Impact of plant domestication on spermosphere and rhizosphere microbiome composition

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Chapter 6

The spermosphere microbiome of wild and domesticated common bean (*Phaseolus vulgaris*)

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Abstract

Plants have a significant influence on the diversity and activity of soil microbial communities. Already during imbibition and germination, plant seeds release exudates that promote microbial growth. Although this so-called spermosphere effect is widely studied, little is known about the impact of plant domestication on spermosphere microbiome assembly. Here, we sampled the spermosphere from germinating seeds of seven wild and modern accessions of common bean (*Phaseolus vulgaris*) and assessed the bacterial community composition by 16S rRNA sequencing. Compared to bulk soil we observed a decrease in α-diversity of the bacterial community in the spermosphere of all bean accessions. Also for the β-diversity, a significant difference was observed between the bean spermosphere and bulk soil. Proteobacteria, Actinobacteria and Firmicutes were enriched in the spermosphere. Small but significant differences in β-diversity were detected between the spermosphere microbiomes of wild and modern bean accessions, suggesting a genotype-dependent effect on microbiome assembly already at this early plant developmental stage. Comparative seed exudate profiling further showed that the spermosphere of modern bean accession M5 contained higher levels of glutamate and glutamine as compared to that of wild accession A2. Subsequent in vitro assays indicated that these single amino acids did not enrich for the specific bacterial taxa found at higher abundance in the spermosphere of modern bean accessions. Collectively, these results suggest that domestication affects spermosphere microbiome assembly of common bean and that more complex mixtures of yet unknown exudate constituents are driving spermosphere microbiome assembly.

Keywords: spermosphere, microbiome, root exudates, glutamate, Actinobacteria

Introduction

Early in plant development, during seed imbibition and germination, exudates are released in the surrounding soil creating a narrow zone with enhanced microbial activity known as the spermosphere (Nelson *et al*., 2004; Schiltz *et al*., 2015; Lemanceau *et al*., 2017). In the spermosphere, a broad diversity of compounds can be found such as sugars, amino acids, phenolic compounds and volatiles (Nelson, 1990; 2018). The composition of exudates in the spermosphere is determined by abiotic factors such as temperature, oxygen tension, soil moisture, seed coat integrity and genetic traits (Short and Lacy, 1976; Schlub and Schmitthenner, 1978; Nelson, 2018). Different plant species and even cultivars from the same species can differ in seed exudation profiles (Kageyama and Nelson, 2003; Singh and Mehrotra, 1980). Consequently, seed exudation may actively influence the spermosphere microbiome composition and activity in a plant genotype-dependent manner. For instance, two contrasting tomato recombinant inbred lines (RIL) populations derived from an interspecific cross of cultivated tomato and a wild tomato relative, differed in the proportion of indigenous fluorescent *Pseudomonas* species in the spermosphere (Simon *et al*., 2001). Similarly, a host genotype-dependent effect was found for the fungal community composition in the spermosphere (Barret *et al*., 2015). To date, however, little is known about the diversity, succession, and activities of indigenous microbial communities in the spermosphere and whether the process of plant domestication influenced spermosphere microbiome assembly. Finally, it is unknown how this short-lived, dynamic spermosphere community affects plant growth, development and health.

The objectives of this study were to i) study the impact of the spermosphere of common bean (*Phaseolus vulgaris*) on bacterial diversity and abundance, and to ii) decipher differences in spermosphere microbiome assembly between modern bean cultivars and wild relatives. To this end, we selected wild and modern accessions of common bean for

which significant differences in the rhizobacterial community assembly were documented earlier (Pérez-Jaramillo *et al*., 2017). Seeds from the common bean accessions were sown in agricultural soil collected from the Colombian highlands and spermosphere soil samples were collected at 48h following seed imbibition and germination. The bacterial communities were characterized through 16S rRNA amplicon sequencing and cultivation-dependent methods. We also conducted comparative chemical profiling of seed exudates of a wild and a modern bean accession and assessed the effect of individual differential amino acids on soil bacterial community composition.

Materials and Methods

Soil and plant material

The agricultural soil used for the assays was collected in a common bean producing farm in the rural area of the municipality of El Carmen de Viboral, Colombia, as described in Pérez-Jaramillo *et al.* (2017). Briefly, the soil was air dried, sieved (2-mm mesh) and stored at 4°C for further use. The seeds of two wild accessions (A1 and A2) and five modern cultivars of common bean (M1 to M5) were selected for this study. The plant material was kindly provided by the Genetic Resources Program at the International Centre for Tropical Agriculture—CIAT—in Palmira, Colombia. A complete description of these bean accessions can be found in Pérez-Jaramillo *et al.* (2017)

Sampling of soil from the spermosphere compartment

Seeds of wild and modern accessions of common bean without cracks or other visible damages were selected. Seed-surface disinfection was applied to the seeds through immersion during 3 minutes in sodium hypochlorite 0.5% (v/v), followed by four washes with ample sterile distilled water. After disinfection, the seeds were air dried in a laminar flow cabinet. For each bean accession, surface-sterilized seeds were sown in 12-well

plates with 3g of the agricultural soil and one seed per well. For each bean accession, 6 replicates were used. Wells with soil but without seeds served as the bulk soil control. After sowing the seeds at a depth of 0.5 cm, 1mL of sterile distilled water was added to each well. This initial watering step initiated seed imbibition. The plates were kept at 25 °C in the dark during 48-72 hours, depending on the differences in germination rates between the seven bean accessions. Once the radicle protruded, the seeds were harvested and replaced by a new seed of the same accession. This first imbibition and germination cycle was done to pre-condition the soil with seed exudates in order to activate the soil microbial communities. In the second cycle, a new seed was sown in the pre-conditioned soil and 1 mL of sterile distilled water was added. The plates were kept at 25 °C in the dark during 48-72 hours. Once the radicle protruded, a round hole puncher (1,5 cm diameter) was used to extract the germinating seed with adhering spermosphere soil from each well. The seed with spermosphere soil was transferred to a 15 mL tube containing 5 ml of LifeGuard Soil Preservation Solution (MoBio Laboratories). The 15mL tubes were thoroughly vortexed at maximum speed during 15 min, and the solution was kept at -20°C for further use.

DNA isolation, sequencing and preparation of the OTU table

For each bean accession and the control (bulk soil), four replicates were used for DNA extraction with MoBio kits (MoBio Laboratories, Carlsbad, CA, USA) as previously described (Pérez-Jaramillo *et al*., 2017). Subsequently, the V3-V4 region of the 16S rRNA was amplified and sequenced by Illumina Myseq (Illumina, 2013). The sequence data analysis was performed by the Hydra pipeline implemented in Snakemake (Köster and Rahmann, 2012) and the obtained OTU table was filtered using QIIME 1 (1.9.1) custom scripts (Kuczynski *et al*., 2012). The Bacteria domain was extracted using the command *split_otu_table_by_taxonomy.py* and singletons, doubletons, chloroplast and

mitochondria sequences were discarded with the command *filter_otus_from_otu_table.py*, obtaining a filtered OTU table for further analysis.

Bacterial diversity, differential abundance and core microbiome analysis

To calculate α-diversity, we rarefied (Brewer and Williamson, 1994; Gotelli and Colwell, 2001) the OTU table up to 45,360 reads (i.e. lowest sequencing depth obtained from a sample) per sample for all spermosphere and bulk soil samples using the script *alpha_rarefaction.py* retrieved from QIIME 1 (1.9.1). Chao1 and Shannon diversity indices were calculated using the *alpha_diversity.py* command and the data obtained was tested for normality and homoscedasticity. To compare α-diversity values by bean accession, the non-parametric Kruskal-Wallis test was applied using R (R Core Team, 2015). To calculate β diversity, the unrarefied OTU table was normalized using a cumulative-sum scaling (CSS) method (Paulson *et al*., 2013). Bray-Curtis dissimilarities followed by Constrained Analysis of Principal Coordinates were calculated with the Phyloseq package (McMurdie and Holmes, 2013) (v.1.22.3). The nonparametric *adonis* test was used to assess the percentage of variation explained by the habitat (bulk soil, spermosphere) and domestication status (wild, modern) along with its statistical significance using Vegan (Oksanen *et al*., 2016) (v.2.4-6), all performed in R. The QIIME command *compute_core_microbiome.py* was applied to the rarefied OTU table in order to obtain a list of OTUs observed in the spermosphere of all bean accessions. Core OTUs with a relative abundance <0.5% and unclassified taxa were not included in the figure. Donut plots were built using the R package ggplot2 (Wickham, 2009) (v. 2_2.2.1). To assess the spermosphere effect on microbiome composition, the rarefied OTU table was analyzed with the STAMP software (v.2.1.3) (Parks *et al*., 2014). Welch's t-tests followed by BH corrections were performed at phylum, family and genus level between spermosphere samples grouped by the domestication status. Bacterial phyla, families and

genera significantly overrepresented ($P < 0.05$) in the spermosphere or in the bulk soil microbiome are depicted using extended error bars. Finally, Welch's t-tests followed by BH corrections were performed to assess if bacterial genera were differentially abundant in the spermosphere of wild and modern bean.

