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Chapter 2

Rhizobacterial community structure differences among sorghum cultivars in different growth stages and soils

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Abstract

Plant genotype selects the rhizosphere microbiome. The success of plant-microbe interactions is dependent on factors that directly or indirectly influence the plant rhizosphere microbial composition. We investigated the rhizosphere bacterial community composition of seven different sorghum cultivars in two different soil types (abandoned (CF) and agricultural (VD)). The rhizosphere bacterial community was evaluated at four different plant growth stages: emergence of the second (day 10) and third leaves (day 20), the transition between the vegetative and reproductive stages (day 35), and the emergence of the last visible leaf (day 50). At early stages (days 10 and 20), the sorghum rhizosphere bacterial community composition was mainly driven by soil type, whereas at late stages (days 35 and 50), the bacterial community composition was also affected by the sorghum genotype. Although this effect of sorghum genotype was small, different sorghum cultivars assembled significantly different bacterial community compositions. In CF soil, the striga-resistant cultivar had significantly higher relative abundances of *Acidobacteria* GP1, *Burkholderia*, *Cupriavidus* (*Burkholderiaceae*), *Acidovorax* and *Albidiferax* (*Comamonadaceae*) than the other six cultivars. This study is the first to simultaneously investigate the contributions of plant genotype, plant growth stage and soil type in shaping the sorghum rhizosphere bacterial community.

Introduction

Interactions between plants and soil-borne microbes influence a wide range of biogeochemical processes, including organic matter mineralization (Fontaine *et al.*, 2007) and the cycling of biologically critical elements such as carbon, nitrogen and potassium (Mendes *et al.*, 2014). The rhizosphere, defined as the narrow zone of adjacent soil that is influenced by the plant roots (Hiltner, 1904), is home to numerous microorganisms and thus is one of the most dynamic interfaces on earth (Philippot *et al.*, 2013). Soil microbes drive plant diversity and productivity (van der Heijden *et al.*, 2008) and influence plant health, nutrient acquisition and growth (Mendes *et al.*, 2014, Cipriano *et al.*, 2016).

Several biotic and abiotic factors affect the structure of the rhizosphere microbial community, such as soil characteristics (Singh *et al.*, 2007, Kuramae *et al.*, 2012), land use history (Debenport *et al.*, 2015), plant species (Burns *et al.*, 2015, Lima *et al.*, 2015), plant genotype and plant development stage (Inceoglu *et al.*, 2010, Marques *et al.*, 2014). Soil shapes the rhizosphere microbial community through physical and chemical traits, including moisture, nutrient availability, texture and pH (Marschner *et al.*, 2004, Fang *et al.*, 2005, Cassman *et al.*, 2016, Taketani *et al.*, 2017), as well as soil management practices (Lima *et al.*, 2015). Plants in turn influence rhizosphere microbial community composition by producing root exudates, which may differ according to plant genotype and developmental stage (Bais *et al.*, 2006, van Overbeek & van Elsas, 2008, van Dam & Bouwmeester, 2016). The relative contributions of factors such as soil type, plant genotype and growth stage to rhizosphere microbial community composition have been reported for different plant species. These studies include the effect of plant growth stage on the rhizosphere microbial assemblies of *Arabidopsis thaliana* (Chaparro *et al.*, 2014) and maize (Li *et al.*, 2014); the effects of soil and plant on the rhizosphere microbial community structures of maize, soybean (Miethling *et al.*, 2000, Buyer *et al.*, 2002) and native legumes (Lima *et al.*, 2015); the effect of plant genotype and plant growth stage on the composition of the rhizosphere microbial communities of potato (van Overbeek & van Elsas, 2008, Inceoglu *et al.*, 2010) and sweet potato (Marques *et al.*, 2014); the effects of plant genotype and soil traits as modifiers of the maize rhizosphere microbial community (Aira *et al.*, 2010, Bakker *et al.*, 2015); and the effect of soil type, plant genotype and plant growth stages on the rhizosphere bacterial communities of soybean (Xu *et al.*, 2009) and maize (Chiarini *et al.*, 1998).

However, research on the composition of the rhizosphere bacterial community of sorghum is relatively scarce (Acosta-Martínez *et al.*, 2010), and no study has simultaneously investigated the contributions of plant genotype, plant growth stage and soil type in shaping sorghum rhizosphere

bacterial community composition. Sorghum is an important staple food crop and the fifth most cultivated cereal in the world, with a presence in approximately 47 countries (Ramu *et al.*, 2013). With nutritional properties similar to maize (Sauvant, 2004) but superior drought resistance (Dutra *et al.*, 2013), sorghum is a promising substitute for maize crops, particularly in arid regions.

Sorghum-breeding programs aim to increase yield and improve plant quality by selecting plants with desired phenotypes (Singh & Lohithaswa, 2007), such as resistance to pathogens or characteristics for grain, silage and forage. Thus, characterization of the rhizosphere bacterial community composition of different sorghum cultivars is of extreme importance for plant breeding programs to develop cultivars with superior rhizomicrobes that mitigate biotic and abiotic stresses. Breeding of plants based on a combination of functional genes and plant responsiveness to beneficial microorganism interactions is expected to produce plants with more robust disease protection (Dangl *et al.*, 2013, Schlaeppli & Bulgarelli, 2015). Therefore, the rhizosphere plant microbiome should be an important component of plant breeding programs.

Directed selection of plant genotypes that enhance populations of beneficial rhizobacteria may confer protection against pathogens (Mazzola *et al.*, 2004, Mendes *et al.*, 2011) as well as abiotic stress (Coleman-Derr & Tringe, 2014). However, to guarantee good plant performance across variable locations, plant breeding programs should take into account the interaction of a particular cultivar with the soil microbiome in a broad range of environments (Bakker *et al.*, 2012). Hence, characterization of the bacterial community in the sorghum rhizosphere at different plant growth stages would contribute to biotechnological and agricultural applications aiming to enhance sorghum growth and yield (Ramond *et al.*, 2013). Although some authors have discussed the effects of factors such as soil type, plant growth stage and cultivar as drivers of the soil microbial community, investigations of these factors have generally not been integrated in the same experimental set or analysis. The failure to consider these factors simultaneously might reduce the accuracy of determining the contributions of factors in driving rhizosphere microbial composition. Thus, in this study, we aimed to (i) determine the relative simultaneous contributions of sorghum genotype, developmental stage and soil type to the structure of the rhizosphere bacterial community and (ii) to assess the rhizosphere bacterial taxonomic compositions of different sorghum cultivars.

Material and Methods

Soil sampling

Two different soil types from The Netherlands were used in this study as microbiome sources: Arenosol soil collected from Clue Field (CF) (52° 03' 37.91"N and 5° 45' 7.074"E) and Gleyic Podzol soil collected from a field in Vredepeel (VD) (51° 32' 25.8"N and 5° 51' 15.1" E). CF is an abandoned soil; the last crop was harvested in 1995 (Bezemer *et al.*, 2010). By contrast, VD is an arable agricultural field that has been in cultivation since 1955. In the four years before sampling, VD was cropped with potato and rye (2010), carrot (2011), and maize and rye (2012–2014) under normal agricultural practices (Korthals *et al.*, 2014). At each field site, soil samples were collected (0–20 cm topsoil layer) from five equidistant points 50 m from each other, sieved through a 4-mm mesh, and homogenized. Each soil was physically and chemically analyzed.

Sorghum cultivars

To assess the sorghum rhizosphere bacterial community assemblies, seven cultivars with different characteristics and origins were selected: BRS330, a hybrid grain of *Sorghum bicolor*; BRS509, a sweet hybrid of *S. bicolor*; BRS655, a hybrid silage type of *S. bicolor*; BRS802, a hybrid grazing type of *S. bicolor*; CMSxS912, a variety of *S. sudanense*; SRN-39, a grain type of *S. bicolor*; and Shanqui-Red, a landrace grain type of *S. bicolor*. The seeds of cultivars BRS330, BRS509, BRS655, BRS802 and CMSxS912 originated from Embrapa (Brazil), and the seeds of cultivars SRN-39 and Shanqui-Red originated from Africa and China, respectively.

Mesocosm experiment

Plastic pots (6.5 L) were filled with 6.0 L of either CF or VD soil. The experimental design comprised two soil types, seven sorghum cultivars and four plant growth stages assembled in triplicate, resulting in a total of 168 randomly distributed experimental units. Fifteen seeds of each sorghum cultivar were directly sown in each pot and grown in a greenhouse under controlled photoperiod and temperature conditions (16/8 h light/dark and temperature of 22 °C/17 °C day/night). The plantlets were thinned to five seedlings per pot at day 5. During the experiment, the rhizosphere soil was sampled at four different stages of plant growth. At the emergence of the second (day 10) and third (day 20) leaves, the plants were completely removed from the pots, and 5 g of rhizosphere soil was collected with sterile brushes. At the transition from the vegetative to reproductive stages (day 35) and at the last emergence of a visible leaf immediately before the flowering stage (day 50), rhizosphere samples

were collected with a cylindrical auger (6 × 150 mm). The pots were randomly rearranged after each sample collection time point. Rhizosphere soil was sampled at a depth of 0–15 cm from soil loosely adhering to seminal roots as well as soil brushed off the seminal root surface. Bulk soil was sampled from pots without plants. The rhizosphere and bulk soil samples were immediately stored at -80 °C until total genomic DNA extraction. At the end of the mesocosm experiment (harvest time, day 50), the shoots and roots of the plants were harvested for measurement of dry weight and for macro- and micronutrient analyses (Table S1).

