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ORIGINAL ARTICLE

Linking rhizosphere microbiome composition of wild and domesticated *Phaseolus vulgaris* to genotypic and root phenotypic traits

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Plant domestication was a pivotal accomplishment in human history, but also led to a reduction in genetic diversity of crop species compared to their wild ancestors. How this reduced genetic diversity affected plant–microbe interactions belowground is largely unknown. Here, we investigated the genetic relatedness, root phenotypic traits and rhizobacterial community composition of modern and wild accessions of common bean (*Phaseolus vulgaris*) grown in agricultural soil from the highlands of Colombia, one of the centers of common bean diversification. Diversity Array Technology-based genotyping and phenotyping of local common bean accessions showed significant genetic and root architectural differences between wild and modern accessions, with a higher specific root length for the wild accessions. Canonical Correspondence Analysis indicated that the divergence in rhizobacterial community composition between wild and modern bean accessions is associated with differences in specific root length. Along the bean genotypic trajectory, going from wild to modern, we observed a gradual decrease in relative abundance of Bacteroidetes, mainly *Chitinophagaceae* and *Cytophagaceae*, and an increase in relative abundance of Actinobacteria and Proteobacteria, in particular *Nocardioidaceae* and *Rhizobiaceae*, respectively. Collectively, these results establish a link between common bean domestication, specific root morphological traits and rhizobacterial community assembly.
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Introduction

The rhizosphere microbiome has a profound impact on plant health and growth by providing key functions involved in nutrient acquisition, abiotic stress tolerance and protection against pathogen infection (Mendes *et al.*, 2011, 2013; Bulgarelli *et al.*, 2013). Edaphic factors and plant genotype shape, to a certain extent, the composition and metabolic activities of the bacterial communities in the rhizosphere (Berg and Smalla, 2009; Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012; Philippot *et al.*, 2013). The effects of the plant genotype on rhizosphere microbiome composition has been proposed to be, at least in part, mediated by quantitative and qualitative differences in root exudate composition (Lakshmanan *et al.*, 2012; Badri *et al.*, 2013; Carvalhais *et al.*, 2013; Lebeis *et al.*, 2015). Hence, the composition of a particular rhizosphere microbial assemblage is dependent on the plant species (Turner *et al.*, 2013; Ofek *et al.*, 2014) and even on the cultivar of a given plant species (Peiffer *et al.*, 2013).

Plant domestication was essential to human history but also resulted in a significant reduction in genetic diversity of crop species as compared to their wild ancestors (Doebley *et al.*, 2006). Whether this reduction in genetic diversity affected specific root morphological traits and microbial diversity and activity in the rhizosphere is still largely unknown. To date, a limited number of studies have indicated that rhizosphere microbiome assembly may have

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been affected in modern cultivars of plants as compared to their wild ancestors (Bulgarelli *et al.*, 2015; Leff *et al.*, 2016; Pérez-Jaramillo *et al.*, 2016). In this context, wild relatives and also landraces have been proposed to provide valuable new insight into plant traits and genes associated with microbiome assembly, allowing an integral role of microbiome assembly in future plant breeding programs. For most economically important food crops, however, little knowledge is available on the impact of plant domestication on root traits and rhizosphere microbiome assembly. Here, we determined the genetic relatedness and root morphological traits of wild and modern accessions of common bean (*Phaseolus vulgaris*) and analyzed their rhizosphere microbiome composition. Common bean is the most important legume crop for low-income farmers in Latin America and Africa (Broughton *et al.*, 2003; Akibode and Maredia, 2011). Wild common bean originated in central Mexico and spread throughout Central and South America (Gepts, 1998; Bitocchi *et al.*, 2012; Desiderio *et al.*, 2013). This wide distribution led to geographical isolation of wild common bean and resulted in well-characterized genetic pools (Gepts and Bliss, 1985). A vast collection of available accessions, ranging from wild relatives to highly productive modern varieties, makes common bean a good model system to investigate the impact of domestication on root phenotypic traits and on rhizobacterial community composition of an economically important food crop. Furthermore, common bean and other leguminous plant species provide excellent experimental systems to study the intertwined relationships between nodulation and rhizosphere microbiome assembly (Zgadzaj *et al.*, 2016).

In this study, we adopted the approach of 'going back to the roots' (Pérez-Jaramillo *et al.*, 2016) and selected eight Colombian accessions of common bean, including wild relatives, landraces and modern cultivars and characterized their genetic relatedness by Diversity Array Technology (Jaccoud *et al.*, 2001). Subsequently, the selected bean accessions were grown in agricultural soil collected from the highlands of Antioquia, Colombia. Colombian mountains are considered an important center of common bean diversification where wild and landraces of common bean from the two main genetic pools (Mesoamerican and Andean) can still be found in their natural habitats (Gepts and Bliss, 1986). The selected common bean accessions were subjected to phenotypic analyses of different root traits as well as rhizobacterial community analyses by 16S rRNA amplicon sequencing.

Materials and methods

Selection of common bean accessions and Colombian soil

Two wild, three landraces and three improved varieties (modern cultivars) of common bean (*Phaseolus vulgaris*) were selected based on the following characteristics: they belong to the Colombian Mesoamerican genetic pool; landraces and modern accessions are the same race, they exhibit the Mesoamerican phaseolin protein type; they originate from the same altitudinal range; and they have the same growth type (that is, climbing instead of bushy). The latter characteristic is the case for all selected accessions, except for accession G5773, which is a bushy commercial variety widely distributed and commonly used in Latin America and Africa. The seeds were kindly provided by the Genetic Resources Program at the International Center for Tropical Agriculture—CIAT—in Palmira, Colombia. The plant passport is given in Supplementary Table S1. The soil used in this study was collected from an agricultural field in the rural area of the municipality of El Carmen de Viboral (Antioquia—Colombia, 6°4′55″ N, 75°20′3″W). Common bean has been cropped in this region for decades and soil conditions are optimal for the growth of several common bean varieties. The soil was collected at three random sites in the field from a depth up to 30 cm, air dried, passed through a 2-mm mesh sieve to remove (plant) debris and stored for further use. Physicochemical analyses were performed in the Soil Science Laboratory from the National University of Colombia in Medellín, using standard procedures (Supplementary Table S2).

Genotyping of common bean accessions

The bean seeds were surface-sterilized and germinated on filter paper wetted with sterile tap water. After 2–5 days, germinated seeds were transferred to 500-ml pots filled with agricultural soil. For each bean accession, two seedlings were transplanted to a pot (1 pot per accession), arranged randomly in a growth chamber (25 °C, 16 h daylight) and watered every day. After 10 days, the youngest leaf of each plant was collected and DNA was isolated with the PowerPlant Pro DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA). The manufacturer's instructions were followed and the yield and quality of the DNA was assessed via agarose gel electrophoresis and a Qubit 2.0 fluorimeter (ThermoFisher Scientific, Waltham, MA, USA). Genome profiling was performed using the complexity genome reduction method developed by Diversity Arrays Technology Pty Ltd (DArT P/L, Bruce, Australia) (Jaccoud *et al.*, 2001). A proprietary analytical pipeline developed by DArT P/L was used to produce single nucleotide polymorphisms (SNP) tables; in total 10 732 SNPs were obtained. The SNP calling was performed using a custom R script (R Core Team, 2015) and after filtering, a total of 7527 SNPs were retained for further analysis.

