental in plant breeding programs and for improving our future crop production systems in a changing environment.

Acknowledgments

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Supplementary materials

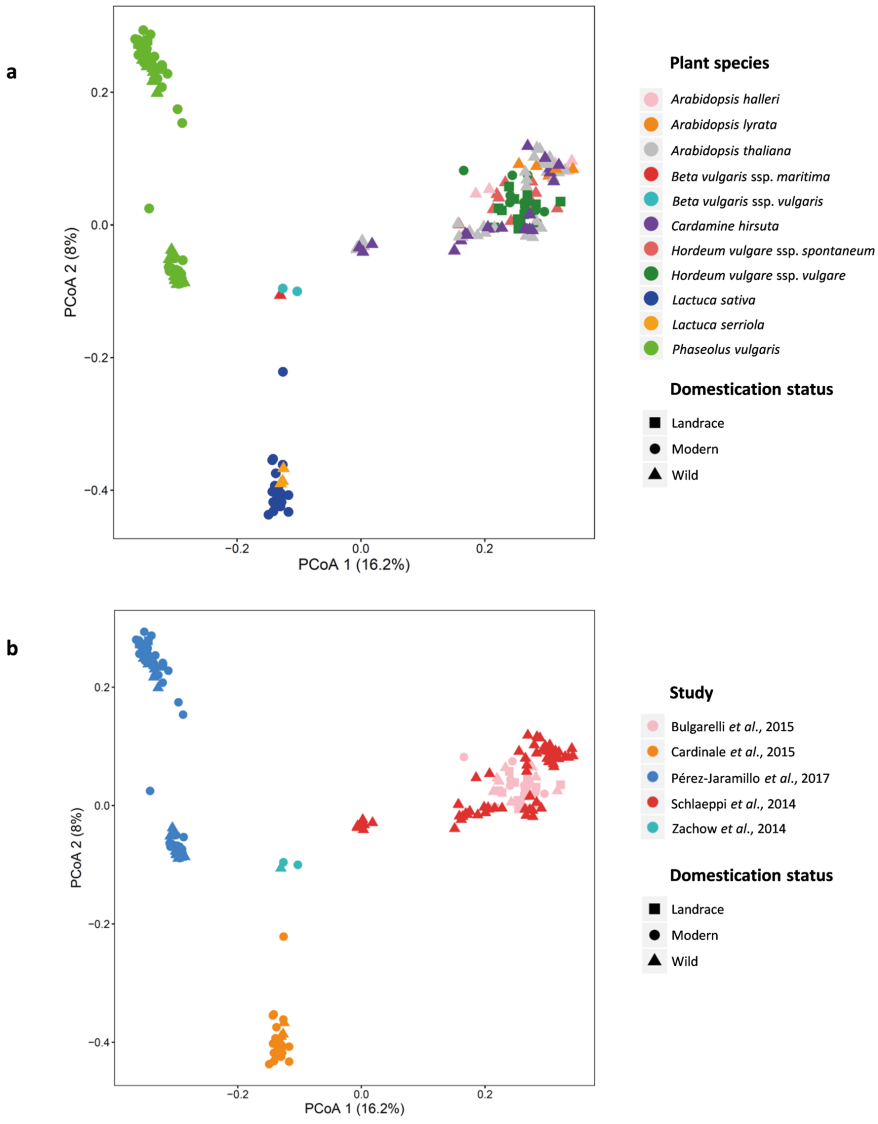

Fig. S1. Rhizosphere bacterial community composition across studies of wild, landrace and modern plants. Principal Coordinate Analysis (PCoA) of Bray-Curtis dissimilarities of 16S rRNA data. **a)** PCoA with samples colored by plant species. b) PCoA with samples colored by study. The source of the data (study) was the main explaining variable as assessed by PERMANOVA (29.1%; P<0.005).