DNA extraction and 16S rRNA partial gene sequencing

DNA was extracted from 0.25 g of each soil sample using a PowerSoil DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). DNA quality was verified by agarose gel (1.5%) electrophoresis in 1X TBE (Tris-borate-EDTA) buffer. The 16S rRNA partial gene was amplified using the primer set 515F and 806R (V3-V4 region) (Bergmann *et al.*, 2011). PCR was performed using 0.2 µL (0.056 U) of FastStart *Taq* Polymerase (Roche Applied Sciences, Indianapolis, IN, USA), 2.5 µL of dNTP (2 mM each), 0.25 µL of each primer and 1.0 µL of DNA template. The PCR conditions were as follows: initial denaturation at 95 °C (5 min); 35 amplification cycles of denaturation at 95 °C (30 s), annealing at 53 °C (30 s), and extension at 72 °C (60 s); and a final extension at 72 °C (10 min). Negative controls contained water instead of DNA, and positive controls contained DNA from *Escherichia coli*. The PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). The quality of the PCR products was assessed before and after purification in agarose gel (1.5%) electrophoresis in 1X TBE buffer. The PCR amplicons were quantified using a Quant-iT™ dsDNA Broad-Range Assay Kit (Invitrogen, Carlsbad, CA, USA) and Gen5 data analysis software (BioTek Technology). The samples were sequenced on the Ion Torrent platform (Macrogen Inc., South Korea).

16S rRNA amplicon data processing

Forward and reverse primer sequences were removed from each sample library FASTQ file using Flexbar version 2.5 (Dodt *et al.*, 2012). Sequences were filtered for quality criteria (Phred quality score of 25 and minimum sequence length of 150 bp) using FASTQ-MCF (Aronesty, 2011). The filtered FASTQ files were converted to FASTA format and concatenated into a single file. All reads were clustered into operational taxonomic units (OTUs considering an evolutionary distance of 97%) using UPARSE (Edgar, 2010) in VSEARCH version 1.0.10 (Flouri *et al.*, 2015). Chimeric sequences

were detected using the UCHIME algorithm (Edgar *et al.*, 2011) implemented in VSEARCH. 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 using RDP Classifier version 2.10 (Cole *et al.*, 2014). All steps were implemented in a Snakemake workflow (Köster & Rahmann, 2012).

Statistical analysis

To evaluate the effects of the factors soil, plant growth stage, and cultivar on sorghum rhizosphere bacterial communities, the bacterial abundance data were subjected to Hellinger transformation (Legendre & Gallagher, 2001) using the package “vegan” version 2.4.0 (Oksanen *et al.*, 2016). Between-class analysis (BCA) based on principal component analysis (PCA) was subsequently performed using the package “ade4” (Dray & Dufour, 2007). A Monte Carlo test with 999 permutations provided statistical significance of the applied tests. This analysis allowed us to identify the relative contribution of each factor in explaining the total variability of the bacterial community structure.

To infer how the rhizosphere bacterial community co-varied with the factors soil, cultivar and plant growth stage, the Hellinger-transformed data were used, and the co-variance was measured by the RV coefficient by multiple factor analysis (MFA) using the package “FactoMineR” (Lê *et al.*, 2008) in R version 3.1.3. To evaluate the effect of the factors soil and cultivar on sorghum rhizosphere bacterial communities in each plant growth stage, two different tests were performed. BCA was performed as described above, and a multivariate non-parametric statistical test (two-way PERMANOVA) was performed in PAST (Paleontological Statistics Software) (Hammer *et al.*, 2001) using Bray-Curtis distance matrices with 999 permutations. This analysis aided the identification of the main driver of microbial community structure at each stage of plant growth.

The variation of the rhizosphere bacterial community was evaluated together with soil type, plant growth stage, and cultivar in a global principal component analysis (GPCA) after normalization by MFA, which consisted of the ordination of each group of variables and posterior transformation by the first eigenvector. For each group of variables that was active in the construction of the factorial axes, the other two groups of variables were considered supplementary variables and were not taken into account in the analysis. To identify the bacterial taxa significantly responsible for the dissimilarities in the GPCA-MFA analysis ($p < 0.05$), ascending hierarchical classification (AHC) was

performed using the FactoMineR package. To control the false discovery rate (FDR), p-values were adjusted. The bacterial taxa significantly responsible for the dissimilarities were identified via AHC.

To explore the dissimilarity between the treatments within each factor, BCA was performed using the package “ade4” (Dray & Dufour, 2007).

Strigolactone analysis

Sorghum seeds were surface sterilized in bleach (2%) for 10 min and washed 3 times with sterile demineralized water. The seeds were subsequently pre-germinated on Petri dishes for 48 h at 25 °C in the dark. Three germinated seeds of each of the seven sorghum cultivars were planted in 0.5 L plastic pots filled with sterilized sand and grown for 3 weeks. The plants were fertilized with 50% Hoagland nutrient solution (v/v) containing 100% phosphate (P) for the first 14 days. To remove P, the pots were washed with 1 L of 50% Hoagland nutrient solution without P. After one week under P deficiency, 1 L of 50% Hoagland nutrient solution without P was applied to drain accumulated exudates from the pot. The root exudate that accumulated during the subsequent 48 h was collected by passing 1 L of nutrient solution without P through the pot. After passing the exudates through an SPE C18 column (500 mg), strigolactones were eluted with 4 mL of acetone, and 0.1 nmol/mL GR24, a synthetic strigolactone, was added to each sample as an internal standard for quantification. After evaporating the acetone to dryness, the residue was dissolved in 4 mL of hexane. For further purification, the samples were loaded on a 200 mg silica gel Grace Pure SPE column, and the column was eluted with 2 mL of 10:90 hexane:ethyl acetate. After evaporating the solvent to dryness, the residue was dissolved in 200 μ L of 25:75 acetonitrile:water and filtered through a 0.45- μ m Minisart SRP filter. Strigolactones were measured by ultra high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) in multiple ion monitoring mode (MRM) according to the method described by Kohlen *et al.* (2011) with minor modifications. The retention times and masses of authentic standards (5-deoxystrigol, ent-2'-epi-5-deoxystrigol (or 4-deoxyorobanchol), orobanchol, ent-2'-epi-orobanchol, and sorgomol) were used to identify the detected strigolactones. Data analysis was performed using MassLynx 4.1 and TargetLynx software (Waters).

Results

Soil and plant characteristics

Total N, S and P contents were higher in the CF abandoned soil than the VD agricultural soil, whereas K, Ca, Mg, and Na contents and cationic exchange capacity (CEC) were higher in VD than in CF

soil. The organic matter content was similar in the two soils, whereas the pH, C:N ratio and texture (clay, silt and sand content) were slightly different (Table S1).

Drivers of the sorghum rhizosphere bacterial community

The number of sequenced reads covered an average of 90% of the bacterial diversity as determined by Good's coverage (Table S2).

Different statistical approaches were applied to test the significance of the three evaluated factors, i.e., cultivar, plant growth stage and soil type, as drivers of sorghum rhizosphere bacterial community composition. Between classes analysis (BCA) revealed that soil, plant growth stage and cultivar explained 15.83% ($p=0.001$), 5.19% ($p=0.001$), and 4.25% ($p=0.085$) of the dissimilarity between the rhizosphere bacterial communities, respectively. Similar results were obtained by co-inertia analysis (RV coefficient), which revealed that soil type, plant growth stage and cultivar co-varied with the rhizosphere bacterial community by 68.30%, 14.18% and 9.69%, respectively (Table 1). When the factors were examined simultaneously, both statistical analyses indicated that the factor soil strongly determined the rhizosphere bacterial community composition, followed by plant growth stage and cultivar.

Table 1. Inertia co-variance between the factors soil type, plant growth stage and cultivar with the rhizosphere bacterial community.

	Soil type	Growth stage	Cultivar	Bacteria
Soil type	100.00%			
Time Point	0.00%	100.00%		
Cultivar	0.00%	18.90%	100.00%	
Bacteria	68.30%	14.18%	9.69%	100.00%

The variations of soil type and cultivar and their interaction as drivers of rhizosphere bacterial composition over different plant growth stages were examined by two-way PERMANOVA. Until day 20, soil drove the majority of the observed shifts in the structure of the rhizosphere bacterial community. At day 35, soil ($F=19.98$; $p<0.001$) and cultivar ($F=1.56$; $p=0.02$) significantly drove sorghum rhizosphere bacterial composition. At day 50, soil ($F=12.35$; $p<0.001$), cultivar ($F=2.34$; $p<0.001$), and their interaction ($F=1.58$; $p=0.01$) had significant effects on the rhizosphere bacterial community (Table 2).