Plant genetic diversity

An identity-by-state distance matrix was constructed in PLINK (v. 1.9) (Chang *et al.*, 2015) and a neighbor-joining phylogenetic tree was created using the Phylip software package (v.3.695). For the quantitative assessment of the number of groups in the panel, a Bayesian clustering analysis was performed using the model-based approach implemented in the STRUCTURE software (Pritchard *et al.*, 2000). This approach uses multi-locus genotypic data to assign individuals to clusters or groups (K) without prior knowledge of their population affinities and assumes loci in Hardy–Weinberg equilibrium. The software was ran considering K-values ranging from 1 to 6 (hypothetical number of groups) with an admixture model with correlated allele frequencies. Each run was implemented with 20 000 burnin iterations followed by 200 000 MCMC (Markov Chain Monte Carlo) iterations for accurate parameter estimates. Five independent runs for each K were performed. The number of genetic groups was estimated using the STRUCTURE HARVESTER software (Earl, 2012), by the Evanno criterion (Evanno *et al.*, 2005). A multidimensional scaling analysis was also performed using PLINK. The inbreeding coefficient and occurrence of homozygous segments were computed using the commands '*–het*' and '*—homozyg*' in PLINK. The number of homozygous regions as well as their genomic locations was determined for each bean accession. Similarity of bean accession G51283K1 to the other accessions was determined by computing pairwise identity-by-state. The genome was divided into 109 blocks and within each block pairwise identity-by-state was calculated for all bean accessions; zero is completely different and two is completely identical. The accession G51283K1 was compared with the whole genomes of G22304 and landrace G23998 as wild accessions, with modern accessions G5773 and G51695 as modern accessions and with landrace G50632I1. All the genetic diversity and homozygosity analyses were performed in PLINK (v1.9) and visualized in R.

Root morphology

Seeds were germinated as described above and transferred to 3 l pots filled with the agricultural soil described above. Three plants per genotype were used. The plants were grown under ambient environmental conditions, with an average temperature of 25 °C and 12 h of daylight. When the V4 stage (3rd trifoliate leaf) was reached, the plants were carefully harvested and the root system was gently washed with tap water until no more soil particles were attached to the roots. Subsequently the entire root system was dyed with methylene blue, laid out on a Scanjet G4050 Scanner (Hewlett-Packard, Palo Alto, CA, USA) and scanned with a resolution of 600 dpi. The images were then analyzed with the software WinRHIZO (Regent Instruments Inc., Quebec, QC, Canada), and several root measurements were recorded (Supplementary Table S3). After scanning, roots were dried and root dry weight (rdw) measured. Subsequently, we computed the specific root length (SRL) using the equation *rl*/*rdw*, and the root tissue density (D), using the equation *rdw*/*rv* (Martín-Robles *et al.*, 2015). These parameters were calculated, normality and homogeneity of variances were checked using Shapiro–Wilk test and Levene's test, respectively, and one way ANOVA and *post hoc* tests were used to assess differences in root morphology between the bean accessions.

Rhizospheric soil collection and DNA isolation

The same procedure for seed germination described above was followed. Seedlings were transferred to 3l pots containing the agricultural soil. For each accession, four replicates were used with one plant per replicate pot. The plants were arranged randomly in a greenhouse with ambient environmental conditions with an average temperature of 25 °C and 12 h of daylight. Four pots with the same amount of soil but without plants were used as controls and served as bulk soil samples. Plants were collected at flowering to synchronize microbiome analyses for all accessions at the same phenological growth stage. Rhizospheric soil was collected according to the method of Lundberg *et al.* (2012). Briefly, the entire root system was sampled from the pots, soil loosely attached to the roots was removed and subsequently the entire root system was divided in three parts and each was transferred to a 15 ml tube containing 5 ml of LifeGuard Soil Preservation Solution (Mo Bio Laboratories). The tubes were vigorously shaken, the roots were removed and at least 1 g (wet weight) of rhizospheric soil was recovered per sample for DNA isolation. For the bulk soils, approximately 1 g of soil was collected from each control pot and also submerged in 5 ml of LifeGuard solution. Root dry weight, number of days to reach flowering and the total numbers of nodules per root system were scored. To obtain rhizospheric DNA, a RNA PowerSoil DNA Elution Accessory Kit (Mo Bio Laboratories) was used according to manufacturer's instructions after a previous step for RNA extraction and elution with a RNA PowerSoil Total RNA Isolation Kit (Mo Bio Laboratories). Each obtained DNA sample was then cleaned with the PowerClean DNA Clean-Up Kit (Mo Bio Laboratories). Agarose gel electrophoresis and a ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) were used to check DNA yield and quality. DNA samples were stored at − 80 °C until further use.

16S rRNA amplicon sequencing

The DNA extracted from the rhizosphere was used for amplification and sequencing of the 16S

rRNA, targeting the variable V3-V4 regions (Forward Primer =5′-CCTACGGGNGGCWGCAG-3′
Reverse Primer = 5′-GACTACHVGGGTATCTAATCC-3′) resulting in amplicons of approximately ~460 bp. Dual indices and Illumina sequencing adapters using the Nextera XT Index Kit were attached to the V3–V4 amplicons. Subsequently, library quantification, normalization and pooling were performed and MiSeq v3 reagent kits were used to finally load the samples for MiSeq sequencing. For more info please refer to the guidelines of Illumina MiSeq System (Illumina, 2013).

The RDP extension to PANDASeq (Masella *et al.*, 2012), named Assembler (Cole *et al.*, 2014), was used to merge paired-end reads with a minimum overlap of 10 bp and at least a Phred score of 25. Primer sequences were removed from the per sample FASTQ files using Flexbar version 2.5 (Dodt *et al.*, 2012). Sequences were converted to FASTA format and concatenated into a single file. All reads were clustered into operational taxonomic units (OTUs) using the UPARSE strategy by de-replication, sorting by abundance with at least two sequences and clustering using the UCLUST smallmem algorithm (Edgar, 2010). These steps were performed with VSEARCH version 1.0.10 (Rognes *et al.*, 2015), which is an open-source and 64-bit multi-threaded compatible alternative to USEARCH. Next, chimeric sequences were detected using the UCHIME algorithm implemented in VSEARCH (Edgar *et al.*, 2011). All reads before the dereplication step were mapped to OTUs using the usearch_global method implemented in VSEARCH to create an OTU table and converted to BIOM-Format 1.3.1 (McDonald *et al.*, 2012). Finally, taxonomic information for each OTU was added to the BIOM file by using the RDP Classifier version 2.10 (Cole *et al.*, 2014). All steps were implemented in a Snakemake workflow (Köster and Rahmann, 2012). The OTU table was filtered using QIIME (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 and chloroplast sequences were discarded with the command *filter_otus_from_otu_table.py,* obtaining a filtered OTU table for further analysis.