Culture-dependent characterization of the bacterial diversity in the spermosphere

After extracting the spermosphere soil, an aliquot of 500 mg was suspended in 0.1 M phosphate buffer. After 5min of rigorous vortexing, serial dilutions of the soil suspension were made and 100 µL aliquots of 10^{-5}, 10^{-6}, and 10^{-7} dilutions were plated onto $1/10^{th}$ strength R2A agar plates. The plates were incubated at 25 ºC for 1 week and isolation of individual colonies was performed based on morphology, colour and size. A total of 631 isolates was purified and kept in R2A broth with 40% (v/v) of glycerol at -80 ºC for further use. For identification purposes, the collection was replicated and the V1-V4 16S rRNA region was sequenced at BaseClear (Leiden, Netherlands). Multiple alignment of sequences larger than 400 bp was performed using Muscle v3.7 (Edgar, 2004) with the default parameters to create an approximate maximum-likelihood tree in FastTree v2.1.8. with default parameters (Price *et al*., 2009). The tree was visualized with the online application iTOL (Letunic and Bork, 2016).

Exudate extraction from seeds

Seeds without cracks or visible damages in the seed coat were selected for seed exudate extractions. Due to seeds limitations of several accessions, only wild accession A2 and modern accession M5 were used for exudate extraction. Firstly, seeds were disinfected as described above. From the last wash 20 µL of water were plated in TSA and PDA petri dishes and incubated 48h to confirm disinfection. One gram of disinfected seeds (i.e. approximately 6 seeds) was transferred to sterile petri dishes containing sterile filter paper

(Whatman no. 1). For each bean accession, 4 petri dishes with 1 g of seeds were used. The filter paper was wetted with 200 µL sterile distilled water to saturation point and incubated at 24ºC for 24 hours. After this time, the seeds were transferred to a new sterile plate with sterile filter paper (Whatman no. 1), and wetted with 200 µL sterile distilled water to saturation point and incubated at 24ºC for another 24 hours. The filter papers were processed individually to harvest the exudates. 1 mL of a Methanol:Water solution (70:30) was added to each of the filter papers and incubated during 1 hour on an orbital shaker (100 rpm). The resulting extract was filtered (2 µm) and dried under a flow of Nitrogen gas. Once the methanol was evaporated, the samples were freeze-dried for three days under vacuum (−80 °C collector temperature; Labconco Free Zone 12 L Freeze Dry System, USA). Dried samples were re-suspended in 200 µL of MilliQ water, filtered through a 13-mm-diameter 0.2 µM PTFE syringe filter and poured in glass vials for chemical analyses. Organic acids were analysed by HPLC (Ultimate 3000, ThermoFisher Scientific, USA) equiped with a UV diode array detector. The sugar analyses were performed by HPLC with electro-chemical detection, whereas amino acid analyses were performed by LC-MS/MS (Agilent, USA). Differences in the amount of exudates between the wild and the modern accessions were determined by the Wilcoxon signed-rank test ($P < 0.05$) in R.

Effect of glutamate on bacterial community assembly

The agricultural soil used for spermosphere microbiome analyses (described above) was used to test the effect of glutamate on bacterial community assembly. Four treatments were evaluated as follows: T1 consisted of M9 minimal media (12.8 g $Na_2HPO_4 \cdot 7H_2O$, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl in 1L of distilled water); T2 consisted of M9 minimal media, 10 mM of glutamate, 1 mM of $MgSO_4$, and glucose 20%; T3 consisted of M9 minimal media, 1 mM of $MgSO_4$, and glucose 20%; and T4 consisted of M9 minimal

media, 10 mM of cysteine, 1 mM of $MgSO_4$, and glucose 20%. Treatment T1 was considered the baseline control, because only had minimal medium without glucose or any type of amino acid. Treatment T2 is the glutamate treatment. Treatment T3 is the glucose control, in order to observe the impact on soil bacterial communities of the glucose alone. Finally, T4 is the amino acid control treatment, which consisted of cysteine, glucose and minimal media. Cysteine was included as a control amino acid due to the low quantity observed in seed exudation profiles, and the assumption that its impact on bacterial communities would be not significant. The experimental unit consisted of a 50 mL conical flask, and to each flask 3 g of the agricultural soil were added. As 4 replicates per treatment were used, in total 16 conical flasks were used for the experiment. At time 0, the respective treatments were applied to the flasks and these were kept in an orbital shaker at 150rpm and 25 ˚C. After 24 hours, the content of the flasks was transferred to 50 mL tubes carefully labelled and centrifuged at 3,500 rpm for 5 min. After centrifugation, the supernatant was removed and the treatments were re-applied, i.e, to each 50 mL tube with soil a new addition of the respective treatment solution was applied, and after vigorous mixing, the soil solutions were transferred back to their respective conical flasks for a new incubation period of 24 h. This enrichment procedure was repeated five times followed by soil DNA extraction, amplification and sequencing of the V3-V4 region of the 16S rRNA, sequence analysis and OTU table preparation, the same procedures as described above for the spermosphere microbiome analyses. The OTU table was rarefied up to 11,790 reads and bar plots with the relative abundance of phyla and families were built based on the normalized OTU table. Also a Bray-Curtis dissimilarity matrix was calculated and used for Principal Coordinate analysis with Phyloseq. The nonparametric *adonis* test was used to assess the percentage of variation explained by the glutamate treatment along with its statistical significance using Vegan package, all performed in R.

Results

Spermosphere microbiome assembly is plant genotype specific

16S rRNA sequencing of the spermosphere and bulk soil samples yielded approximately 2.2 million reads after quality filtering, representing 8,263 bacterial operational taxonomic units (OTUs) at 97% sequence similarity. For the α-diversity, we observed significantly lower chao1 and Shannon indices for the spermosphere as compared to the bulk soil (Fig. 1a and b). Regarding the β-diversity, Bray-Curtis metrics and Constrained Principal Coordinate Analysis (CAP) revealed a significant effect of the spermosphere and the domestication status of the bean accessions (Fig. 1c and d). The structure of the bacterial communities differed between the spermosphere and the bulk soil samples: the percentage of variability explained by the habitat (spermosphere versus bulk soil) was 28.2% (PERMANOVA, P=0.001) (Fig. 1c). Furthermore, a subtle but statistically significant difference was found between the spermosphere microbiome composition of wild and modern bean accessions with 5.2% of the total variation explained by the domestication status (wild versus modern; PERMANOVA, P=0.001) (Fig. 1d).

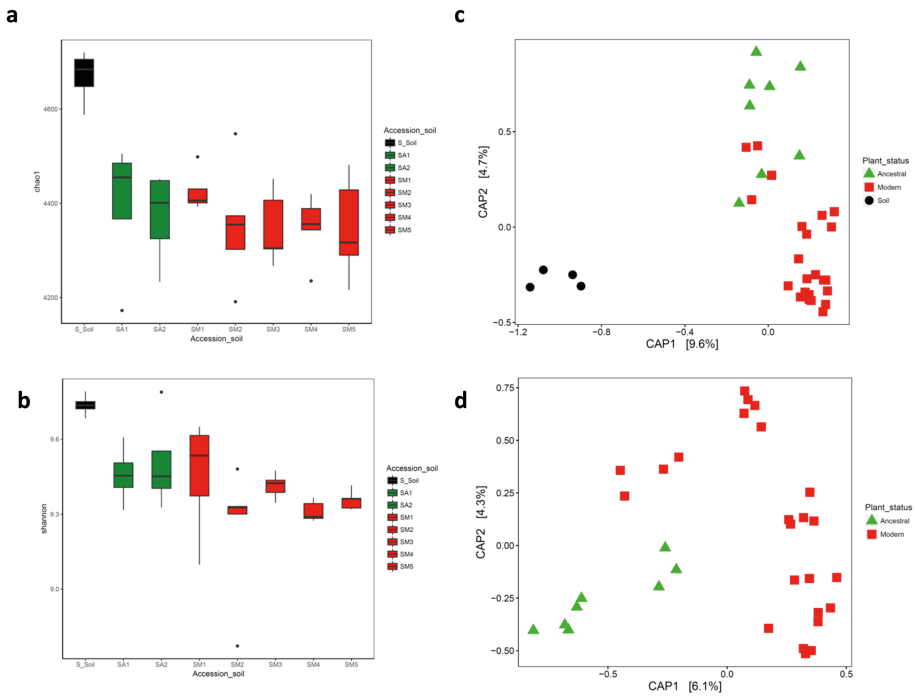

Fig. 1. Bacterial diversity and community structure of spermosphere and bulk soil samples. (a) chao 1 and (b) Shannon were calculated for all bean accessions and the bulk soils samples. The data was rarefied up to 45360 counts per sample. Additionally, Constrained Principal Coordinate Analysis (CAP) of 16S rRNA diversity in the spermosphere of the seven common bean accessions were performed, with (c) and without (d) 16S rRNA diversity in the bulk soil, respectively. Green color was assigned to ancestral accessions, red color was assigned to modern accessions and black color was assigned to bulk soil samples.