Table 2. Two-way PERMANOVA testing the effect of the factors soil, cultivar and the interaction between the both factors within each plant growth stage.

Plant growth stage	Factors	Sum of squares	Df	F	P
10 days	Soil type	1.77	1	12.14	<0.001
	Cultivar	0.86	6	0.99	0.50
	Soil type *Cultivar	0.87	6	0.99	0.48
	Residue	4.08	28		
20 days	Soil type	2.15	1	22.28	<0.001
	Cultivar	0.72	6	1.25	0.15
	Soil type *Cultivar	0.72	6	1.24	0.15
	Residue	2.70	28		
35 days	Soil type	1.80	1	19.98	<0.001
	Cultivar	0.84	6	1.56	0.02
	Soil type *Cultivar	0.73	6	1.36	0.08
	Residue	2.52	28		
50 days	Soil type	1.51	1	12.35	<0.001
	Cultivar	1.71	6	2.34	<0.001
	Soil type *Cultivar	1.16	6	1.58	0.01
	Residue	3.42	28		

To better understand the contribution of the factors soil and cultivar on the total variation of the bacterial community across plant growth stages, BCA was performed for each growth stage. Although the contribution of soil type to the total variation of the rhizosphere bacterial community composition within plant growth stages was significant ($p=0.001$), BCA showed that this contribution (given by the percentage of inertia) explained a smaller proportion of the community structure on day 50 (15.63%). Interestingly, the cultivar effect became a significant ($p=0.001$) contributor explaining the variance in the rhizosphere bacterial community composition only at day 50, explaining 21.89% of the total variation. At this growth stage, the cultivar effect surpassed the contribution of the factor soil (which at day 50 explained 15.63% of the total variance), although soil remained a significant factor in determining the bacterial community structure (Table 3).

Table 3. Between Classes Analysis (BCA) testing the effect of the factors soil and cultivar within each plant growth stage.

Growth stage (day)	Variables	% Inertia	P-value
10	Soil	20.06	0.001
	Cultivar	13.66	0.68
20	Soil	21.97	0.001
	Cultivar	13.33	0.72
35	Soil	18.42	0.001
	Cultivar	15.66	0.24
50	Soil	15.63	0.001
	Cultivar	21.89	0.001

Bacterial community composition

Bulk soil

In the soils, the most abundant bacterial phyla were Acidobacteria (CF, 26%; VD, 31%), Verrucomicrobia (CF, 19%; VD, 16%) and Proteobacteria (CF, 15%; VD, 14%). The most abundant classes in both soils were *Spartobacteria* (CF, 18%; VD, 15%), *Acidobacteria* subdivisions GP6 (CF, 15%; VD, 14%) and GP4 (CF, 7%; VD, 14%), *Alphaproteobacteria* (CF, 7%; VD, 6%) and *Betaproteobacteria* (CF and VD, 6%). The most abundant taxa that could be assigned at the order level were *Rhizobiales* (CF, 5%; VD, 3%) and *Planctomycetales* (CF, 5%; VD, 4%). At the family level, the most abundant taxa were *Planctomycetaceae* (CF, 5%; VD, 4%), *Bradyrhizobiaceae* (CF, 4%; VD, 2%), *Sphingomonadaceae* (CF and VD, 2%), *Chitinophagaceae* (CF and VD, 2%), and *Xanthomonadaceae* (VD, 2%) (Figure S1). Multiple Factor Analysis (MFA) revealed the bacterial families that most contributed to the dissimilarities between the bulk soils of CF and VD (Figure 1A). Among the groups with relative abundances higher than 1%, unclassified *Spartobacteria*, unclassified *Acidobacteria* GP4 and GP16 and *Xanthomonadaceae* together contributed to 70% of the total dissimilarity between the bacterial communities (family level) in the two soils (Table S3).

Bulk soil versus rhizosphere

Multiple Factor Analysis (MFA) at the family level revealed the rhizosphere effect for both soil types (Figure 1B and 1C). In the treatments with CF soil, the dissimilarity between the bulk soil and the rhizosphere was caused mainly by changes in *Bradyrhizobiaceae*, *Chitinophagaceae*, *Planctomycetaceae*, *Sphingomonadaceae*, *Xanthomonadaceae*, and *Oxalobacteraceae*, as well as organisms that could not be classified at the family level belonging to Acidobacteria subdivisions GP1, GP4, GP6 and GP16, Bacteroidetes, Betaproteobacteria, *Myxococcales*, *Rhizobiales* and *Spartobacteria* (Table S4). In the treatments with VD soil, the distinction between the bulk soil and rhizosphere clusters evidenced by Dim 1 and Dim 2 was related to differences in the abundances of *Bradyrhizobiaceae*, *Burkholderiaceae*, *Oxalobacteraceae*, *Sphingomonadaceae*, *Xanthomonadaceae*, *Planctomycetaceae* and unclassified groups at the family level belonging to Acidobacteria subdivisions GP1, GP4, GP6, GP16, Betaproteobacteria, *Burkholderiales*, *Rhizobiales* and *Spartobacteria* (Table S5).

Rhizosphere CF versus rhizosphere VD soil

MFA at the family level revealed that the cluster evidenced by Dim 1 explained 30.85% of the total rhizosphere bacterial community variation between CF and VD (Figure 1D). Among the bacteria driving the dissimilarity ($p < 0.05$), those with the highest relative abundances included *Bradyrhizobiaceae* (CF, 6%; VD, 3.9%), unclassified *Spartobacteria* (CF, 8.9%; VD, 3.6%), unclassified Betaproteobacteria (CF, 8.2%; VD, 6%), and the unclassified *Acidobacteria* subdivisions GP6 (CF, 8.1%; VD, 5.2%) and GP4 (CF, 4.4%; VD, 5.7%) (Table S6).

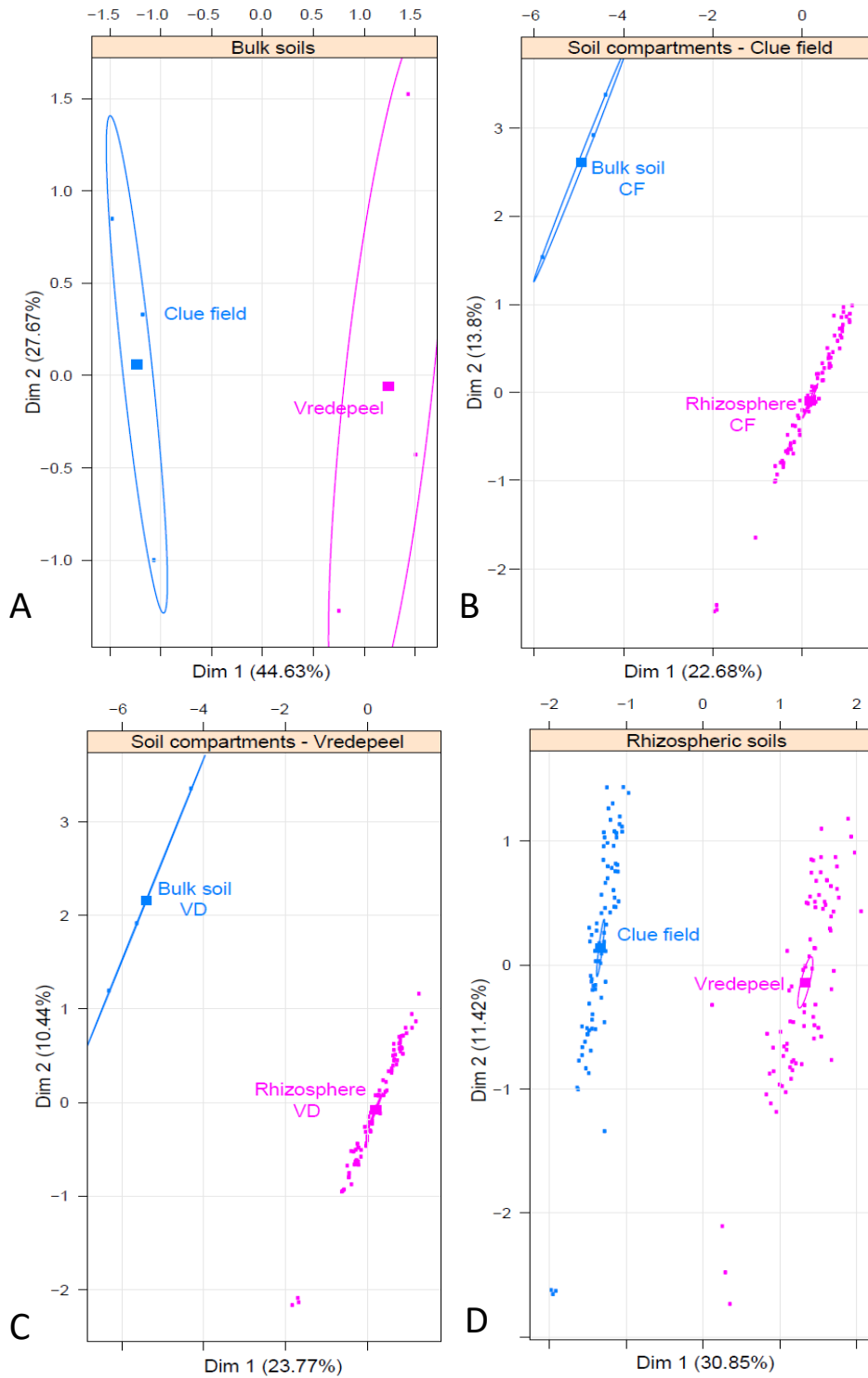


Figure 1. Multiple factor analysis (MFA) with supplementary variables emphasizing the factor soil and showing the bacterial community dissimilarity between (A) bulk soils from Clue Field (CF) and Vredepeel (VD); (B) bulk soil and rhizosphere soil from CF; (C) bulk soil and rhizosphere soil from VD; (D) rhizosphere soil from CF and VD.