Rhizobacterial diversity and link with genotypic and root phenotypic traits

The alpha diversity was calculated using QIIME customs scripts. The command *alpha_rarefaction.py* was used to rarefy the OTU table to counts up to 50 000 reads. This was the lowest sequencing depth obtained from a sample and therefore used as a threshold for rarefaction and alpha diversity calculations (Gotelli and Colwell, 2001). The *alpha_diversity.py* command was applied to rarefied data and observed OTUs, Shannon, Chao1 and Faith's Phylogenetic Diversity metrics were obtained. One-way ANOVA and Tukey HSD were performed in R. For the Beta-diversity calculations, the entire filtered OTU table was used and normalized using the function *cumNorm* from the R package metagenomeSeq (v.1.12) (Paulson *et al.*, 2016). We used a cumulative-sum scaling method, which calculates the scaling factors equal to the sum of counts up to a particular quantile to normalize the read counts in order to avoid the biases generated with current sequencing technologies due to uneven sequencing depth (Paulson *et al.*, 2013). Bray–Curtis dissimilarity matrix was calculated and used it to build Principal Coordinate Analyses and Constrained Principal Coordinate Analysis constrained by phylogenetic group, that is, ancestral (A1 and A2) and modern (M1 to M5), using the function *capscale* retrieved from Vegan package (Oksanen *et al.*, 2016) (v.2.3-2) and implemented in the Phyloseq package (McMurdie and Holmes, 2013) (v.1.10), both in R. The nonparametric *adonis* test was used to assess the percentage of variation explained by the Phylogenetic grouping along with its statistical significance. Permutational multivariate analyses of variance were performed to evaluate the significance of the constrained principal coordinate analyses, both retrieved from Phyloseq and Vegan packages. A Regularized Canonical Correlation Analysis was also performed in order to graphically depict whether the genetic make-up of the bean accessions correlates with their rhizobacterial community structures, using the R package CCA (González and Déjean, 2012). The function rcc was used, which is an extension of the Canonical Correlation Analysis to seek correlations between two data matrices. Subsequently, the function plt.cc was used to generate the plots. Canonical Correspondence Analysis (CCA, Canoco 5.0) was also conducted with a complete set of cumulative-sum scaling normalized counts of the 16S rRNA data and the root morphological traits SRL, Root Density, Root Dry weight and Number of Nodules. The adjusted explained variation and unrestricted permutations were calculated to determine the significant contribution of each variable and Bonferroni corrections were applied to adjust the *P*-values. Constrained ordinations were built using rhizobacterial phyla or families together with plant genotypic and root morphological traits as explanatory variables.

Species abundance distribution and differential abundance analysis

Species abundance distribution models were used to determine whether neutral or niche-based mechanisms were governing the bacterial assembly. We used the command Radfit from the R package Vegan to evaluate broken stick, pre-emption, log-normal, Zipf and Zipf–Mandelbrot rank abundance models and a zero-sum multinomial (ZSM) model using the TeTame2 software (Jabot *et al.*, 2008). The comparison of the models fit was done based on the Akaike Information Criterion using the equation Akaike

Information Criterion = − 2 log-likehood+2 × npar (Mendes *et al*., 2014). Akaike Information Criterion values were compared, being the lowest selected as the best fit to the data (Dumbrell *et al*., 2010). To compare the differences in taxonomic composition and to assess whether some bacterial taxa were

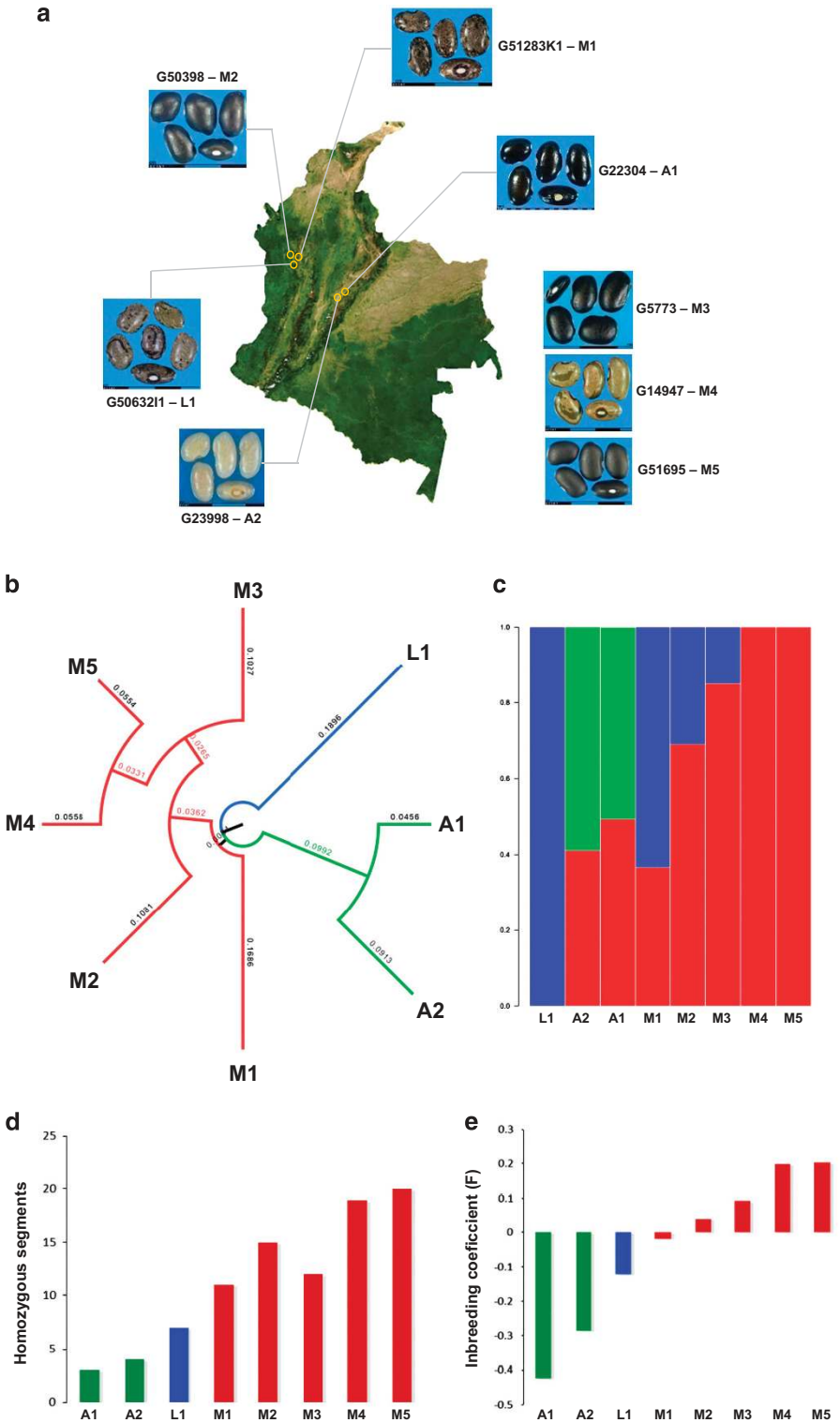


Figure 1 Origin and genetic structure of the common bean accessions. (**a**) Map of Colombia depicting the geographic origin, accession number and classification of the bean accessions based on DArT genotyping performed in this study; (**b**) Neighbor joining and phylogenetic relatedness; (**c**) STRUCTURE analysis ($k = 3$); (**d**) Number of homozygous segments; (**e**) Inbreeding coefficients (F-values). Green color is assigned to ancestral accessions A1 and A2, blue to the landrace accession L1 and red to modern accessions M1–M5. DArT, Diversity Array Technology.