The spermosphere significantly impacts bacterial abundances

From the total of 8,263 clustered OTUs, we found that 1,252 OTUs comprised the core spermosphere microbiome of the seven bean accessions. When classified up to genus level, the core microbiome of 1,252 OTUs represented 17.8% of the total number of OTUs and the overall majority (89%) of all the sequence reads detected. The genus *Sphingomonas* was the most abundant genus in the spermosphere core microbiome

(6.6%), followed by *Pseudarthrobacter* (4.8%), *Burkholderia-Paraburkholderia* (2.5%), *Bradyrhizobium* (2.5%) and *Bacillus* (2.4%) (Fig. 2). Another genus found in the core spermosphere microbiome of common bean was *Rhizobium*, with a relative abundance of 1.6%.

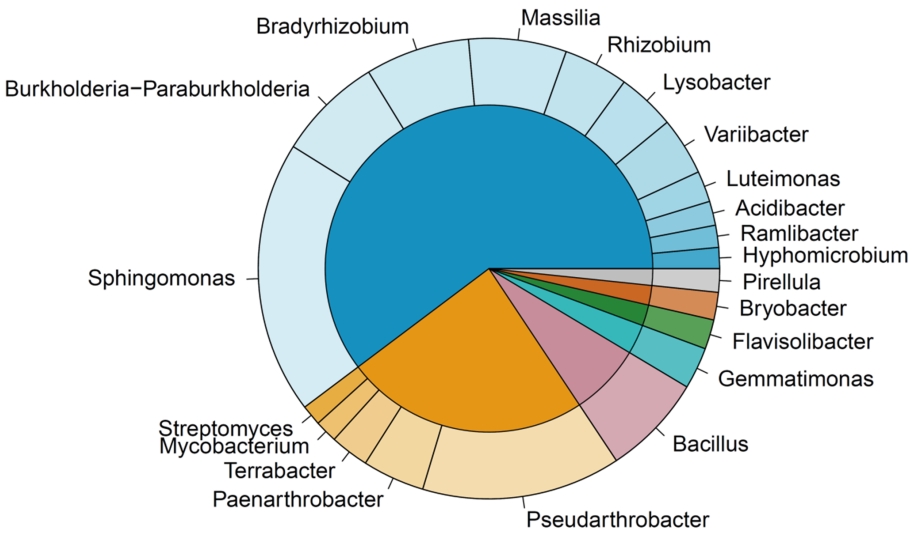

Fig. 2. Core spermosphere microbiome of common bean. The different portions within the inner pie chart represent the bacterial phyla that are part of the spermosphere core microbiome. The outer donut plot represents the genera that are part of the core and each genus assigned to the phylum they belong to. The size of the different pie and donut portions represents the contribution of each phylum/genus to the total relative abundance.

To determine the selective effect of the spermosphere on microbiome composition, the bacterial abundances of spermosphere samples from wild and modern common bean accessions were compared first to bulk soil samples. At Phylum level, a significant increase was observed for Proteobacteria, Firmicutes, Bacteroidetes and Saccharibacteria in the spermosphere of both wild and modern common bean accessions as compared to bulk soil (Fig. S1). Conversely, bacterial phyla such as Chloroflexi, Acidobacteria, Verrucomicrobia and Planctomycetes were significantly more abundant in bulk soil (Fig.

S1). At family level, *Bacillaceae*, *Oxalobacteraceae*, *Burkholderiaceae*, *Rhizobiaceae*, *Xanthomonadaceae*, *Comamonadaceae*, *Methylophilaceae* and *Paenibacillaceae* were more abundant in the spermosphere microbiome of wild bean accessions than in bulk soil (Fig. S2). The spermosphere microbiome of modern bean accessions was enriched with the same bacterial families as well as with *Micrococcaceae*, *Mycobacteriaceae*, *Streptomycetaceae*, *Nocardiaceae Sphingomonadaceae*, and *Sphingobacteriaceae* (Fig. S3). At genus level, *Bacillus, Burkholderia-Paraburkholderia, Rhizobium, Massilia*, *Lysobacter, Paenarthrobacter, Luteimonas, Ramlibacter* and *Paenibacillus* were enriched in the spermosphere of both wild and modern bean accessions as compared to bulk soil (Figs. S4 and S5). The spermosphere of modern accessions was also enriched with *Sphingomonas*, *Pseudarthrobacter, Paenarthrobacter, Mycobacterium, Arenimonas, and Streptomyces* (Fig. S5). Comparing abundances at bacterial family and genus levels between the spermosphere of wild and modern common bean accessions showed that the families *Streptomycetaceae, Mycobacteriaceae* and *Nocardiaceae* were enriched in the spermosphere of the modern common bean accessions (Fig. 3a). At genus level, *Paenarthrobacter*, *Streptomyces*, *Mycobacterium*, *Rhodococcus* and *Geodermatophilus*, all from the phylum Actinobacteria, were enriched in the spermosphere of modern common bean (Fig. 3b).

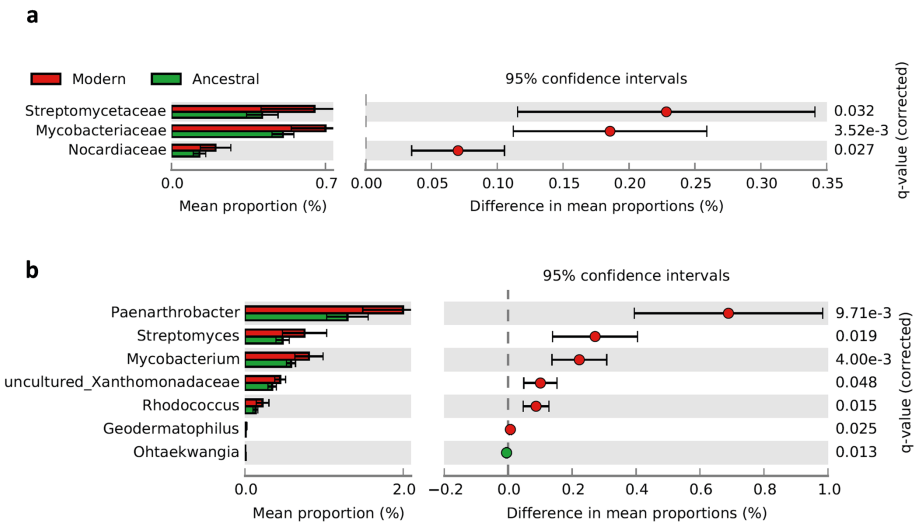

Fig. 3. Relative abundance of bacterial families and genera in the spermosphere of wild and modern bean accessions. Welch's t-tests followed by BH corrections were performed. Only families and genera differentially abundant are shown in the extended error bar plots ($P < 0{,}05$).

Bacterial isolation captured the majority of abundant core microbiome genera

The culture-dependent isolation and 16S rRNA characterization of bacteria from the bean spermosphere showed that from the core microbiome described above, only the Proteobacterial genera *Variibacter*, *Luteimonas*, *Acidibacter* and *Hyphomicrobium* were not isolated on the agar medium used together with a few genera from Acidobacteria, Planctomycetes and Gematimonadetes phyla. Amongst the 596 sequenced isolates, we found in total 67 genera belonging to the Actinobacteria, Proteobacteria, Firmicutes and Bacteroidetes phyla (Fig. S5). Actinobacteria was the most isolated phylum with a total of 306 isolates and 24 genera. *Streptomyces*, *Terrabacter, Arthrobacter, Nocardioides* and *Phycicoccus* were the most abundant genera amongst the Actinobacterial isolates with 66, 47, 24, 19 and 19 isolates obtained, respectively. The second most isolated phylum was Proteobacteria with a total of 185 isolates and 34 genera. *Lysobacter*, *Massilia*, *Burkholderia-Paraburkholderia*, *Rhizobium* and *Sphingomonas* were the most abundant genera within Proteobacteria, comprising 33, 26, 18, 16 and 11 isolates per

genus, respectively. From the Firmicutes, a total of 100 isolates were recovered belonging to 5 genera, with *Paenibacillus* (58 isolates) and *Bacillus* (41) representing the majority. Finally, from the phylum Bacteroidetes, 4 isolates were recovered and classified as *Dyadobacter*, *Flavisolibacter*, *Pedobacter* and *Terrimonas*. Additionally, when the V3-V4 region sequences from the 16S rRNA spermosphere dataset were matched with the sequences from the V1-V4 region from the isolates, we observed that more than 66.6% of the genera identified with the cultivation-independent approach as part of the core (relative abundance >0.5%) were successfully recovered with the cultivation-dependent approach (Table 1).