Influence of plant growth stage on the rhizosphere bacterial community

A clear cluster distinction was observed for both soils by analysis of the symmetric variation of the rhizosphere bacterial community over time using plant growth stage as the active factor (MFA). In the treatments with CF soil, the day 10 and day 50 clusters differed significantly ($p < 0.05$) from each other and from the other clusters (Figure 2A). The bacterial family groups responsible for the significant dissimilarity at day 10 compared with the other growth stages were *Oxalobacteraceae* (4.7%), *Sphingobacteriaceae* (1.3%), and an unclassified Verrucomicrobia from subdivision 3 (1.2%). At day 50, the bacterial family groups that significantly differed from the other growth stages were *Bradyrhizobiaceae* (4.6%), *Chitinophagaceae* (3.2%), *Comamonadaceae* (1.7%), *Opitutaceae* (1.2%), *Oxalobacteraceae* (1.6%), *Planctomycetaceae* (3.7%), *Sphingomonadaceae* (1.8%), *Xanthomonadaceae* (1.3%), Acidobacteria subdivisions GP6 (11.8%) and GP16 (2.3%) and a group that could not be classified at the family level that included unclassified Proteobacteria (1.3%), unclassified *Burkholderiales* (1%) and Verrucomicrobia subdivision 3 (1.8%) (Table S7). In the treatments with VD soil, the rhizosphere microbial communities at day 10, day 20, and day 50 were significantly dissimilar (Figure 2B). *Sphingomonadaceae* and *Sphingobacteriaceae* were responsible for the dissimilarity at day 10 (3.8 and 2.3%, respectively) and day 50 (1.2 and 0.6%). The groups *Oxalobacteraceae* (6.7 and 3.2, respectively), *Xanthomonadaceae* (5.5 and 2.7), Acidobacteria subdivision GP6 (2.9 and 7.4), unclassified Proteobacteria (2.2 and 1.5), unclassified *Burkholderiales* (1.8 and 1.3), *Caulobacteraceae* (1.3 and 0.6), *Polyangiaceae* (1.2 and 0.7), *Planctomycetaceae* (1.2 and 2.8) and Verrucomicrobia unclassified subdivision 3 (0.7 and 1.4) were responsible for the significant dissimilarity at day 20 and day 50. Unclassified *Myxococcales* (3.6%) and *Comamonadaceae* (2.9%) were significantly dissimilar at day 20, whereas unclassified *Spartobacteria* (4.8%) were significantly dissimilar at day 50 (Table S8).

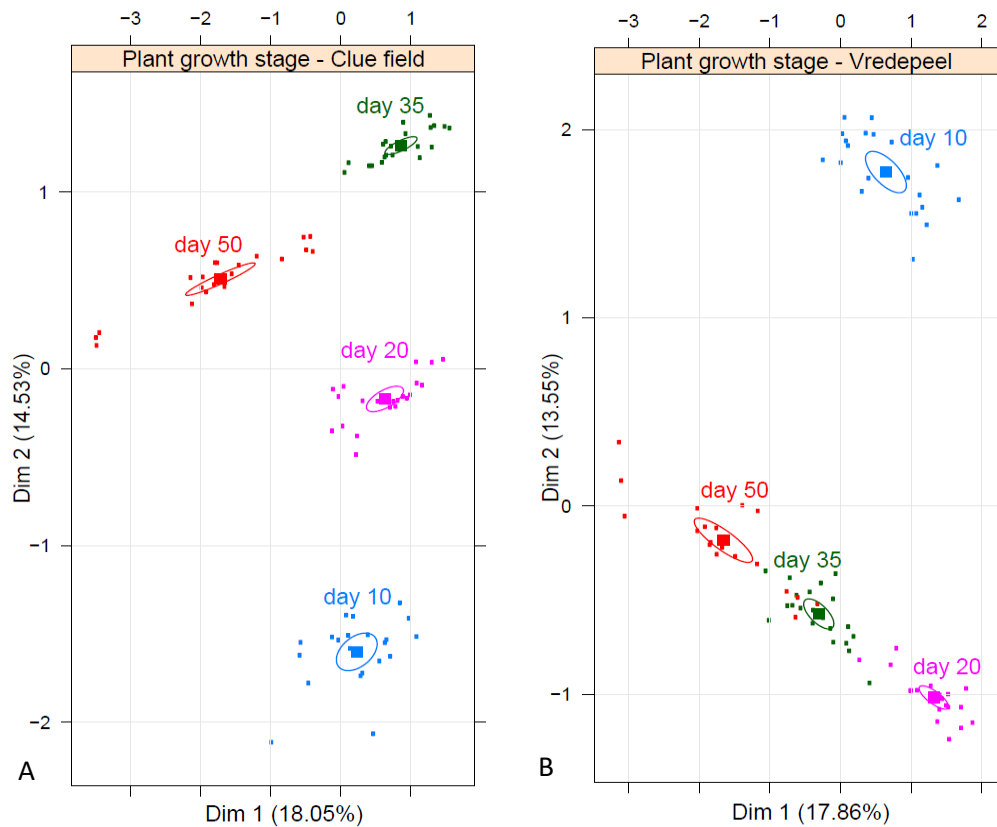


Figure 2. Multiple factor analysis (MFA) with supplementary variables emphasizing the factor plant growth stage and showing the rhizosphere bacterial community dissimilarity in (A) Clue Field (CF) soil and (B) Vredepeel (VD) soil.

Influence of cultivar in shaping the rhizosphere bacterial community

MFA with cultivar as the active variable demonstrated that cultivar had an effect on the dissimilarity of the rhizosphere bacterial community only in CF soil (Figure 3A) and not in VD soil (Figure 3B). In the treatments with CF soil, the rhizosphere bacterial community of SRN-39 (C6) was significant dissimilar ($p < 0.05$) from those of the other cultivars. The bacterial family groups responsible for this dissimilarity were *Comamonadaceae* (3.4%), *Burkholderiaceae* (3.6%) and *Acidobacteria* subdivision GP1 (3.7%) (Table S9). *Burkholderia* and *Cupriavidus* were the genera responsible for the higher relative abundance of *Burkholderiaceae*, whereas *Acidovorax* and *Albidiferax* were the responsible genera of *Comamonadaceae* (Table S10).