differentially abundant, we conducted a three-step analysis in which we assessed separately the read counts based on Phylum, Family and OTU level. For Phylum and Family level, custom R commands were used in order to aggregate all the reads according to the level chosen. For the OTU level analysis, the function *calculateEffectiveSamples* from the metagenomeSeq R package 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 Phylum, Family and OTU level, we used normalized tables applying the cumulative-sum scaling normalization as described above. 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 0.1 at Phylum and Family level, and 0.05 at OTU level. Volcano plots were built to graphically represent the results of the moderated *t*-tests using the R package ggplot2 (v.2.0.0) (Wickham, 2009). To graphically represent the results obtained at Phylum and Family level, a script developed by Bulgarelli *et al.* (2015) was adapted, in which relative abundance of read counts per mil was used, as well as box plot representations using the R package ggplot2. Taxa above 5‰ relative abundance were plotted for Phylum and Family level analysis. Treemap (v.3.7.3) was used to visualize the significantly abundant OTU's, the annotated 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 mil of the raw read counts.

Data access and bioinformatic analyses

The sequence data are deposited at the European Nucleotide Archive under accession number PRJEB19467. Data, scripts and codes used for statistical and bioinformatic analyses are available at: https://doi.org/10.5281/zenodo.580027 and https://doi.org/10.5281/zenodo.556538, respectively.

Results and discussion

Genetic relatedness of common bean accessions

Diversity Array Technology analysis resulted in 7527 SNPs as genetic markers for the eight selected local common bean accessions. Phylogenetic and Bayesian clustering approaches as well as multidimensional scaling allowed us to decipher the divergence among the selected bean accessions (Figures 1b and c; Supplementary Figure S1). Bean accessions G22304 and G23998, originally selected for this study as wild and landrace, respectively, showed strong genetic concordance and were classified as wild or 'Ancestral' accessions A1 and A2, respectively (Figure 1 and Supplementary Table S1). For the accession G51283K1, selected originally as a wild, a genome-wide comparison showed that it is more similar to the modern bean accessions than to the wild accessions (Supplementary Figure S2). We postulate that G51283K1 is probably a weedy accession, that is, the product of a cross between wild common bean and a modern cultivar (Toro *et al.*, 1990). Hence, accession G51283K1 was classified together with G50398, G14947, G51695 and G5773, as 'Modern'. Hereinafter, these five accessions are referred to as M1–M5, respectively (Figure 1 and Supplementary Table S1). Accession G50632I1, selected as a landrace, did not show significant similarity with any of the other bean accessions and was named L1 (Figures 1b and c; Supplementary Figure S3). Inference of the genetic diversity of the selected bean accessions further supported our classification: accessions M1–M5 have a higher number of homozygous segments and higher inbreeding coefficients than landrace L1 and wild accessions A1 and A2 (Figures 1d and e). Accessions A1 and A2 originate from the same geographic area where several wild relatives of common bean have been collected (Blair *et al.*, 2012) (Figure 1a). The proximity between collection sites might partly explain the genotypic similarities between these two wild accessions.

Root phenotypic traits of wild and modern bean accessions

Wild accessions A1 and A2 had a similar specific root length (SRL: ratio of root length and dry weight) and a similar root density (ratio of dry weight and volume), different from the other bean accessions (Figure 2). These results confirm and extend earlier results found for wild common bean as compared to cultivars (Martín-Robles *et al.*, 2015). Taken together, a high SRL and small diameter point to thinner roots and may provide a higher efficiency of water search and uptake, traits that are important for wild beans to prosper and survive in the dry native habitats (Toro *et al.* 1990,Comas *et al.*, 2013). When harvested at flowering stage, significant differences were observed between the bean accessions in the number of days to reach flowering, the root dry weight and the number of nodules (ANOVA, $P<0.005$; Supplementary Figure S4). Consistent with previous findings (Toro *et al.* 1990), bean plants of the ancestral group require more days to reach flowering than the modern accessions. Only A2 presented a significant higher number of nodules per root system, while no significant differences were found

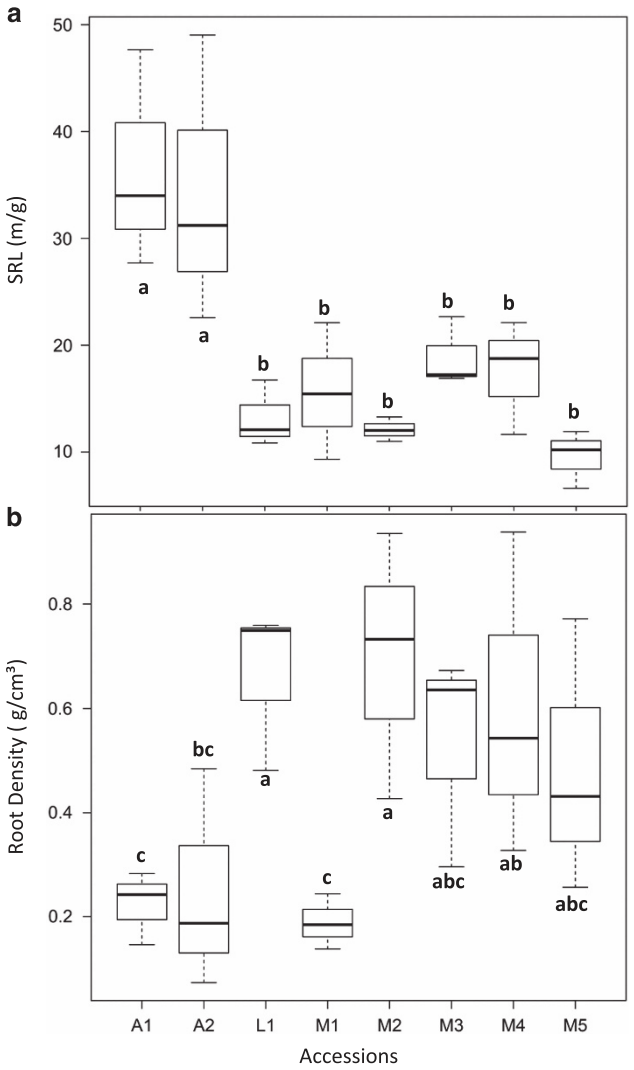


Figure 2 Root morphology parameters of common bean accessions. (**a**) Specific Root Length is the product of root length divided by the root dry weight, and (**b**) Root density is the product of root dry weight divided by root volume. Root length and root volume were determined by WinRHIZO. Statistically significant differences between group means for SRL and for Root Density were determined by one-way ANOVA ($P<0.05$). Three replicates per accession were used. Different letters indicate statistically significant differences (Fisher LSD test).

between the other accessions (Supplementary Figure S4c).