Seed exudate composition of wild and modern bean accessions

Seed exudates were collected after 24 and 48 hours incubation and, due to limited availability of seeds from all accessions, quantification of amino acids, sugars and organic acids was performed for seeds of wild accession A2 and of modern bean accession M5. In general, the seed exudation profiles observed for modern accession M5 differed from those of wild accession A2 (Fig. 4). Regarding the exudation of amino acids, differences in decreasing order were observed for glutamate, aspartate, asparagine and alanine. For instance, the amount of glutamate in seed exudates of accession M5 was approximately 10 times higher than for accession A2. Also for several other amino acids, including glutamine, valine, threonine, among others, significant differences were observed between the wild and modern bean accessions (Figs. 4a and b). Similarly, the amount of fructose, glucose and sucrose was significantly higher for accession M5 than for accession A2 both at 24 and at 48h (Figs. 4c and d). No differences were observed in the amounts of organic acids (fumaric and malic acid) detected (Fig. 4e).

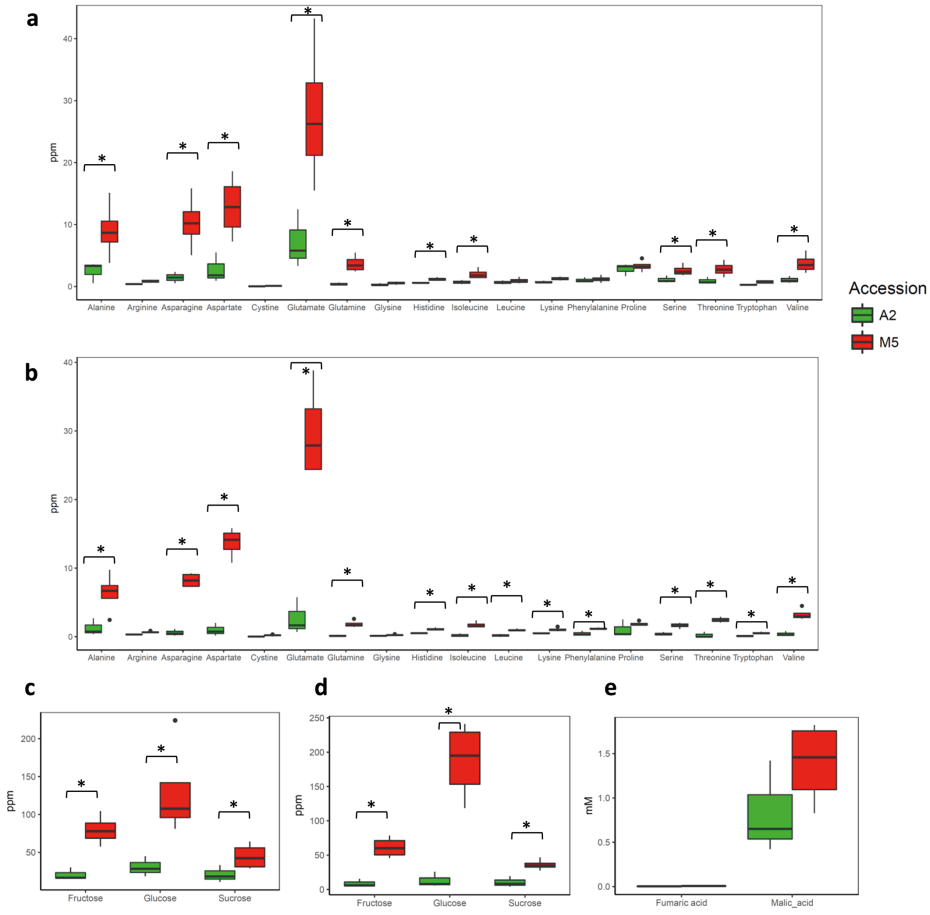

Fig. 4. Aminoacid, sugar and organic acid exudation by seeds of wild and modern common bean accessions A2 and M5. Box plots shows the amount of aminoacids (a and b) sugars (c and d) and organic acids (e), at 24h (a and c) and 48h (b, d and e) after water imbibition. Significant differences in the amount of exudates between the wild (A2 - green) and the modern (M5 - red) accessions were determined by the Wilcoxon signed-rank test ($P < 0,05$)

No clear effect of glutamate on bacterial community composition

Based on the observations that glutamate was the most abundant amino acid released 24 and 48h after seed imbibition, and that Actinobacterial taxa were significantly more abundant in the spermosphere of several modern bean accessions, we hypothesized that

glutamate enriches for Actinobacterial taxa. To begin to test this hypothesis, the same agricultural soil used for the spermosphere microbiome analyses was incubated successively in minimal media supplemented (or not) with glutamate. The results showed that soil samples treated with minimal media (M9) presented a very similar bacteria community composition (Fig. 5a) while soil samples treated with M9 supplemented with glucose, glucose + glutamate, and glucose + cysteine, presented high sample intra- and inter-variability (Fig. 5b). Therefore, the selective impact of glutamate on spermosphere microbiome composition was not apparent. Furthermore, it was observed that the addition of glucose, glutamate or cysteine led to a reduction of the relative abundance of the Actinobacteria as compared to the minimal medium and no specific and statistically significant effects of glutamate on the relative abundance of this phylum were found (Fig. S6).

The dominant bacterial phylum in all treatments was Proteobacteria with several abundant bacterial families dominating the community. In the samples enriched with glutamate, cysteine and glucose, most of the Proteobacterial sequences belonged to the *Pseudomonadaceae* family, while in the treatment with minimal media (M9) most of Proteobacterial sequences were assigned to the *Oxalobacteraceae* family (Figs. S6a and b). The family *Paenibacillaceae* (Firmicutes) was also found enriched as a consequence of the different treatments, especially in the treatment with cysteine. The family *Flavobacteriaceae* was found enriched in all the samples of the glucose treatment and also in a few samples from the glutamate and cysteine treatments; and finally, the family *Rhizobiaceae* was found enriched in almost all the samples from all the treatments; however, this enrichment was very pronounced in some samples, while for other samples within the same treatment the enrichment was minimal. In conclusion, the effect of glutamate on the relative abundance of members of the Actinobacteria phylum was not resolved with the experimental design used in this study.

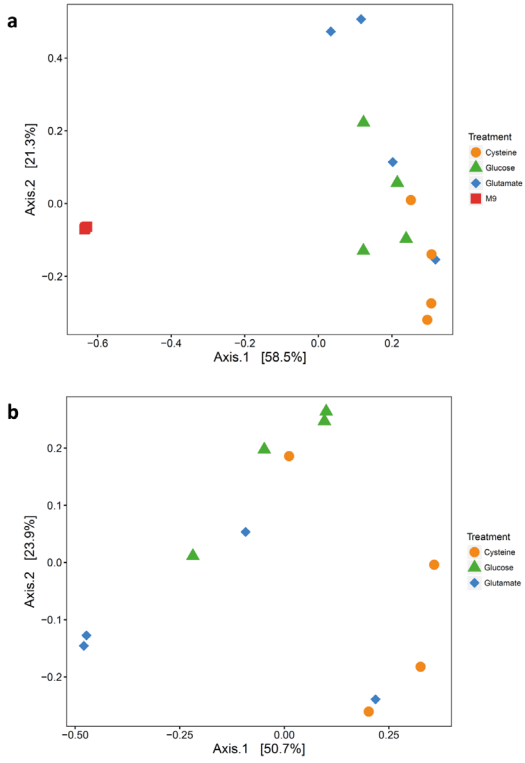

Fig. 5. Bacterial community structure of soil samples exposed to glutamate. To decipher the effect of glutamate on soil bacterial community assembly, four different treatments (n=4) were applied as follows: Soil enriched with M9 or minimal media (red boxes); soil enriched with cystein and glucose (yellow circles); soil enriched with glutamate and glucose (blue diamonds); and finally soil enriched with glucose alone (green triangles). Principal coordinate analysis of bray distances from MiSeq 16S sequence data were calculated, with (a) and without minimal media (M9) samples (b).

Table 1. Bacterial genera from the core isolated through cultivation methods. Taxonomic classification and the number of isolates obtained are shown.