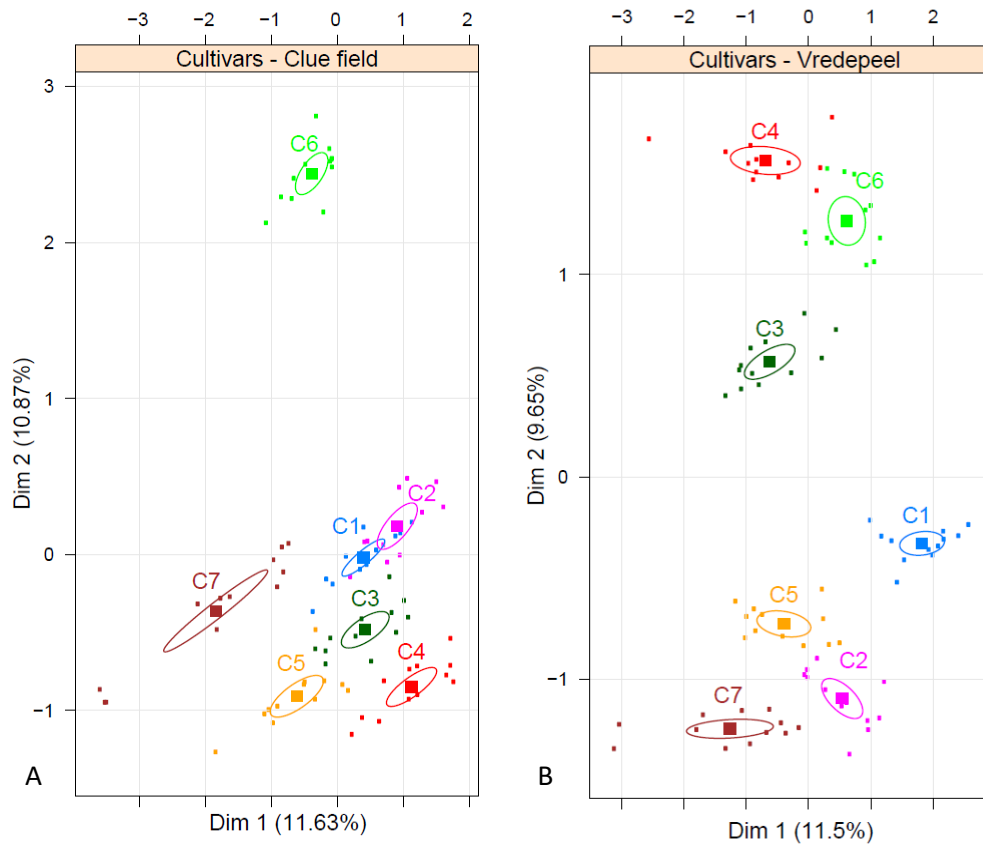


Figure 3. Multiple factor analysis (MFA) with supplementary variables emphasizing the factor cultivar and showing the rhizosphere bacterial community dissimilarity among cultivars: C1 = Hybrid grain (BRS330), C2 = Sweet hybrid (BRS509), C3 = Hybrid silage (BRS655), C4 = Hybrid grazing (BRS802), C5 = *Sorghum sudanense* (CMSxS912), C6 = grain (SRN-39) and C7 = grain (Shanqui-Red) in (A) Clue Field (CF) and (B) Vredepeel (VD).

Strigolactone profile

Sorgomol was produced by cultivars BRS330, BRS509, BRS655, BRS802 and CMSxS912 and was highly exuded by BRS655 compared with the other cultivars. All cultivars produced 5-deoxystrigol, which was highly exuded by BRS509 and Shanqui-Red and minimally produced by SRN-39. Orobanchol was exuded by SRN-39 at levels 300 to 1100 times higher than those of the other six cultivars (Figure 4).

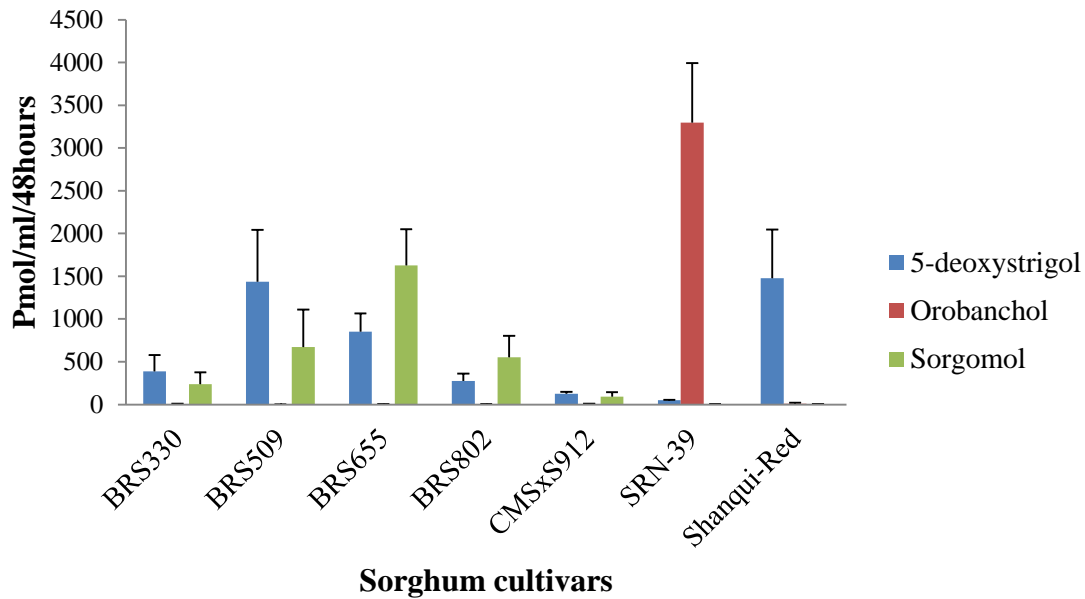


Figure 4. Strigolactone profile in different sorghum cultivars. The bars represent the mean values of biological replicates ($n=3$) \pm (SE).

Discussion

The bacterial taxonomic compositions of the rhizosphere communities of seven *Sorghum* genotypes at different growth stages and cultivated in two different soils were assessed by high-throughput 16S rRNA gene fragment sequencing. Simultaneous evaluation of the three factors revealed that soil type was the main driver of sorghum rhizosphere bacterial community composition, with a co-variance of 68.30%, followed by plant growth stage and plant cultivar, which contributed co-variances of 14.18% and 9.69%, respectively. Although there are no previous reports of the effect of these factors on sorghum rhizosphere bacterial community composition, some studies in different plant species corroborate our findings. For example, in a study of the composition of the soybean rhizosphere bacterial community using denaturing gradient gel electrophoresis (DGGE), Xu *et al.* (2009) found that soil played a major role in shaping the rhizosphere bacterial community composition, with plant growth stage as the second main factor. DGGE analyses also demonstrated that soil type and plant growth stage had stronger effects on potato rhizosphere bacterial assembly than genotype (van Overbeek & van Elsas, 2008, Inceoglu *et al.*, 2010). Using culture-dependent methods to evaluate the microbial colonization of maize roots, Chiarini *et al.* (1998) observed that soil type and plant development had a strong influence on the rhizosphere microbial community, whereas cultivar showed no effect. Although partially corroborating our results, these studies did not evaluate these factors simultaneously, and the techniques applied to assess the bacterial community structure (i.e.,

culture-dependent and DGGE) are rather low resolution compared with the next-generation sequencing approach applied in the present study.

In addition, although soil was the major contributor driving bacterial community composition in the sorghum rhizosphere at all evaluated stages of growth, an effect of plant genotype on the composition of the rhizosphere bacterial community was observed only after day 35. Similar results were reported by Inceoglu *et al.* (2010) for the effect of potato genotype and growth stage on the rhizosphere *Betaproteobacteria* community, with no effect of cultivar in the earlier stage of plant growth but an obvious effect in later stages. The exudates released at different growth stages can vary among different cultivars, thus affecting the rhizosphere microbial community composition (Micallef *et al.*, 2009, Inceoglu *et al.*, 2010). In a study of the rhizosphere microbiome of *Arabidopsis* throughout plant development, Chaparro *et al.* (2013) suggested that young plants exude sugars that are used by a wide diversity of microorganisms, whereas at later stages, plants release more specific exudates, such as phenolic compounds, possibly to select more specific microbes. Our results and those of previous studies suggest that the interaction between the plant and soil bacterial community is stochastic at earlier stages of sorghum growth and becomes more deterministic over time with the release of more complex compounds by the roots. The apparent lag in the effect of cultivar might also be attributable to the resilience and resistance of the soil microbial community.

The two soils (CF and VD) used as microbial sources for this study had different initial bacterial communities. Among the groups responsible for this dissimilarity were *Acidobacteria* GP4, which had higher abundance in VD compared with CF, and *Bradyrhizobiaceae*, which had higher abundance in CF compared with VD. In the rhizosphere, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes* were considerably enriched, whereas *Acidobacteria* (GP4, GP6 and GP16) and *Verrucomicrobia* (*Spartobacteria*) had much lower relative abundances compared with both VD and CF bulk soils. Similar to our findings, studies based on 16S rRNA sequencing showed an enrichment of a specific subset of *Proteobacteria* (including *Xanthomonadaceae*, *Oxalobacteraceae*, *Burkholderiaceae* and *Sphingomonadaceae*) and *Bacteroidetes* (*Chitinophagaceae* and *Flavobacteriaceae*) in the rhizosphere (Li *et al.*, 2014) and a lower proportion of *Acidobacteria* (Kielak *et al.*, 2009) and *Verrucomicrobia* (Lima *et al.*, 2015) in the rhizosphere compared with bulk soil.

Among the bacterial groups with significant dissimilarity across plant growth stages, members of *Proteobacteria* and *Bacteroidetes* showed the highest abundance in the earlier stages of plant growth, whereas members of *Acidobacteria* and *Verrucomicrobia* showed the highest

abundance during the last stage of plant growth. Differences in the exudates released during different growth stages among cultivars can affect the rhizosphere microbial community composition (Garbeva *et al.*, 2004, Singh *et al.*, 2007, Berg & Smalla, 2009, Inceoglu *et al.*, 2010). In a study of the rhizosphere microbiome in potato, Pfeiffer *et al.* (2017) suggested that a stable core microbiome over plant growth stages could be related to a similar pattern of plant exudates over time, whereas dynamic core microbiome members may respond to changes in root exudates over plant development.