The common bean rhizobacterial diversity

As root exudation profiles may change due to specific root architectural features (Marschner *et al.*, 2002), we hypothesized that the observed contrasting root morphologies and the genetic divergence between accessions may affect the rhizobacterial community composition. Plants were harvested at flowering to synchronize microbiome analyses for all bean accessions at the same phenological stage. Through sequencing of the V3–V4 region of the 16S rRNA, 2.4 million quality reads were recovered, identifying 12 293OTUs at 97% sequence similarity (Supplementary Table S4). For the α-diversity, we observed a significant reduction in the rhizosphere of all bean accessions as compared to the bulk soil (ANOVA, $P<0.05$) (Supplementary Figures S5 and S6). Between accessions, however, we did not find significant differences in the diversity indexes, except for the number of observed OTUs which was higher for M1 than for A1 (Tukey HSD, $P<0.05$). Bray–Curtis metrics and Constrained Analyses of Principal Coordinates further showed that the microhabitat (soil, rhizosphere) explained 30.2% of the β-diversity, that is, the total variability in bacterial community structure between groups (PERMANOVA, $P<0.001$). Accordingly, a significant separation between rhizosphere and bulk soil was observed (PERMANOVA $P<0.005$) (Figure 3a). This selective pressure of the rhizosphere on microbiome composition is well known (Lakshmanan *et al.*, 2012; Badri *et al.*, 2013) and most likely driven by the quantity and quality of root exudates in combination with different growth rates, substrate utilization spectra and competitive abilities of the rhizobacterial genera. The results further showed that 13.5% of the total variability in rhizobacterial community composition was explained by the bean genotype (PERMANOVA, $P<0.001$). The constrained analysis of the principal coordinates by phylogenetic group was significant (PERMANOVA, $P<0.005$) (Figure 3b). A Regularized Canonical Correlation Analysis further confirmed that the genetic make-up of the wild bean accessions correlates with their rhizobacterial community composition (Supplementary Figure S7). These results are in accordance with previous findings on maize and barley, where the impact of the plant genotype shapes host-dependent rhizosphere bacterial communities (Peiffer *et al.*, 2013; Bulgarelli *et al.*, 2015).

Niche-based processes in rhizobacterial community assembly

The SAD models and the comparison of Akaike Information Criterion values showed that the rhizobacterial species abundance in the rhizosphere of all accessions and in the bulk soil are explained by niche-based distributions (Supplementary Figure S8 and Supplementary Table S5). In the case of the rhizosphere environment, root exudation is a strong modulating factor of rhizobacterial communities, where several taxa can thrive and become highly abundant while other community members exhibit low abundance (Jones *et al.*, 2009). We also tested a neutral model in order to generate a SAD to be compared with the other niche-based models. With this model, a parameter (m) which accounts for the immigration rate into local communities from a regional pool is obtained (Etienne, 2005). Values closer to 1 indicate no dispersal limitation. The m values for bulk soil samples were closer to 1 as

compared to those of the rhizosphere samples, suggesting a possible effect of neutral-driven processes in the bulk soil and at the same time a stronger niche-driven process in the rhizosphere of all the bean accessions tested (Supplementary Table S6).

Linking rhizobacterial community composition with the common bean genotype

To determine which rhizobacterial taxa were affected in a bean genotype-dependent manner, a Zero-Inflated Gaussian model was used to assess the differential abundance. At phylum level, all eight bean accessions presented an enrichment of Proteobacteria and a lower abundance of Acidobacteria as compared to the bulk soil (Supplementary Figures S9a). The phyla Bacteroidetes and Verrucomicrobia were significantly more abundant in the rhizosphere of the wild bean accessions, whereas the Actinobacteria were more abundant in the rhizosphere of the modern bean accessions (FDR < 0.1; Figures 4a and b). At family level, a significant increase in the relative abundance of the *Rhizobiaceae* and *Sphingomonadaceae* was observed for all bean accessions as compared to the bulk soil (Supplementary Figures S9b). Following the bean genotypic trajectory from A1 through M5 (based on inbreeding coefficient and homozygosity), we observed a gradual decrease in the relative abundance of *Chitinophagaceae* and *Cytophagaceae*, both of the Bacteroidetes phylum (FDR < 0.1; Figures 4c and d). Following this same trajectory, gradual increases in relative abundance were observed for the *Nocardiodaceae* (Actinobacteria) and *Rhizobiaceae* (Proteobacteria) (Figures 4g and h) and to some extent also for the *Streptomycetae* (Figure 4f). For *Bacillaceae*, however, no specific pattern in relative abundance was observed along the bean genotypic trajectory; this family was significantly more abundant only in the M5 rhizosphere (Figure 4e).