Genus	Number of Isolates
Bacillus	41
Bradyrhizobium	8
Burkholderia-Paraburkholderia	18
Flavisolibacter	1
Lysobacter	33
Massilia	26
Mycobacterium	5
Paenarthrobacter	24
Pseudarthrobacter	17
Ramlibacter	7
Rhizobium	16
Sphingomonas	11
Streptomyces	66
Terrabacter	47

Discussion

In this study we characterized the spermosphere microbiome of wild and modern accessions of common bean grown in an agricultural soil from the Colombian highlands, a centre of common bean diversification. Firstly, we observed a spermosphere effect of seeds of wild and domesticated common bean accessions (*Phaseolus vulgaris*) on soil bacterial richness and evenness, represented by an overall lower bacterial diversity of the spermosphere compartment as compared to bulk soil. This reduction in bacterial diversity suggests that even during this short developmental stage, only 48 hours after sowing, a subset of soil bacterial taxa is enriched around the germinating bean seeds. This reduction in bacterial diversity in the spermosphere was also observed for plant species of the Brassicaceae family (Barret *et al*., 2015). Similarly, reduction of the bacterial richness has also been observed for the rhizosphere, the endosphere and the phyllosphere (Bulgarelli *et al*., 2015; Edwards *et al*., 2015; Ruiz-Pérez *et al*., 2016). The results of our study further showed that the domestication status has an impact, albeit subtle, on the

spermosphere microbiome composition of common bean. These results confirm and extend earlier observations we made for the rhizosphere microbiome of common bean (Pérez-Jaramillo *et al*. 2017). For wild and modern common bean accessions, the genera *Bacillus, Burkholderia-Paraburkholderia, Rhizobium, Massilia*, *Lysobacter, Paenarthrobacter, Luteimonas, Ramlibacter* and *Paenibacillus* were enriched as compared to bulk soil samples. The genera *Bacillus* and *Paenibacillus* have been described as colonizers of common bean seeds (López-López *et al*., 2010). Also the genus *Lysobacter* is common in agricultural soils (Puopolo *et al*. 2018) and known by its ability to produce a diverse set of secondary metabolites with antimicrobial properties that may aid in the control of soil-borne pathogens (Hayward *et al*., 2010; de Bruijn *et al*., 2015; Gómez-Expósito *et al*., 2015). Finally, the genera *Luteimonas* and *Ramlibacter* have also been found associated with different plant compartments (Pfeiffer *et al*., 2016; Wemheuer *et al*., 2017), however, their role as spermosphere abundant taxa remains to be elucidated. The most remarkable difference was the higher relative abundance of several Actinobacterial families in the spermosphere of modern bean accessions as compared to soil. At genus level, these differences in Actinobacterial taxa were mainly explained by a higher abundance of *Pseudarthrobacter*, *Paenarthrobacter* and *Streptomyces*. The genera *Pseudarthrobacter* and *Paenarthrobacter*, formerly classified within the genus *Arthrobacter*, are commonly found as natural soil inhabitants (Busse, 2016). Similarly, the genus *Streptomyces* is recognized as a conspicuous soil bacteria, many species within the genus are part of the plant microbiome and several strains have been reported as plant growth-promoting and effective biocontrol bacteria (Manteca and Sanchez, 2009; Viaene *et al*., 2016).

To what extent, seed endophytic bacteria (Berg and Raaijmakers, 2018) contributed to the genotype-dependent effect on the spermosphere bacterial microbiome composition is not yet known. The bacterial phyla Proteobacteria, Firmicutes and Actinobacteria have

been found as the main seed-associated bacteria for several plant species as well as diverse taxa within the fungal classes Dothideomycetes and Tremellomycetes (López-Velasco *et al*., 2013; Barret *et al*., 2015; Klaedtke *et al*., 2015; Rybakova *et al*., 2017). *Bacillus* and *Paenibacillus* have been also described as seed endophytic (López-López *et al*., 2010). Their high abundance in the spermosphere microbiome as compared to bulk soil may suggest that these seed endophytic bacteria rapidly colonized the spermosphere after imbibition, or that soil populations rapidly colonized the carbon rich spermosphere compartment. The first hypothesis is supported by the low abundances of both genera in bulk soil samples as well as by their very low abundances in rhizosphere samples (Pérez-Jaramillo *et al*., 2017). Most of these microbes are alive, and once the seed germinates, actively colonize the protruding seedling (López-Velasco *et al*., 2013; Adam *et al*., 2016). Under axenic conditions, the seed microbiome and the spermosphere microbiome might share a significant proportion of these endophytic microbial taxa. However, when the seed germinates in soil, the spermosphere microbiome is mainly composed by soil bacteria (Buyer *et al*., 1999). Although the impact of endophytic communities on spermosphere microbiome composition was not evaluated experimentally in our study, we postulate that the spermosphere compartment of common bean is colonized primarily by a subset of soil bacteria and that a small contribution of the endophytic microbiome may reinforce genotype-specific effects of the seeds on the spermosphere microbiome.

The pairwise comparison of the bacterial abundances in the spermosphere of wild and the modern common bean accessions showed that Actinobacterial families and genera were enriched in modern common bean. Despite that Actinobacteria is commonly found associated with plants, it is yet unclear why the association was more prominent with the spermosphere of modern bean. In a recent meta-analysis, we described that rhizospheres of wild plant species are enriched in Bacteroidetes, while Proteobacteria and Actinobacteria are enriched in the rhizosphere of modern plant cultivars (Pérez-Jaramillo

et al., 2018). As a possible explanation we previously proposed that both Actinobacteria and Proteobacteria may benefit more from the type and/or quantity of exudates that modern plants release (Pérez-Jaramillo *et al*., 2018). High competition for resources and space occurs within the spermosphere, and only microorganisms with traits for successful spermosphere competence may succeed. In this sense, fast-growing copiotrophic taxa possess a competitive advantage in the spermosphere compartment as compared to other resident soil bacteria (Lemanceau *et al*., 2017). In the bean spermosphere microbiome, a significant increase in members of the bacterial phyla Proteobacteria, Firmicutes and Bacteroidetes were observed. These groups have been associated with a copiotrophic life strategy (Fierer *et al*., 2007; Uksa *et al*., 2015; Ho *et al*., 2017). Conversely, Chloroflexi, Plactomycetes, Acidobacteria and Verrucomicrobia, generally regarded as oligotrophs (Ho *et al*., 2017), were significantly overrepresented in bulk soil samples. The most abundant genera in the core microbiome of the bean spermosphere belonged to the phylum Proteobacteria, represented by the genera *Sphingomonas*, *Burkholderia-Paraburkholderia, Bradyrhizobium, Massilia* and *Rhizobium*. The genus *Sphingomonas* has been reported as part of the core microbiome of the roots and phyllosphere of other plant species (Chen *et al*., 2018; Hamonts *et al*., 2018). The genus *Paraburkholderia*, also part of the core, is a recent genus that includes former non-pathogenic environmental *Burkholderia* (Eberl and Vandamme, 2016). This genus has been found associated with legume plants as nitrogen fixing bacteria and also was found as the predominant genus within the nodules of common bean plants grown in natural soils (Dall'Agnol *et al*., 2016). The occurrence of *Paraburkholderia* in the core spermosphere microbiome of common bean, together with *Bradyrhizobium* and *Rhizobium*, which are bacterial genera commonly associated with common bean nodules (Aguilar *et al*., 2004; Wang *et al*., 2016), suggests that bacterial genera with nitrogen fixing capabilities are actively recruited to the bean spermosphere compartment, irrespective of the domestication status.

Finally, the genus *Massilia* has been found colonizing seeds and roots of several plant species, and several beneficial traits related with biological control have been described, such as the production of siderophores and extracellular lytic enzymes (Ofek *et al*., 2012). In synthesis, we postulate that the main taxa in the spermosphere core microbiome of common bean are copiotrophic bacteria that may use seed exudates as a carbon source, can establish symbiotic associations with the plant and have the potential to inhibit soil-borne plant pathogens.

In the original definition by Onorato Verona (1958), the spermosphere is regarded as a zone of intense microbial activity around a germinating seed, as a result of carbon deposition (Nelson, 2004). Therefore, the spermosphere microbiome assembly relies, at least in part, on the composition of the seed exudates released by seeds during germination (Lemanceaeu *et al*., 2017). For roots, differences on exudation profiles between wild and modern plant genotypes have been already reported. For instance, wild and primitive genotypes of wheat showed differences in root exudation as compared to modern wheat varieties. Accordingly, higher contents of fructose, galactose and *myo*-inositol were observed in wild and primitive genotypes, while higher contents of isomaltose, sucrose, hexadecanoic acid, octadecanoic acid and 1-octaconasol were detected in modern wheat varieties (Iannucci *et al*., 2017). In this study we observed that common bean seeds released several amino acids, organic acids and sugars during imbibition and germination. The main difference in seed exudation profiles between wild and modern accessions was related with the quantity of the compounds released by the seed. We observed higher quantities of various exudate constituents by the modern accession M5 as compared to wild accession A2. It is important to emphasize that the collection of seed exudates was performed *in vitro*, while the characterization of the spermosphere microbiome was performed directly in soil. The exudate composition observed for the two accessions after 48 hours may not directly correspond to the exudate composition in the soil after the same

time, and in consequence a particular spermosphere microbiome composition may not be the consequence of a particular set of exudates detected in vitro. This may have affected the results of the assay in which soil was treated with different amino acids minimal media and glucose as a carbon source. As glutamate was the most abundant amino acid released by modern bean accessions *in vitro*, and Actinobacterial taxa were significantly more abundant in the spermosphere microbiome, we hypothesized that glutamate could have a positive effect on the abundance of actinobacterial taxa. What we observed was that the Phylum Actinobacteria responded negatively to the treatments, while Proteobacteria and to a lesser extent Firmicutes, responded positively. Most probably, the concentration of glucose was optimal for the growth of competitive and fast growing taxa within Proteobacteria and Firmicutes. In order to accurately evaluate the effect of glutamate on soil bacterial communities, an optimization of glucose and amino acid levels in the culture media is needed. Furthermore, it is pivotal to validate whether glutamate is also a more abundant seed exudate in the spermosphere of modern accessions germinating in soil.