Cultivar had little effect on sorghum rhizosphere bacterial community composition. However, in CF soil, SRN-39 had significantly higher relative abundances of Acidobacteria GP1, *Burkholderia*, *Cupriavidus* (*Burkholderiaceae*), *Acidovorax* and *Albidiferax* (*Comamonadaceae*) than the other six genotypes. In VD soil, cultivar had no effect on sorghum rhizosphere bacterial community composition. Corroborating our findings, Rasche *et al.* (2006) observed that the impact of plant variety on the structure of the potato rhizosphere microbial community was strongly dependent on soil type. The *Acidovorax* genus includes species characterized as iron oxidizers, whereas species belonging to the *Albidiferax* genus are described as iron reducers (Brown *et al.*, 2015). The cultivar SRN-39 exhibited less iron uptake in shoots and roots than the other cultivars (Table S11). The cause of the higher relative abundances of these groups in the SRN-39 rhizosphere in CF remains unclear. However, we hypothesize that sorghum root exudates play a role in establishing this specific rhizosphere microbial composition. Indeed, it has been suggested that specific exudates of different sorghum genotypes may influence rhizosphere microbial community composition (Henry, 2000, Funnell-Harris *et al.*, 2008). Different sorghum cultivars release different strigolactones, such as orobanchol, 5-deoxystrigol and sorgomol (Czarnta *et al.*, 2003, Mohamed *et al.*, 2016). Orobanchol and 5-deoxystrigol strongly induce hyphal branching in *Gigaspora margarita* (Akiyama *et al.*, 2010). Sorghum cultivar SRN-39 has a high level of orobanchol and a much lower level of 5-deoxystrigol in its root exudate (Gobena *et al.*, 2017), conferring resistance to the root parasitic weed *Striga hermonthica* (Del.) Benth. By contrast, the highly striga-susceptible cultivar Shanqui-Red contains a high level of 5-deoxystrigol and a very low level of orobanchol in its root exudate (Mohamed *et al.*, 2016). Our strigolactone analyses of the seven sorghum genotypes confirmed that SRN-39 produced orobanchol at levels 300 to 1100 times higher than the other six genotypes (Figure 4). Taking into account the high level of orobanchol produced by SRN-39, we postulate that the high production of orobanchol contributed to the high abundances of certain bacterial groups in the rhizosphere of SRN-39 cultivated in CF soil. Plants produce higher amounts of strigolactones in less-fertile soils (Jamil *et al.*, 2014). VD soil is more than twice as fertile as CF soil as assessed by base saturation (Table S1), which might explain why the effect of the SRN-39

cultivar on the microbial community was not significant in VD soil. However, further studies are needed to confirm this hypothesis and to exclude effects of other possible differences in the root exudate compositions of these sorghum genotypes.

In conclusion, this work provides evidence that soil is the main factor driving sorghum rhizosphere bacterial community composition, followed by plant growth stage and genotype. An effect of genotype on the microbial community only became apparent at later stages of growth. Additionally, although cultivar was not the main driver of sorghum rhizosphere bacterial community changes, cultivar SRN-39, which has a distinct strigolactone composition in its root exudate, selects its own rhizosphere bacterial community composition, dependent on the soil microbial pool. Further investigations will reveal the mechanism underlying this specific microbial recruitment process.

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Supplementary Materials

Table S1. Soil physical and chemical properties of Clue Field and Vredepeel.

Parameter	Unit	Clue Field	Vredepeel
N total	mg/Kg	1220	970
C : N ratio		18	22
N supply capacity	Kg/ha	43	24
S total	mg/Kg	240	190
P	mg/Kg	5.4	4.6
K	mg/Kg	18	209
Ca	Kg/ha	107	188
Mg	mg/Kg	43	108
Na	mg/Kg	6	26
pH		5.1	5.4
OM	%	3.7	3.7
C inorganic	%	0.03	0.06
SB	Cmolc/dm ³	0.42	1.53
V	%	9.3	25.6
Clay	%	3	1
Silt	%	4	5
Sand	%	89	90
CEC	mmol+/Kg	46	60

P, K, Ca, Mg, Na = available; OM= Organic matter; SB: Sum of bases; V: Base saturation; CEC = Cation exchange capacity; N supply capacity = N expected to be mineralized based on N-total, C/N ratio and soil life.

Table S2. Goods coverage (Alpha diversity index) in seven sorghum cultivars (BRS330, BRS509, BRS655, BRS802, CMSxS912, SRN-39 and Shanqui-Red); in four different growth stages (days 10, 20, 35 and 50); in the Bulk and rhizosphere of Clue Field (CF) and Vredepeel (VD) soil.

Soil	Bulk soil	Rhizosphere				
		Cultivar	Day 10	Day 20	Day 35	Day 50
CF	0.90 ± 0.05	BRS330	0.93 ± 0.06	0.93 ± 0	0.92 ± 0.01	0.93 ± 0.02
		BRS509	0.93 ± 0.01	0.94 ± 0.01	0.93 ± 0.01	0.94 ± 0.01
		BRS655	0.94 ± 0.04	0.93 ± 0	0.93 ± 0.01	0.88 ± 0.05
		BRS802	0.93 ± 0.04	0.95 ± 0.01	0.91 ± 0.05	0.94 ± 0.03
		CMSxS912	0.71 ± 0.33	0.92 ± 0.04	0.94 ± 0.04	0.91 ± 0.02
		SRN-39	0.95 ± 0	0.94 ± 0	0.94 ± 0.03	0.9 ± 0.06
		Shanqui-Red	0.92 ± 0.03	0.95 ± 0.04	0.93 ± 0	0.87 ± 0.03
VD	0.88 ± 0.03	BRS330	0.89 ± 0.03	0.92 ± 0.02	0.9 ± 0.01	0.9 ± 0.02
		BRS509	0.87 ± 0.04	0.88 ± 0.03	0.88 ± 0.01	0.83 ± 0.05
		BRS655	0.89 ± 0.03	0.92 ± 0.01	0.91 ± 0.02	0.89 ± 0.04
		BRS802	0.93 ± 0.05	0.91 ± 0.02	0.92 ± 0.01	0.79 ± 0.18
		CMSxS912	0.9 ± 0.01	0.86 ± 0.07	0.88 ± 0.05	0.88 ± 0.02
		SRN-39	0.85 ± 0.12	0.92 ± 0.01	0.92 ± 0.04	0.95 ± 0.04
		Shanqui-Red	0.91 ± 0.01	0.92 ± 0.01	0.9 ± 0.01	0.89 ± 0.03

Table S3. Summary of MFA analysis of the bacterial contribution to the dissimilarity between the bulk soil of Clue Field (CF) and Vredepeel (VD).

Bacterial group			Contribution (%)		Rel. abundance	
Phylum	Class	Family	Dim. 1	Dim. 2	CF	VD
Acidobacteria	Acidobacteria_Gp4	uncl_Acidobacteria_Gp4	1.64	0.14	7.1	13.7
	Acidobacteria_Gp16	uncl_Acidobacteria_Gp16	0.73	1.05	2.9	2.3
Proteobacteria	Gammaproteobacteria	Xanthomonadaceae	1.15	1.52	1.0	2.2
Verrucomicrobia	Spartobacteria	uncl_Spartobacteria	0.82	0.57	18.3	14.5

Dim. 1 = Dimension 1 (Horizontal axis); Dim. 2 = Dimension 2 (Vertical axis).

Table S4. Summary of MFA analysis of the bacterial contribution to the dissimilarity between the bulk soil (BK) and rhizosphere (RH) in Clue Field soil.

Bacterial group			Contribution (%)		Rel. abundance	
Phylum	Class	Family	Dim. 1	Dim. 2	BK	RH
Acidobacteria	Acidobacteria_Gp1	uncl_Acidobacteria_Gp1	1.76	0.86	1.2	2.7
	Acidobacteria_Gp4	uncl_Acidobacteria_Gp4	1.76	1.58	7.1	4.4
	Acidobacteria_Gp6	uncl_Acidobacteria_Gp6	1.93	1.54	14.8	8.1
	Acidobacteria_Gp16	uncl_Acidobacteria_Gp16	1.42	0.92	2.9	1.7
Bacteroidetes	Sphingobacteriia	<i>Chitinophagaceae</i>	1.75	1.35	2.2	4.0
	uncl_Bacteroidetes	uncl_Bacteroidetes	1.20	1.13	1.6	2.3
Planctomycetes	Planctomycetia	<i>Planctomycetaceae</i>	1.86	1.35	4.8	2.6
Proteobacteria	Alphaproteobacteria	<i>Bradyrhizobiaceae</i>	1.42	1.29	3.7	6.0
		<i>Sphingomonadaceae</i>	1.13	1.50	1.6	2.2
		uncl_Rhizobiales	1.55	1.19	1.6	2.7
	Betaproteobacteria	<i>Oxalobacteraceae</i>	0.95	0.49	1.2	3.2
		uncl_Betaproteobacteria	1.82	1.03	4.4	8.2
	Deltaproteobacteria	uncl_Myxococcales	2.02	1.24	1.2	2.3
	Gammaproteobacteria	<i>Xanthomonadaceae</i>	1.52	1.35	1.0	1.7
Verrucomicrobia	Spartobacteria	uncl_Spartobacteria	1.96	1.10	18.3	8.9

Dim. 1 = Dimension 1 (Horizontal axis); Dim. 2 = Dimension 2 (Vertical axis).