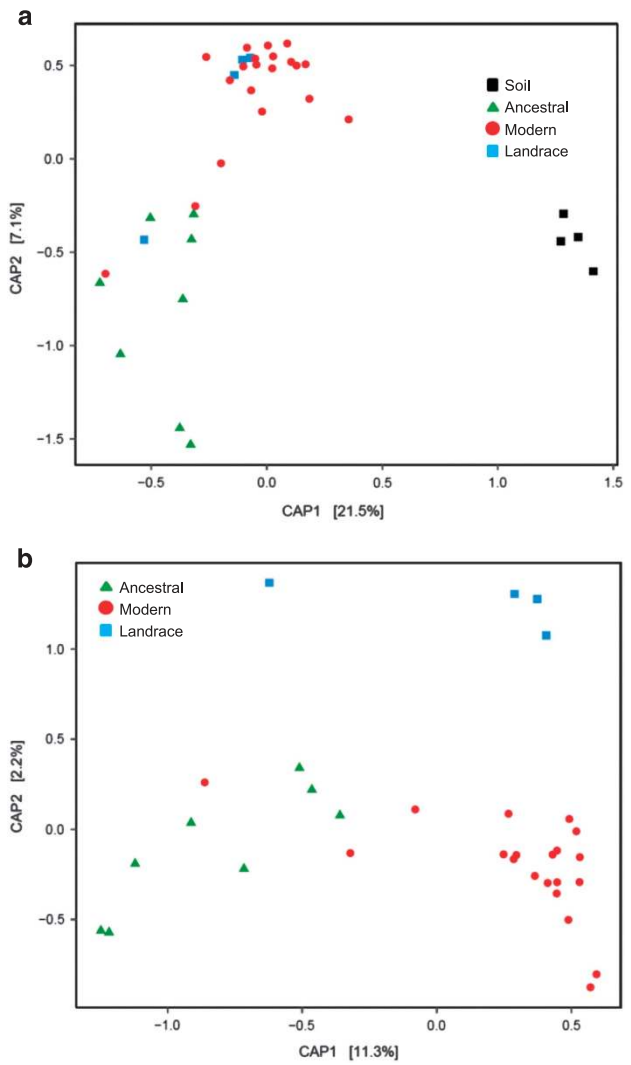
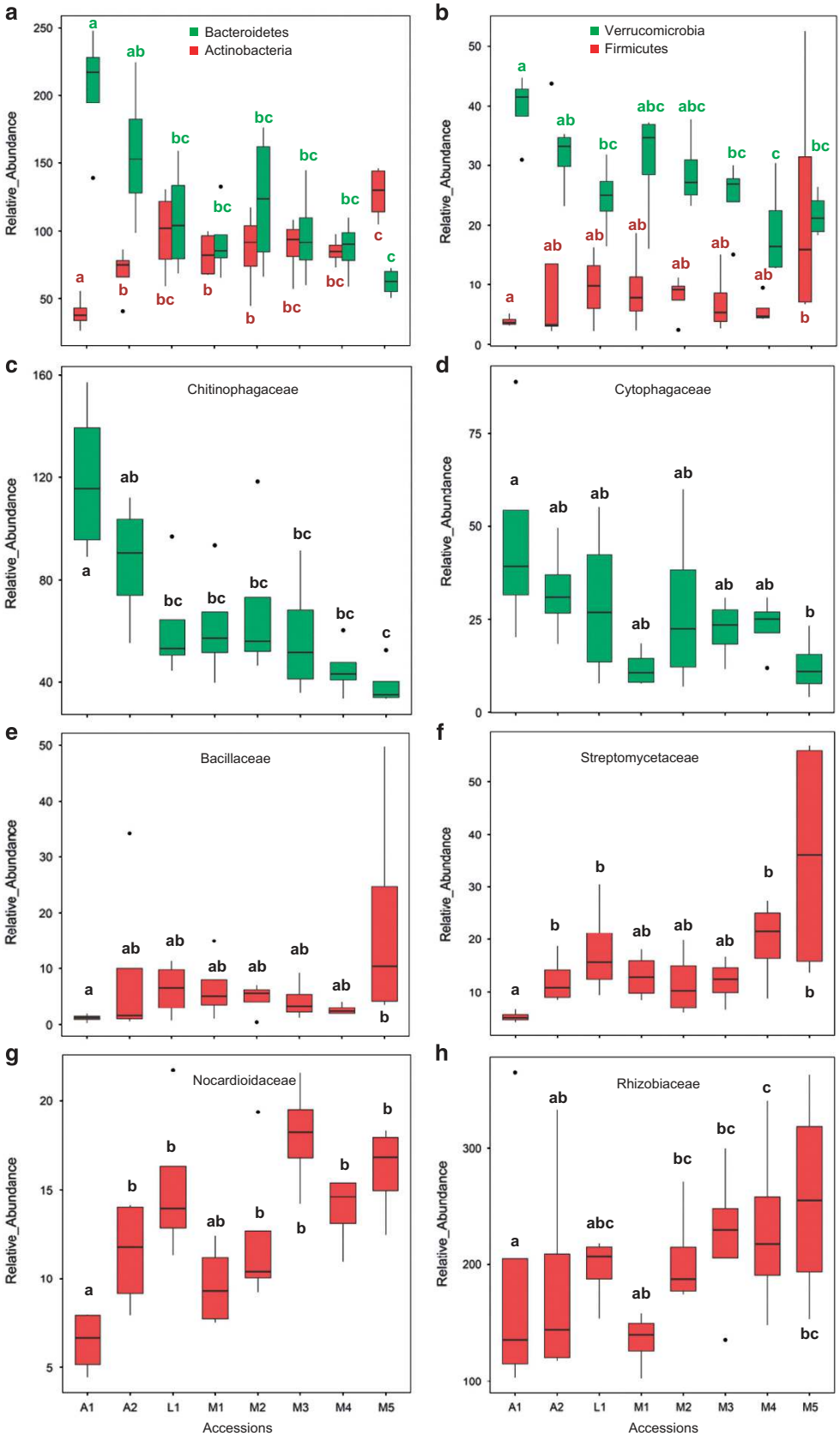


Figure 3 Rhizosphere bacterial community structure of common bean. Constrained Analysis of Principal Coordinates (CAP) of 16S rRNA diversity in the rhizosphere of the eight common bean accessions used in this study, with (**a**) and without (**b**) 16S rRNA diversity in the bulk soil, respectively. CSS transformed reads were used to calculate Bray–Curtis distances and a constrained analysis was performed by microhabitat (**a**) (30.2% of the overall variance; $P<0.005$) and bean group (**b**) (13.47% of the overall variance; $P<0.005$). Statistical significance of the constrained analysis was assessed by Permanova ($P<0.005$). CSS, cumulative-sum scaling.

Next, we zoomed in on specific OTUs that were differentially enriched or depleted among the bean accessions by using the filtered OTU table. Based on the inbreeding coefficients and number of homozygous segments (Figures 1d and e), bean accessions A1 and M5 were the most divergent and therefore compared first to see if this divergence was also reflected in the rhizobacterial community composition. We found 221 OTUs enriched in the A1 rhizosphere and 181 OTUs enriched in the M5 rhizosphere (Figure 5 and Supplementary Table S7). A1 was significantly enriched with representatives of the *Chitinophagaceae* family (25 OTUs). The genus *Dyadobacter* from the *Cytophagaceae* family was particularly enriched in the rhizosphere of A1. For the M5 rhizosphere, three out of the 10 most abundant OTUs were significantly enriched, belonging to *Rhizobium* (OTU9047), *Streptomyces* (OTU7) and Burkholderiales (OTU8). Also enriched in M5 were two highly abundant OTUs of the genus *Arthrobacter* (OTU17 and OTU886) and several OTUs from the family *Nocardioidaceae* (13 OTUs) and the genus *Lysobacter* (8 OTUs). All microbiome comparisons between A1 and the other bean accessions showed similar enrichments (Supplementary Figures S10–S14). Collectively, these analyses showed that OTUs from Bacteroidetes and Verrucomicrobia were enriched in the rhizosphere of accession A1, whereas OTUs from Actinobacteria were consistently enriched in the rhizosphere of accession M5. Similarly, when comparing A2 to M4 and to M5, we observed that OTUs from *Chitinophagaceae* family were consistently enriched in the A2 rhizosphere (Supplementary Figures S15 and

S16). Also when we merged the data of the individual bean accessions into a collective data set for each of the two bean genotypic groups (that is, ancestral and modern), similar overall patterns and differences in rhizobacterial community composition were observed: Bacteroidetes (8 OTUs) and



Verrucomicrobia (5 OTUs) were enriched in the ancestral group, whereas the phylum Actinobacteria (2 OTUs) was enriched in the modern group (Supplementary Figure S17).