Finally, 66.6% of the genera identified as part of the core spermosphere microbiome were succesfully isolated by common culture-dependent methods. Furthermore, it was possible to match MiSeq V3-V4 metagenome sequence data with the V1-V4 sequence data from the isolate collection (Table S1). This isolate repository will be tested in future experiments, either alone or in consortia, for their ability to colonize the spermosphere of common bean accessions and to utilize specific exudate components as well as for their effects on growth and health of common bean.

Conclusions

The spermosphere is an active and dynamic compartment that, despite its short duration, can impact the diversity and abundance of soil bacterial communities. A subtle but significant effect of the domestication of common bean was revealed in the spermosphere

microbiome composition, where modern accessions showed a higher abundance of several Actinobacterial taxa. The spermosphere core microbiome was composed of several copiotrophic bacteria, with several bacteria capable of fixing nitrogen. Additionally, several taxa within the core spermosphere microbiome have been reported as plant growth promoting bacteria and with biocontrol activities. Several bacterial genera found in the core were isolated through culture-dependent methods and the resulting bacterial repository will be the basis for future experiments to validate their differential ability to colonize the spermosphere of wild and modern bean accessions. Finally, it was not possible to validate *in vitro* the role of glutamate, which was the most abundant amino acid detected in seed exudates of the modern bean accession M2. Optimization of the culture media and glucose levels is needed in order to accurately determine the selective impact of glutamate on soil bacterial communities, especially for Actinobacterial taxa.

Supplementary materials

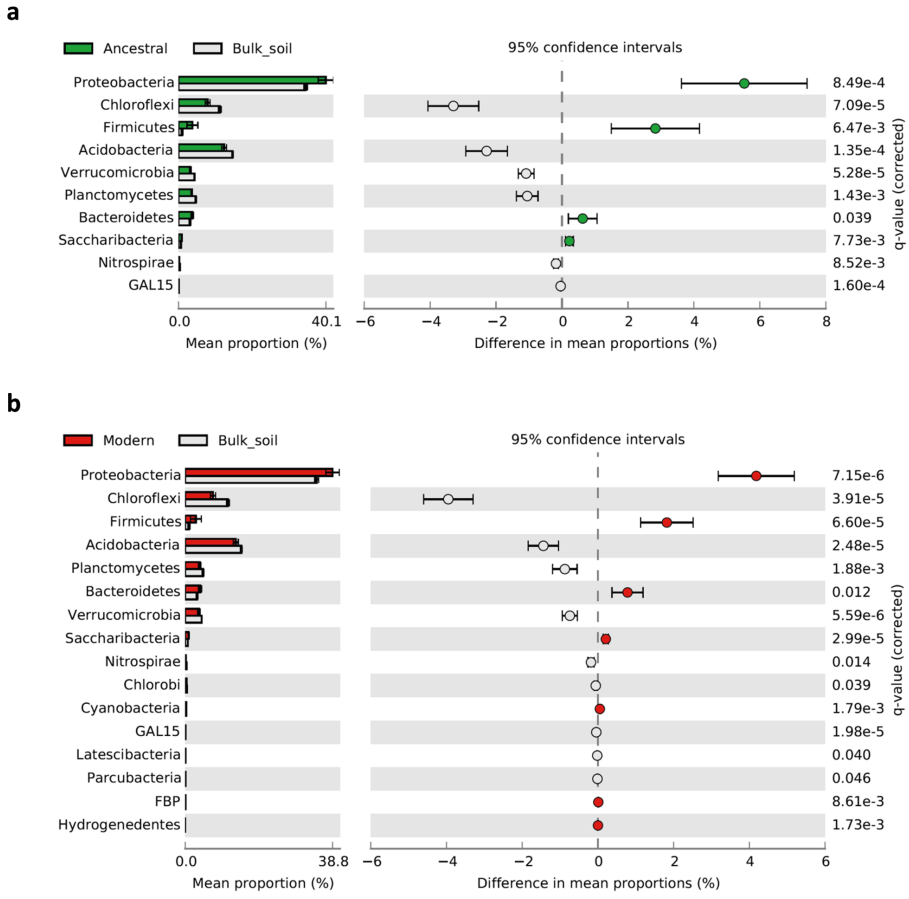

Fig. S1. Differential abundance of bacterial phyla in spermosphere and bulk soil samples. Extended error bar plots showing differentially abundant phyla between (a) the spermosphere microbiome of ancestral and (b) modern common bean accessions and bulk soil samples. Welch's t-tests followed by BH corrections were performed between merged spermosphere samples by domestication status and bulk soil samples. Only differentially abundant Phyla are shown.

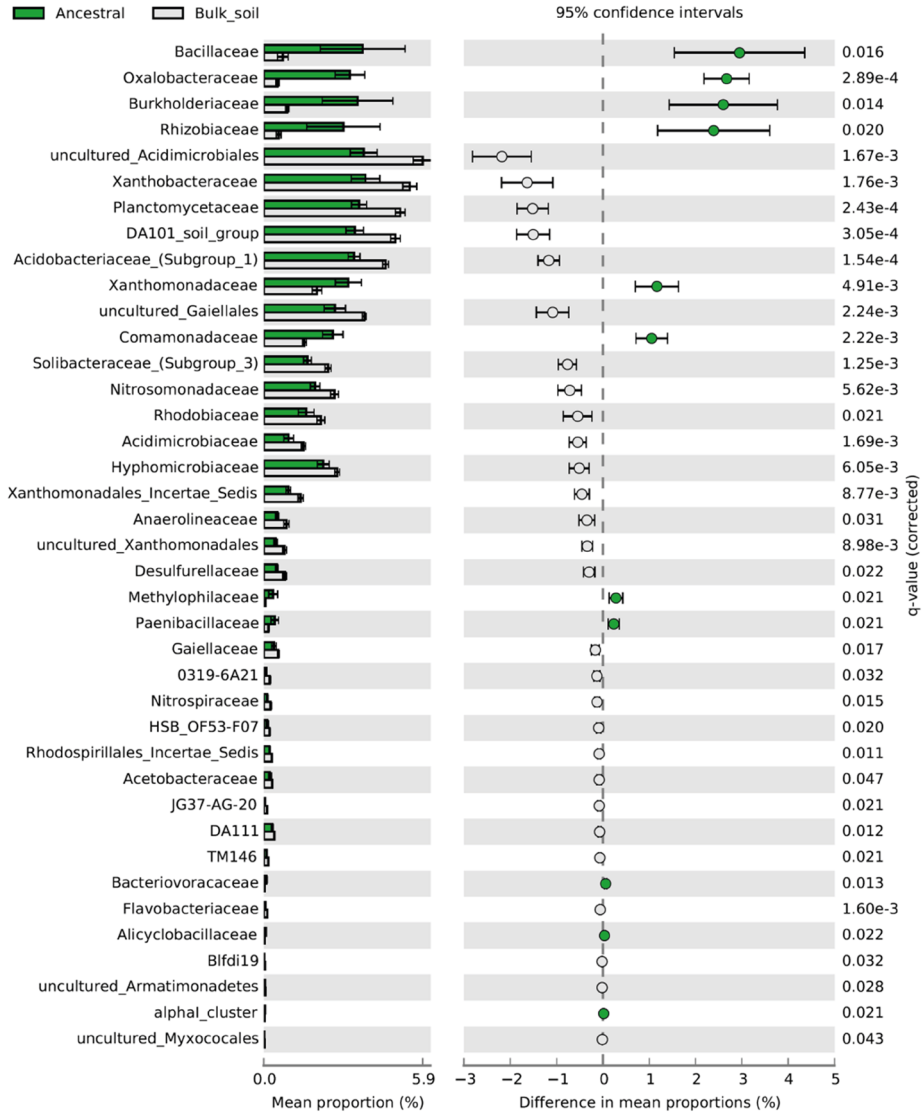

Fig. S2. Differential abundance of bacterial families in the spermosphere of ancestral common bean and bulk soil samples. Extended error bar plots showing differentially abundant families between the spermosphere microbiome of ancestral common bean accessions and bulk soil samples. Welch's t-tests followed by BH corrections were performed. Only differentially abundant families are shown.