Table S5. Summary of MFA analysis of the bacterial contribution to the dissimilarity between the bulk soil (BK) and rhizosphere (RH) in Vredepeel (VD) soil.

Bacterial group			Contribution (%)		Rel. abundance	
Phylum	Class	Family	Dim. 1	Dim. 2	BK	RH
Acidobacteria	Acidobacteria_Gp1	uncl_Acidobacteria_Gp1	1.01	0.74	1.3	1.8
	Acidobacteria_Gp4	uncl_Acidobacteria_Gp4	1.39	1.58	13.7	5.7
	Acidobacteria_Gp6	uncl_Acidobacteria_Gp6	1.11	2.17	13.9	5.2
	Acidobacteria_Gp16	uncl_Acidobacteria_Gp16	1.12	1.79	2.3	1.1
Planctomycetes	Planctomycetia	<i>Planctomycetaceae</i>	0.77	2.54	3.9	2.0
Proteobacteria	Alphaproteobacteria	<i>Bradyrhizobiaceae</i>	1.43	1.24	2.3	3.9
		<i>Sphingomonadaceae</i>	1.41	1.69	1.8	3.1
		uncl_Rhizobiales	1.07	0.57	1.2	2.7
	Betaproteobacteria	<i>Burkholderiaceae</i>	1.17	0.41	0.6	1.1
		<i>Oxalobacteraceae</i>	1.86	0.30	1.3	4.4
		uncl_Burkholderiales	1.45	1.13	0.6	1.5
		uncl_Betaproteobacteria	1.34	1.12	3.4	6.0
Gammaproteobacteria	<i>Xanthomonadaceae</i>	1.96	1.55	2.2	4.2	
Verrucomicrobia	Spartobacteria	uncl_Spartobacteria	1.54	0.97	14.5	3.6

Dim. 1 = Dimension 1 (Horizontal axis); Dim. 2 = Dimension 2 (Vertical axis).

Table S6. Relative abundance of the total rhizosphere bacterial community in Clue field (CF) and Vredepeel (VD).

Bacterial group			Soil	
Phylum	Class	Family	CF	VD
Acidobacteria	Acidobacteria_Gp1	uncl_Acidobacteria_Gp1	2.7 ± 0.001	1.8 ± 0.001
	Acidobacteria_Gp4	uncl_Acidobacteria_Gp4	4.4 ± 0.002	5.7 ± 0.003
	Acidobacteria_Gp6	uncl_Acidobacteria_Gp6	8.1 ± 0.005	5.2 ± 0.004
	Acidobacteria_Gp16	uncl_Acidobacteria_Gp16	1.7 ± 0.001	1.1 ± 0.001
Bacteroidetes	uncl_Bacteroidetes	uncl_Bacteroidetes	2.3 ± 0.001	3.1 ± 0.001
Planctomycetes	Planctomycetia	<i>Planctomycetaceae</i>	2.6 ± 0.002	2 ± 0.001
Proteobacteria	Alphaproteobacteria	<i>Bradyrhizobiaceae</i>	6 ± 0.002	3.9 ± 0.001
		<i>Sphingomonadaceae</i>	2.2 ± 0.001	3.1 ± 0.001
		uncl_Alphaproteobacteria	1.6 ± 0	2.7 ± 0.001
	Betaproteobacteria	<i>Burkholderiaceae</i>	1.7 ± 0.002	1.1 ± 0
		<i>Comamonadaceae</i>	2.2 ± 0.001	2.4 ± 0.001
		<i>Oxalobacteraceae</i>	3.2 ± 0.002	4.4 ± 0.003
		uncl_Betaproteobacteria	8.2 ± 0.003	6 ± 0.002
	Deltaproteobacteria	uncl_Myxococcales	2.3 ± 0.001	2.9 ± 0.001
	Gammaproteobacteria	<i>Xanthomonadaceae</i>	1.7 ± 0.001	4.2 ± 0.002
	uncl_Proteobacteria	uncl_Proteobacteria	1.5 ± 0	1.8 ± 0.001
Verrucomicrobia	Spartobacteria	uncl_Spartobacteria	8.9 ± 0.006	3.6 ± 0.002

The values are means of replicates (n=84) ± (SE). Numbers in bold represent the relative abundance of the groups significant dissimilar (p<0.05) among the treatments.

Table S7. Relative abundance of rhizosphere bacterial community at four different plant growth stages (days 10, 20, 35 and 50) in Clue Field soil.

Bacterial group			Growth stage			
Phylum	Class	Family	day 10	day 20	day 35	day 50
Acidobacteria	Acidobacteria_Gp16	uncl_Acidobacteria_Gp16	1.5 ± 0.002	1.4 ± 0.002	1.5 ± 0.002	2.3 ± 0.003
	Acidobacteria_Gp6	uncl_Acidobacteria_Gp6	6.8 ± 0.008	6.9 ± 0.008	7 ± 0.008	11.8 ± 0.013
Bacteroidetes	Sphingobacteriia	<i>Chitinophagaceae</i>	4.3 ± 0.003	4.2 ± 0.003	4.2 ± 0.002	3.2 ± 0.003
		<i>Sphingobacteriaceae</i>	1.3 ± 0.002	1 ± 0.002	0.4 ± 0.001	0.2 ± 0
Planctomycetes	Planctomycetia	<i>Planctomycetaceae</i>	2.2 ± 0.002	2.3 ± 0.002	2.3 ± 0.003	3.7 ± 0.004
Proteobacteria	Alphaproteobacteria	<i>Bradyrhizobiaceae</i>	6.2 ± 0.005	6.6 ± 0.004	6.5 ± 0.004	4.6 ± 0.005
		<i>Sphingomonadaceae</i>	2.3 ± 0.001	2.3 ± 0.002	2.5 ± 0.001	1.8 ± 0.002
		Betaproteobacteria	<i>Comamonadaceae</i>	2.5 ± 0.003	2.5 ± 0.002	2.1 ± 0.002
		<i>Oxalobacteraceae</i>	4.7 ± 0.004	4.1 ± 0.004	2.6 ± 0.002	1.6 ± 0.002
		uncl_Burkholderiales	1.5 ± 0.001	1.6 ± 0.001	1.4 ± 0.001	1 ± 0.001
		Gammaproteobacteria	<i>Xanthomonadaceae</i>	1.9 ± 0.001	1.9 ± 0.001	1.7 ± 0.001
Verrucomicrobia	uncl_Proteobacteria	uncl_Proteobacteria	1.5 ± 0.001	1.7 ± 0.001	1.6 ± 0.001	1.3 ± 0.001
	Opitutae	<i>Opitutaceae</i>	0.6 ± 0.001	0.6 ± 0.001	0.5 ± 0.001	1.2 ± 0.003
	Subdivision3	uncl_Subdivision3	1.2 ± 0.001	1.5 ± 0.001	1.5 ± 0.001	1.8 ± 0.001

The values are means of replicates (n=42) ± (SE). Numbers in bold represent the relative abundance of the groups significant dissimilar (p<0.05) among the treatments.

Table S8. Relative abundance of rhizosphere bacterial community at four different plant growth stages (days 10, 20, 35 and 50) in Vredepeel soil.

Bacterial group			Growth stage				
Phylum	Class	Family	day 10	day 20	day 35	day 50	
Acidobacteria	Acidobacteria_Gp6	uncl_Acidobacteria_Gp6	5.2 ± 0.006	2.9 ± 0.004	5.5 ± 0.005	7.4 ± 0.008	
Bacteroidetes	Sphingobacteriia	<i>Sphingobacteriaceae</i>	1.2 ± 0.001	0.9 ± 0.001	0.6 ± 0	0.6 ± 0.001	
Planctomycetes	Planctomycetia	<i>Planctomycetaceae</i>	2.2 ± 0.003	1.2 ± 0.002	2 ± 0.002	2.8 ± 0.003	
Proteobacteria	Alphaproteobacteria	<i>Sphingomonadaceae</i>	3.8 ± 0.002	3.5 ± 0.002	2.8 ± 0.002	2.3 ± 0.001	
		<i>Caulobacteraceae</i>	1 ± 0.001	1.3 ± 0.001	0.8 ± 0.001	0.6 ± 0.001	
		Betaproteobacteria	<i>Comamonadaceae</i>	2.5 ± 0.002	2.9 ± 0.002	2.2 ± 0.001	2.1 ± 0.002
			<i>Oxalobacteraceae</i>	3.6 ± 0.002	6.7 ± 0.006	4.1 ± 0.004	3.2 ± 0.003
			uncl_Burkholderiales	1.5 ± 0.001	1.8 ± 0.001	1.5 ± 0.001	1.3 ± 0.001
		Deltaproteobacteria	uncl_Myxococcales	2.4 ± 0.002	3.6 ± 0.002	3 ± 0.002	2.6 ± 0.002
	Gammaproteobacteria		<i>Xanthomonadaceae</i>	5 ± 0.003	5.5 ± 0.006	3.5 ± 0.002	2.7 ± 0.002
			<i>Polyangiaceae</i>	1 ± 0.002	1.2 ± 0.001	0.9 ± 0.001	0.7 ± 0.001
			uncl_Proteobacteria	uncl_Proteobacteria	1.7 ± 0.001	2.2 ± 0.001	1.8 ± 0.001
	Verrucomicrobia	Subdivision3	uncl_Subdivision3	0.8 ± 0	0.7 ± 0	1 ± 0.001	1.4 ± 0.001
Spartobacteria		uncl_Spartobacteria	3.4 ± 0.004	2.4 ± 0.004	3.8 ± 0.004	4.8 ± 0.006	

The values are means of replicates (n=42) ± (SE). Numbers in bold represent the relative abundance of the groups significant dissimilar (p<0.05) among the treatments.