Intriguingly, Bacteroidetes have also been reported at higher relative abundance in the rhizosphere of other wild plant species and wild crop relatives, including *Cardamine hirsuta* (Schlaeppi *et al.*, 2014), *Beta vulgaris* subsp. *maritima* (Zachow *et al.*, 2014) and *Hordeum vulgare* subsp. *spontaneum* (Bulgarelli *et al.*, 2015). In human microbiome research, Bacteroidetes have received considerable attention for their association with low carbohydrate diets, lean mice and weight loss in humans (Ley *et al.*, 2006; Turnbaugh *et al.*, 2006; De Filippo *et al.*, 2010; Brown *et al.*, 2012). Considering the increased abundance of Bacteroidetes on the thin roots of wild relatives of common bean and the higher relative abundance of Actinobacteria and Proteobacteria on the thicker roots of modern varieties, it is tempting to

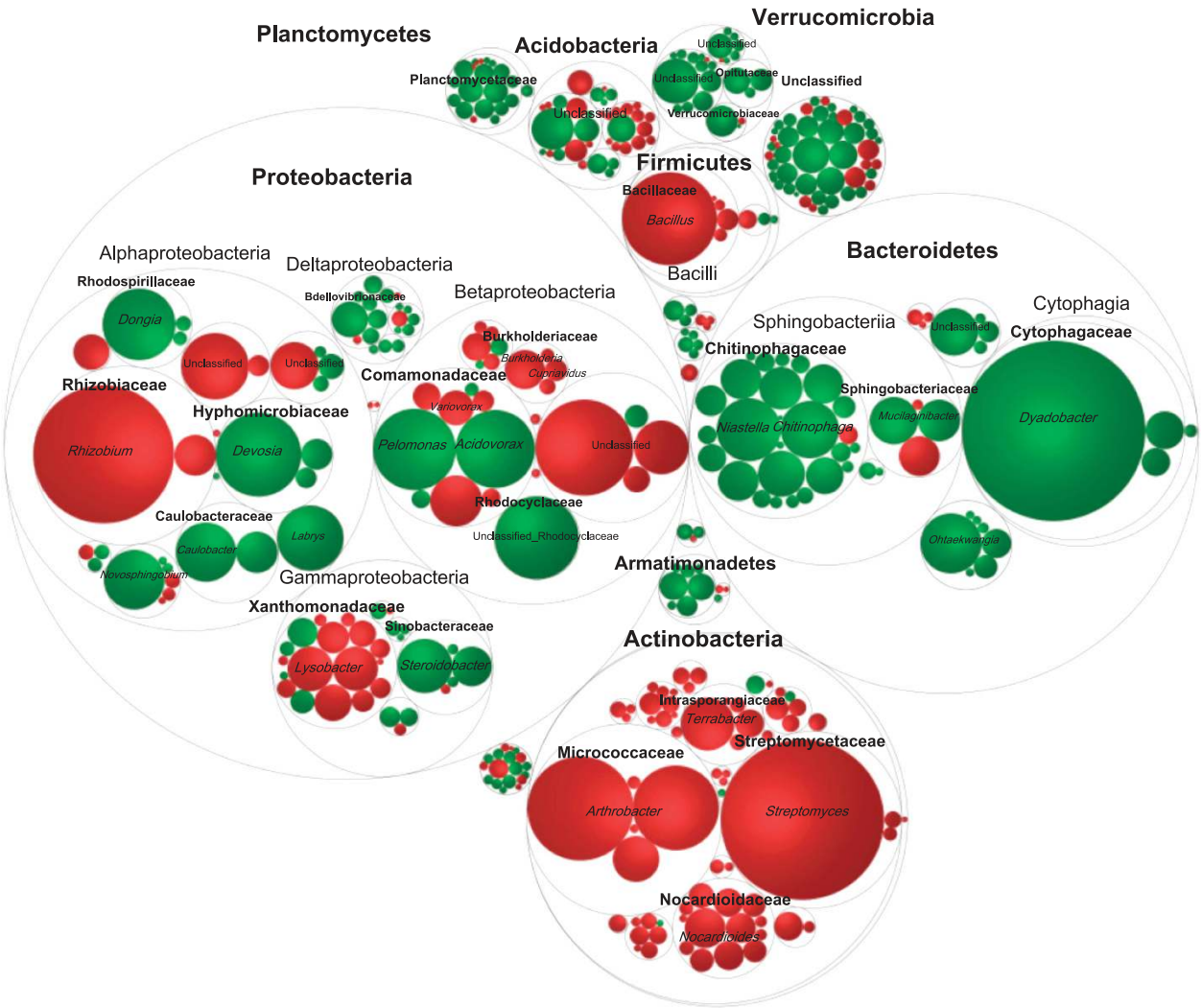


Figure 5 Differential abundance of bacterial OTUs between the wild bean accession A1 and modern bean accession M5. The comparison was made using a zero-inflated Gaussian distribution mixture model followed by moderated *t*-test and a Bayesian approach. Data from four replicates per accession was used. Only OTUs significantly enriched in one of the two accessions are shown (FDR < 0.05). The largest circles represents Phylum level. The inner circles represent Class and Family level. The color of the circles represents the OTUs enriched in the rhizosphere of wild accession A1 (green) or of modern accession M5 (red), with the assigned *Genus* in italics. The size of the circle is the mean read relative abundance of the differentially abundant OTU.

Figure 4 Relative abundance of bacterial phyla and families in the rhizosphere of the different bean accessions. The relative abundance (‰) of the phyla and families of four replicates per accession was used. At phylum level, results are shown for (**a**) Bacteroidetes and Actinobacteria, and (**b**) Firmicutes and Verrucomicrobia. At family level, results are shown for representatives of the Bacteroidetes (*Chitinophagaceae* (**c**) and *Cytophagaceae* (**d**)). For the Firmicutes and Actinobacteria, results are shown for *Bacillaceae* (**e**), *Streptomycetaceae* (**f**) and *Nocardioidaceae* (**g**). For the phylum Proteobacteria, the relative abundance of the *Rhizobiaceae* (**h**) is shown. Different letters indicate significant differences between accessions (moderated *t*-test, FDR < 0.1).

make an analogy of 'lean beans' and 'obese beans'. Whether the association with Bacteroidetes might result in a healthier development for plants as shown for animals, and whether its increased abundance on the roots of the wild relatives is a signature of coevolution remains speculative and opens exciting