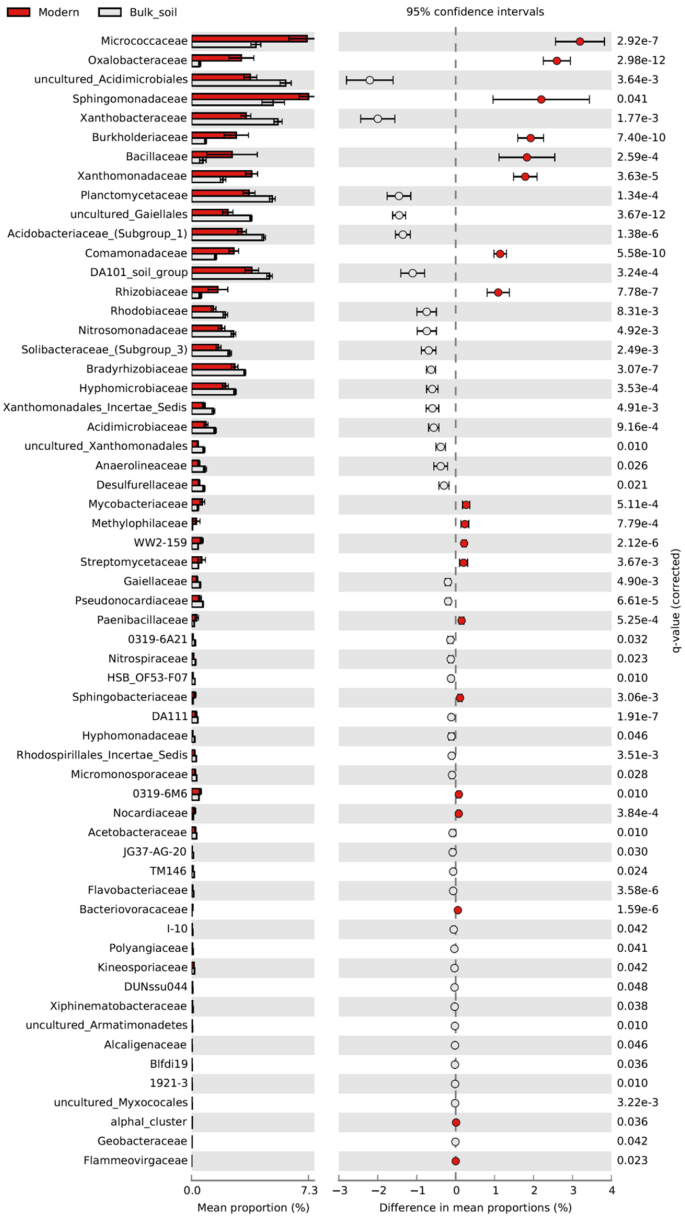

Fig. S3. Differential abundance of bacterial families in the spermosphere of modern common bean and bulk soil samples. Extended error bar plots showing differentially abundant families between the spermosphere microbiome of modern common bean accessions and bulk soil samples. Welch's t-tests followed by BH corrections were performed. Only differentially abundant families are shown.

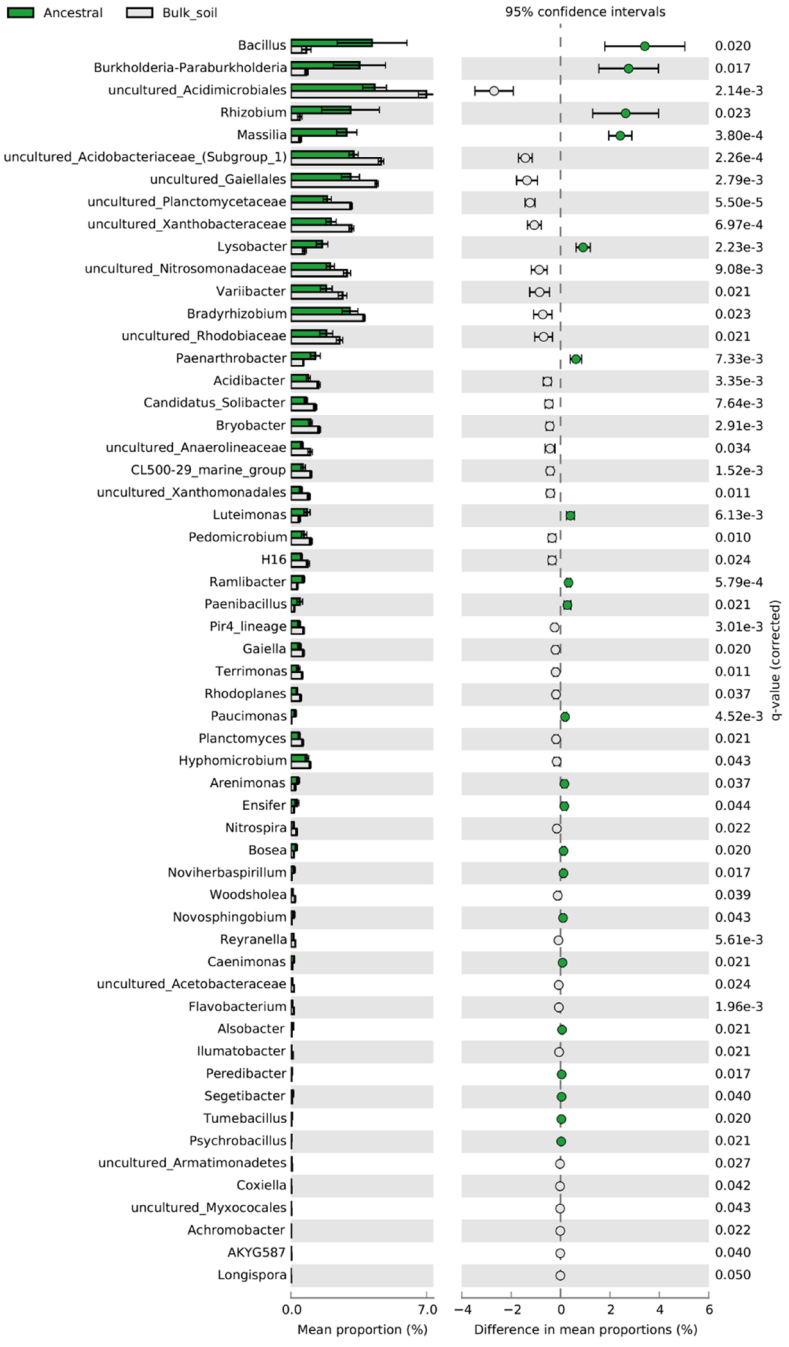

Fig. S4. Differential abundance of bacterial genera in the spermosphere of ancestral common bean and bulk soil samples. Extended error bar plots showing several differentially abundant genera between the spermosphere microbiome of ancestral common bean accessions and bulk soil samples. Welch's t-tests followed by BH corrections were performed.

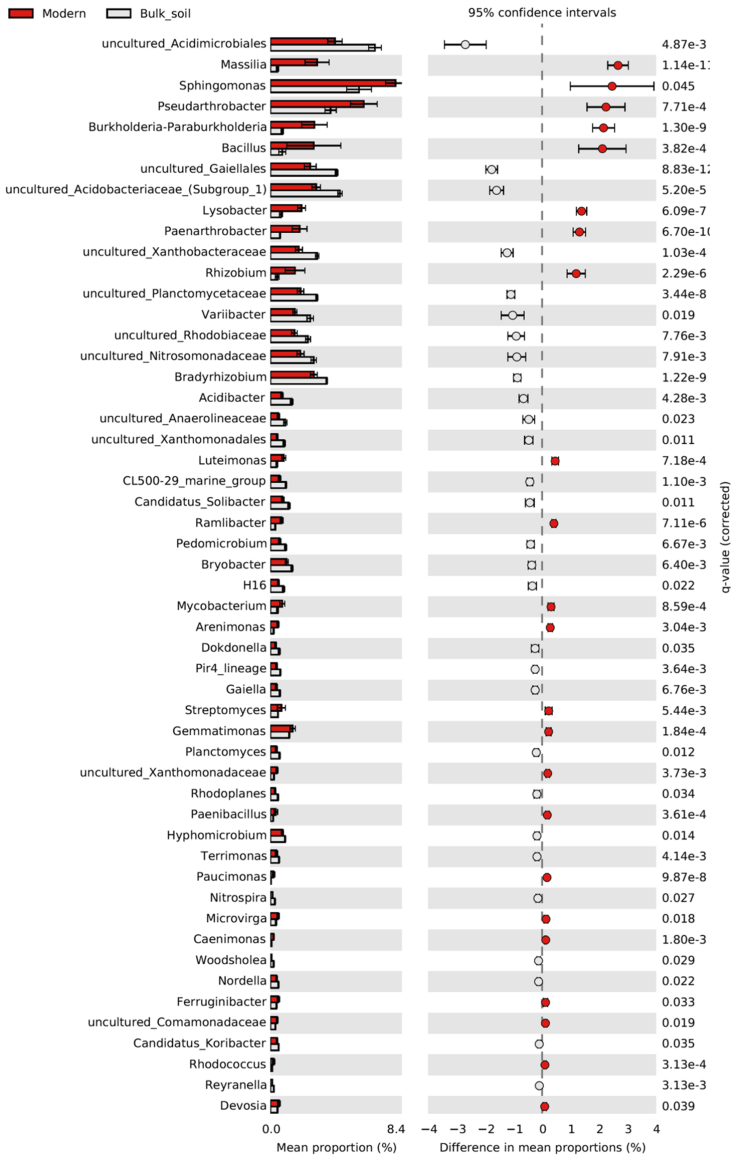

Fig. S5. Differential abundance of bacterial genera in the spermosphere of modern common bean and bulk soil samples. Extended error bar plots showing several differentially abundant genera between the spermosphere microbiome of modern common bean accessions and bulk soil samples. Welch's t-tests followed by BH corrections were performed.