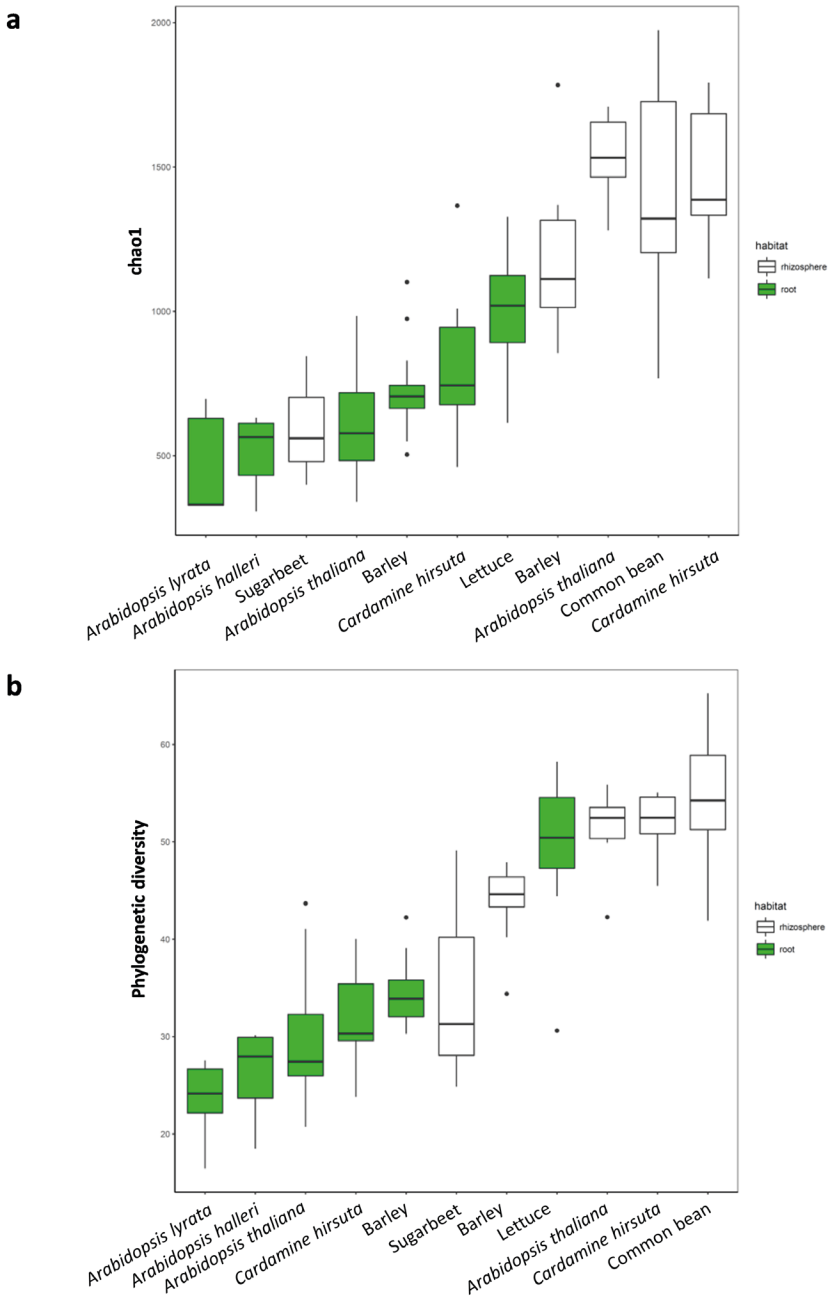

Fig. S2. α-diversity of 16S sequence data of wild, landrace and modern plant species for rhizosphere and roots. (a) Chao1 and **(b)** Phylogenetic diversity for all the plants included in the meta-analysis. Bacterial diversity on/in the roots is less than in the rhizosphere.

Table S1. General information of the datasets used for the meta-analysis

Study	Sequencing Method	Repository	Project code	16S primer pair	Plant species and subspecies	Domestication status	DOI
Zachow *et al.,* 2014	454	NCBI*	PRJNA233435	V4-V5	*Beta vulgaris* ssp. *vulgaris*	Domesticated beet	10.3389/fmicb.2014.00415
					Beta vulgaris ssp. *maritima*	Wild relative	
Schlaeppi *et al.* , 2014	454	ENA**	PRJEB5058	V5-V6-V7	*Cardamine hirsuta*	Wild plant	10.1073/pnas.1321597111
					Arabidopsis halleri	Wild plant	
					Arabidopsis lyrata	Wild plant	
					Arabidopsis thaliana	Wild plant	
Bulgarelli *et al.,* 2015	454	ENA	PRJEB5860	V5-V6-V7	*Hordeum vulgare* ssp. *spontaneum*	Wild relative	10.1016/j.chom.2015.01.011
					Hordeum vulgare ssp. *vulgare*	Domesticated barley	
Cardinale *et al.,* 2015	454	ENA	PRJEB5101	V4	*Lactuca serriola*	Wild relative	10.1111/1462-2920.12686
					Lactuca sativa ssp. *capitata*	Domesticated lettuce	
					Lactuca sativa ssp. *crispa*	Domesticated lettuce	
					Lactuca sativa ssp. *longifolia*	Domesticated lettuce	
					Lactuca sativa ssp. *augustana*	Domesticated lettuce	
Leff *et al.,* 2017	MiSeq	NCBI	SRP075934	V4	*Helianthus annuus*	Wild, landraces and modern accessions included ***	10.1111/nph.14323
Pérez-Jaramillo *et al.,* 2017	MiSeq	ENA	PRJEB19467	V3-V4	*Phaseolus vulgaris*	Wild, landraces and modern accessions included ***	10.1038/ismej.2017.85

*National Center for Biotechnology Information of the United States of America

**European Nucleotide Archive

***Wild; plants that have a ready ability to grow freely in natural ecosystems, with strong dispersal mechanisms; Landrace: locally developed crop varieties by farmers within their own agricultural, horticultural or agri-silvicultural systems; Modern accessions or modern improved varieties are the result of plant breeding in the pursue of higher yields, better quality and more stable production, typically grown on heavily managed agricultural settings.

Table S2. Physicochemical characteristics of the soils used in the studies included in the meta-analysis.

Study	Soil type used for plant growth						Type of experiment
	Texture (%)			Classification	pH	Organic C (%)	
	Clay	Silt	Sand				
Zachow *et al.,* 2014	NA*	NA	NA	Clay	9,5	NA	Field/Pot
Schlaeppi *et al.* , 2014	13,4	37,3	49,3	Sandy Loam	6,95	4,0	Pot trial
Bulgarelli *et al.,* 2015	4,2	4,2	91,6	Sand	7,12	1,0	Pot trial
Cardinale *et al.,* 2015	NA	NA	NA	NA	NA	NA	Field
Leff *et al.,* 2017	NA	NA	NA	Sandy Loam	NA	NA	Field
Pérez-Jaramillo *et al.,* 2017	8	30	62	Clay Loam	5,8	17.9**	Pot trial
*Not available							
*Organic Matter							