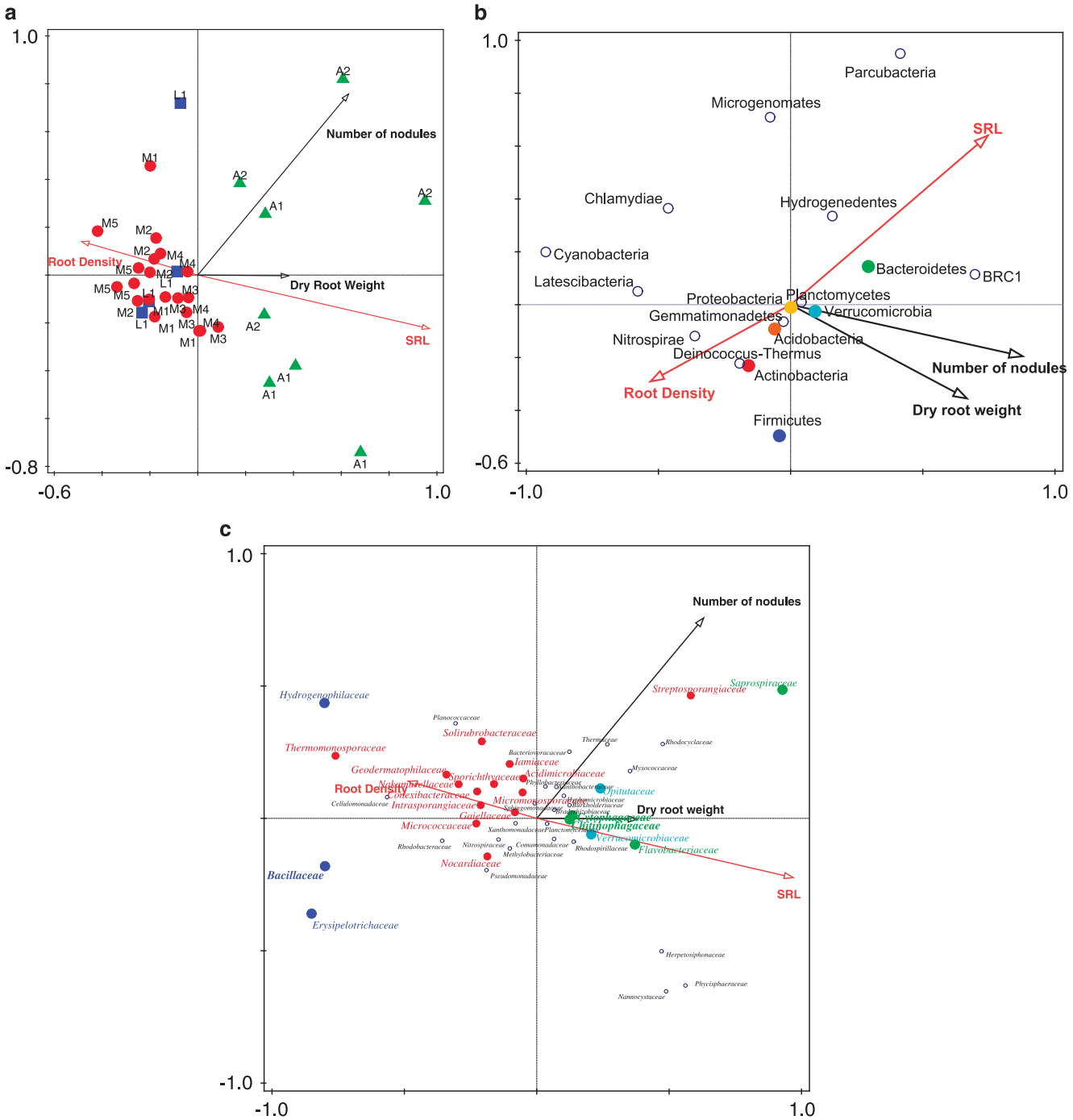


Figure 6 Constrained Canonical Correspondence Analysis of 16S sequence data and root morphological traits. (**a**) Root morphological traits as explanatory variables for the divergence between the overall rhizobacterial community composition of the eight different bean accessions. The rhizobacterial community composition is based on CSS normalized counts. Green triangles represent the replicates of the wild bean accessions A1 and A2, blue squares represent the landrace accession L1, and red circles represent the modern bean accessions M1–M5. The colored arrows represent the root morphological traits: Number of nodules and Dry root weight (black), Specific Root Length (SRL) and Root Density (red). (**b**) Same as in (**a**) depicting the root morphological traits as explanatory variables for the divergence between the different bacterial phyla. Only phyla with a relative abundance higher than 1% are colored: Acidobacteria (orange), Actinobacteria (red), Bacteroidetes (green), Firmicutes (blue), Proteobacteria (yellow) and Verrucomicrobia (light blue). (**c**) Same as in (**b**), depicting the root morphological traits as explanatory variables for the divergence between the bacterial families. Here, only the bacterial families belonging to Actinobacteria (red), Bacteroidetes (green), Verrucomicrobia (light blue) and Firmicutes (blue) were highlighted. CSS, cumulative-sum scaling.

avenues for further research. More specifically, the putative link between the abundance of this bacterial Phylum in the rhizosphere of wild crop relatives and its ability to degrade complex biopolymers (Thomas *et al*., 2011) will be subject of future experiments. Also in terms of plant health, the *Chitinophagaceae* family, which belongs to the Bacteroidetes, has been proposed for their potential role in protection against soil-borne pathogens (Yin *et al*., 2013; Chapelle *et al*., 2016).

Linking rhizobacterial community composition with root phenotypic traits

Canonical Correspondence Analysis revealed that the overall variation in rhizobacterial community composition is explained for 11.4% (Bonferroni adjusted $P = 0.002$) by the different root phenotypic traits (Figure 6a), which resembles the percentage of variation (13.5%) in rhizobacterial composition explained by the common bean genotype (Figure 3b). Among the root morphological and phenological traits included in the analysis, the SRL was responsible for most of the explained variation (Bonferroni adjusted $P = 0.008$) followed by the number of nodules (Bonferroni adjusted $P = 0.016$). The percentages of explanation for the variables Root Density and Root Dry Weight were not statistically significant. Interestingly, the dissimilarities between wild accessions A1 and A2 appears to be largely driven by the number of nodules. When the explanatory variables are used together with the bacterial phyla (Figure 6b) and bacterial families (Figure 6c), the results show that the abundance of Bacteroidetes families is explained by SRL, in contrast to the abundance of Actinobacterial families.

Conclusions

In this study, we found significant associations between the rhizobacterial community composition, the common bean genotype and specific root phenotypic traits. The phyla Bacteroidetes and Verrucomicrobia were consistently more abundant in the rhizosphere of wild common bean accessions, whereas representatives of the phyla Actinobacteria and Proteobacteria were enriched on roots of modern bean accessions. What the impact of the observed shifts in microbiome composition is on growth and health of common bean will be subject of future studies, ultimately providing an answer to the larger question if plant domestication compromised (or not) the beneficial effects of the rhizosphere microbiome. The divergence in rhizobacterial community composition between wild and modern bean accessions suggest a plant genetic basis of rhizosphere microbiome assembly. While these concepts apply also to other important food crops (for example, cereals), only with legumes it is possible to study how nodulation, the rhizosphere microbiota and the relationships between these two types of plant–microbe interactions are intertwined. In our study, only wild bean accession A2 presented a higher number of nodules per root system, while no significant differences were found between the other bean accessions. This suggests that symbiotic nitrogen fixation *per se* may not be the major driver of the root microbiome composition as was elegantly shown recently for *Lotus japonicum* (Zgadzaj *et al*., 2016). These results also imply that other or additional host-derived cues shape the bean rhizosphere microbiota. The relatively small sample size used in our study precludes a statistically robust GWAS analysis but did provide a well differentiated set of traits in wild and modern accessions associated with a number of bacterial taxa. In-depth genetic and phenotypic analyses of a larger population of plant accessions (Kraft *et al*., 2009) will be needed for the identification of genes or molecular markers that ultimately can be used in plant breeding programs for the recruitment of specific plant-beneficial microbial taxa.

Conflict of Interest

The authors declare no conflict of interest.

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