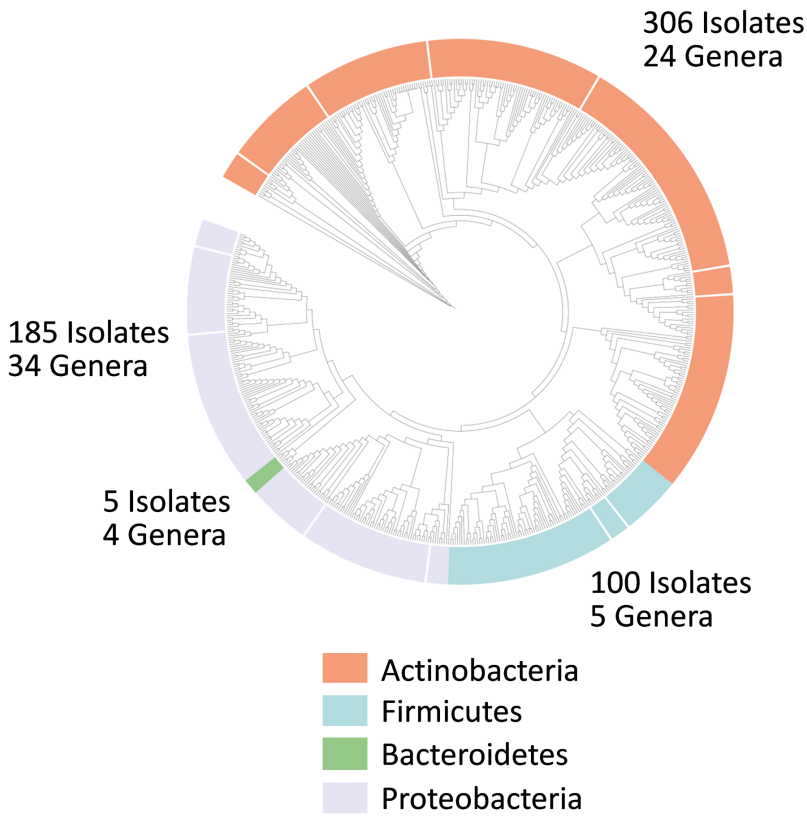

Fig. S6. Phylogenetic tree of bacterial strains isolated from common bean spermosphere. The four bacterial phyla identified are shown with colors within the tree. Additionally, the number of genera and isolates per bacterial phyla are indicated.

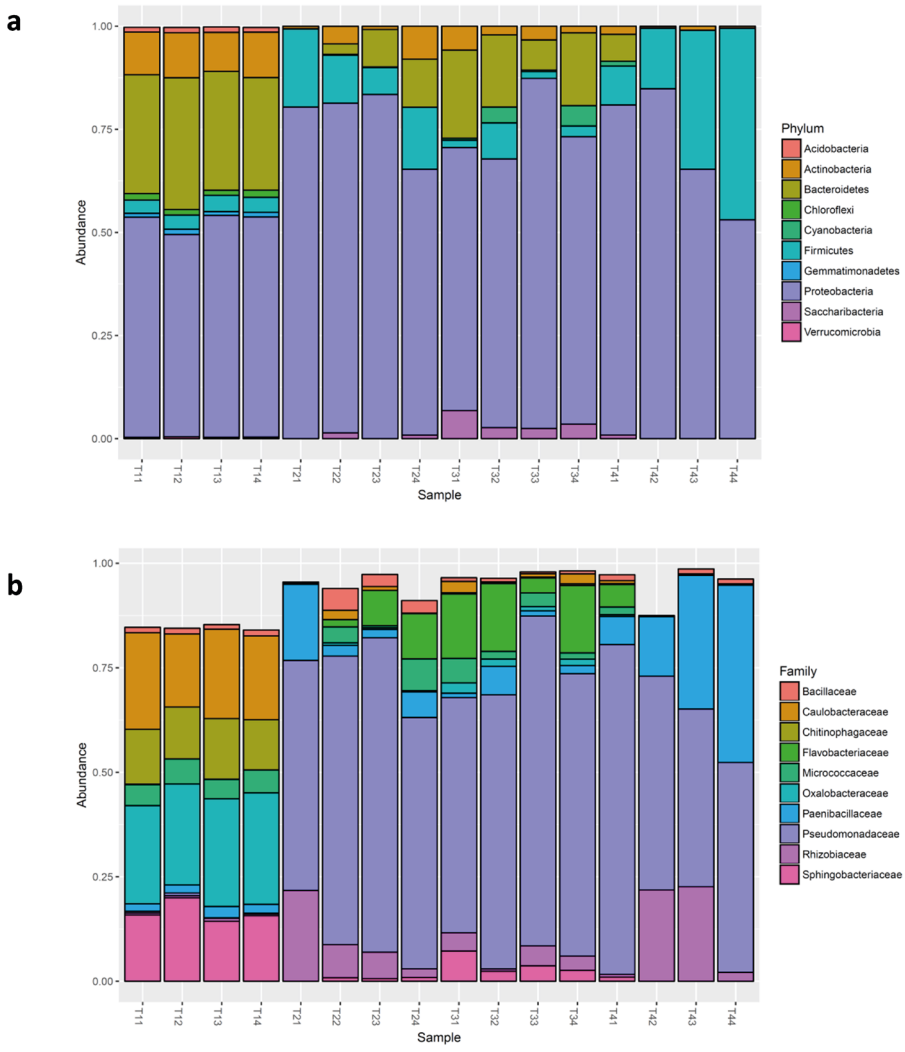

Fig. S7. Bar graphs of the most abundant phylum and families in soil under treatment with different amino acids minimal media and glucose. T1 (minimal media treatment) consisted of M9 minimal media as a control treatment; T2 (glutamate treatment) consisted of M9 minimal media, 10 mM of glutamate, 1 mM of $MgSO_4$, and glucose 20%; T3 (glucose treatment) consisted of M9 minimal media, 1 mM of $MgSO_4$, and glucose 20%; and T4 (cysteine treatment) consisted of M9 minimal media, 10 mM of cysteine, 1 mM of $MgSO_4$, and glucose 20%. The numbers following the treatment correspond to the replicate number.

Table S1. Match of MiSeq V3-V4 spermosphere data and the V1-V4 Sequences from the isolate collection. Taxonomic classification, the number of reads obtained with MiSeq sequencing, and the number of isolates retrieved per OTU_ID is shown.

Phylum	Genus	OTU_ID	Spermosphere MiSeq reads	Isolates matched
Proteobacteria	*Sphingomonas*	OTU_2	67739	1
Actinobacteria	*Pseudarthrobacter*	OTU_4	61652	2
Proteobacteria	*Bradyrhizobium*	OTU_3	27957	1
Gemmatimonadetes	uncultured_*Gemmatimonadaceae*	OTU_18	20328	**
Actinobacteria	*Paenarthrobacter*	OTU_14	19375	1
Proteobacteria	uncultured_*Xanthobacteraceae*	OTU_12	19164	**
Firmicutes	*Bacillus*	OTU_42	16161	2
Proteobacteria	uncultured_*Rhodobiaceae*	OTU_22	13817	**
Actinobacteria	*Terrabacter*	OTU_30	12070	2
Acidobacteria	*RB41*	OTU_37	11979	**
Proteobacteria	*Rhizobium*	OTU_9	8647	1
Proteobacteria	unclassified_*JG34-KF-161*	OTU_6***	6819	2
Proteobacteria	*Devosia*	OTU_20	5333	1
Actinobacteria	*Streptomyces*	OTU_8	4660	1
Proteobacteria	*Rhizobium*	OTU_1	3528	2
Proteobacteria	*Rhizobium*	OTU_253	2004	2
Proteobacteria	unclassified_*Comamonadaceae*	OTU_11	1057	**
Proteobacteria	unclassified_*Comamonadaceae*	OTU_21	972	1
Bacteroidetes	*Dyadobacter*	OTU_5*	826	1
Proteobacteria	unclassified_*Sphingomonadaceae*	OTU_16****	740	1
Bacteroidetes	*Terrimonas*	OTU_1424,OTU_1906,OTU_2003,OTU_202,OTU_979	639	1

*With MiSeq/Sanger matched with several OTUs: OTU_10611,OTU_11077 and OTU_5.
**Not available in the collection
***With MiSeq/Sanger as: Sphingomonadaceae; *Sphingomonas*.
****With MiSeq/Sanger as: Sphingomonadaceae; *Novosphingobium*.