Table S9. Relative abundance of rhizosphere bacteria in the rhizosphere of seven different sorghum cultivars (BRS330, BRS509, BRS655, BRS802, CMSxS912, SRN-39 and Shanqui-Red), in Clue Field soil.

Bacterial group		Cultivar						
Class	Family	BRS330	BRS509	BRS655	BRS802	CMSxS912	SRN-39	Shanqui-Red
Acidobacteria_Gp1	uncl_Acidobacteria_Gp1	2.5 ± 0.002	2.9 ± 0.002	2.2 ± 0.002	2.4 ± 0.002	2.3 ± 0.002	3.7 ± 0.003	3 ± 0.005
Betaproteobacteria	<i>Burkholderiaceae</i>	1.3 ± 0.001	1.3 ± 0.001	1.4 ± 0.001	1.6 ± 0.003	1.3 ± 0.002	3.6 ± 0.016	1.2 ± 0.002
	<i>Comamonadaceae</i>	2.2 ± 0.002	1.9 ± 0.001	2.3 ± 0.003	1.9 ± 0.002	2 ± 0.003	3.4 ± 0.005	1.6 ± 0.003

The values are means of replicates (n=24) ± (SE). Numbers in bold represent the relative abundance of the groups significant dissimilar (p<0.05) among the treatments.

Table S10. Distribution of bacterial groups for different Sorghum cultivars (BRS330, BRS509, BRS655, BRS802, CMSxS912, SRN-39 and Shanqui-Red), in Clue Field soil.

Bacterial group		Cultivar						
Family	Genus	BRS330	BRS509	BRS655	BRS802	CMSxS912	SRN-39	Shanqui-Red
Burkholderiaceae	<i>Burkholderia</i>	12.2 ± 0.3	11.3 ± 0.1	11.2 ± 0.2	13.4 ± 0.2	10 ± 0.2	32.2 ± 1.3	9.6 ± 0.2
	<i>Cupriavidus</i>	3.7 ± 0.1	3.6 ± 0.1	10.2 ± 0.3	33.9 ± 1.3	3.7 ± 0.1	38.8 ± 1.3	6.2 ± 0.2
Comamonadaceae	<i>Acidovorax</i>	12.4 ± 0.2	14.2 ± 0.2	17.6 ± 0.4	12.3 ± 0.3	12 ± 0.2	20.8 ± 0.6	10.7 ± 0.2
	<i>Albidiferax</i>	10.7 ± 0.4	12 ± 0.2	18.7 ± 0.6	8.8 ± 0.1	10.7 ± 0.3	34 ± 0.5	5.2 ± 0.1
	<i>Polaromonas</i>	14 ± 0.3	11 ± 0.3	16.5 ± 0.3	14.2 ± 0.3	19.5 ± 0.6	13.5 ± 0.3	11.4 ± 0.3
	<i>Variovorax</i>	18.5 ± 0.4	14.3 ± 0.1	17.2 ± 0.2	15.4 ± 0.2	13 ± 0.2	11.7 ± 0.1	10 ± 0.2
	uncl_ <i>Comamonadaceae</i>	15.9 ± 0.4	12.3 ± 0.1	13.9 ± 0.2	11.8 ± 0.1	11.1 ± 0.2	25.9 ± 0.4	9.1 ± 0.1

The values are means of replicates (n=24) ± (SE). Numbers in bold represent highest bacterial genus relative abundance among the cultivars.

Table S11. Macro and micronutrients and dry biomass of different sorghum cultivars.

Samples	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	Mo	Al	Cl	Dry biomass (g)
	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	
C1 SHOOT-CF	4.6	3.1	9.5	17.1	3.1	0.7	42.5	81	3.9	33.7	0.18	49.6	4082.5	7.43
C2 SHOOT-CF	3.4	3.2	7.4	15.7	3.3	0.7	24.3	75.1	3.5	31.1	0.13	26	3674.3	9.95
C3 SHOOT-CF	4.1	4.1	9.8	12.6	2.9	0.7	20.1	44.3	3.6	29.6	0.14	15.1	3408	9.85
C4 SHOOT-CF	2.3	2.6	4.8	14.7	3.8	0.7	19.3	80.3	3.6	48.5	0.12	22.7	3780.8	14.6
C5 SHOOT-CF	3	4.2	6.9	13.6	3.7	1.4	20.6	75.2	5.6	71	0.20	14.4	3301.5	5
C6 SHOOT-CF	3.2	2.3	4	12.4	3.4	0.6	10.6	75.2	4.3	28.3	0.08	10.6	2183.3	10
C7 SHOOT-CF	4.2	3.2	7.7	14.6	3.5	0.9	28.7	88.5	5.4	43.5	0.09	15.1	3780.8	4.05
C1 ROOT-CF	7.4	0	1.8	8.5	1.3	1.7	41.2	97.2	30.6	81.5	0.94	376.4	1810.5	3.28
C2 ROOT-CF	6.5	2.3	1.5	8.4	2.9	2.3	3381.8	252.6	40.5	226.3	0.76	2544.9	2112.3	3.43
C3 ROOT-CF	7.7	1.1	5.1	7.4	2.9	2.6	705.2	220.4	35.6	161.7	0.70	1347	2023.5	2.26
C4 ROOT-CF	2.1	0.1	0.7	4	1.3	0.9	84.2	94.4	15.7	101.9	0.26	647.1	1970.3	10.9
C5 ROOT-CF	3.3	0.4	3.4	5.6	1.6	1.2	485.5	207.9	26.4	105.8	0.45	1181.6	2183.3	2.92
C6 ROOT-CF	5.5	0.1	3.4	5.2	3.1	2.1	37.8	142.4	15.2	136.1	0.26	35.7	2538.3	3.45
C7 ROOT-CF	5.6	1.4	1.2	5.4	1.9	1.7	2499.8	238.6	39.5	119.3	0.31	1597	2218.8	2.23
C1 SHOOT-VD	6.7	2	17.2	6.6	3.1	0.6	20.4	29	3	20.4	1.18	9.7	5857.5	7.17
C2 SHOOT-VD	4.6	1.6	17	5.4	3.2	0.4	11	19	2.2	16.4	1.04	9	6354.5	14.29
C3 SHOOT-VD	5	1.9	17.7	4.3	2.2	0.5	13.1	15	2.5	16.8	0.90	7.1	7987.5	12.08

C4 SHOOT-VD	1.6	1.3	13.5	4.2	2.4	0.5	10	18	2.6	20.7	0.58	10.6	5591.3	28.2
C5 SHOOT-VD	2.3	1.9	18	4.6	2.5	0.8	14.8	19.9	3.7	33.7	0.14	17.2	6283.5	12.33
C6 SHOOT-VD	2.3	1.5	13.3	4.6	2.3	0.5	8.2	21.9	2.5	18	0.09	7.8	4792.5	18.6
C7 SHOOT-VD	3.2	2.1	19.6	6.1	3.1	0.7	11.1	23.7	3.7	32.2	0.13	8	3858.3	11.81
C1 ROOT-VD	11.1	1.7	9.6	8	2.5	2.5	1475.3	102.9	40.2	177.8	0.36	2309.5	3283.8	2.91
C2 ROOT-VD	10.3	1.8	9.6	9.6	3.2	3.2	1391.1	104.1	55	171.7	0.34	2571.8	3514.5	3.97
C3 ROOT-VD	10.5	0	7.5	7.8	2.5	2.9	38.2	89.7	29.3	122.1	0.35	168.3	3461.3	3.23
C4 ROOT-VD	8	0	5	7.3	1.9	2.2	26.3	76.4	28.4	97	0.17	136.8	2289.8	7.3
C5 ROOT-VD	7.8	0	4.7	7.1	1.8	2.2	24.3	76.7	29	96	0.18	150.3	3940.5	4.86
C6 ROOT-VD	7.9	0	7.5	7.5	2	2.1	44	85.8	31	101.6	0.18	261.4	2556	4.88
C7 ROOT-VD	7.4	1.4	9.1	7.1	2.6	2.5	1191.6	84.1	38	135.2	0.12	1678	1970.3	6.09

Cultivar: C1 = BRS330, C2 = BRS509, C3 = BRS655, C4 = BRS802, C5 = CMSxS912, C6 = SRN-39 and C7 = Shanqui-Red.

Soils: CF = Clue field soil and VD = Vredepeel soil.

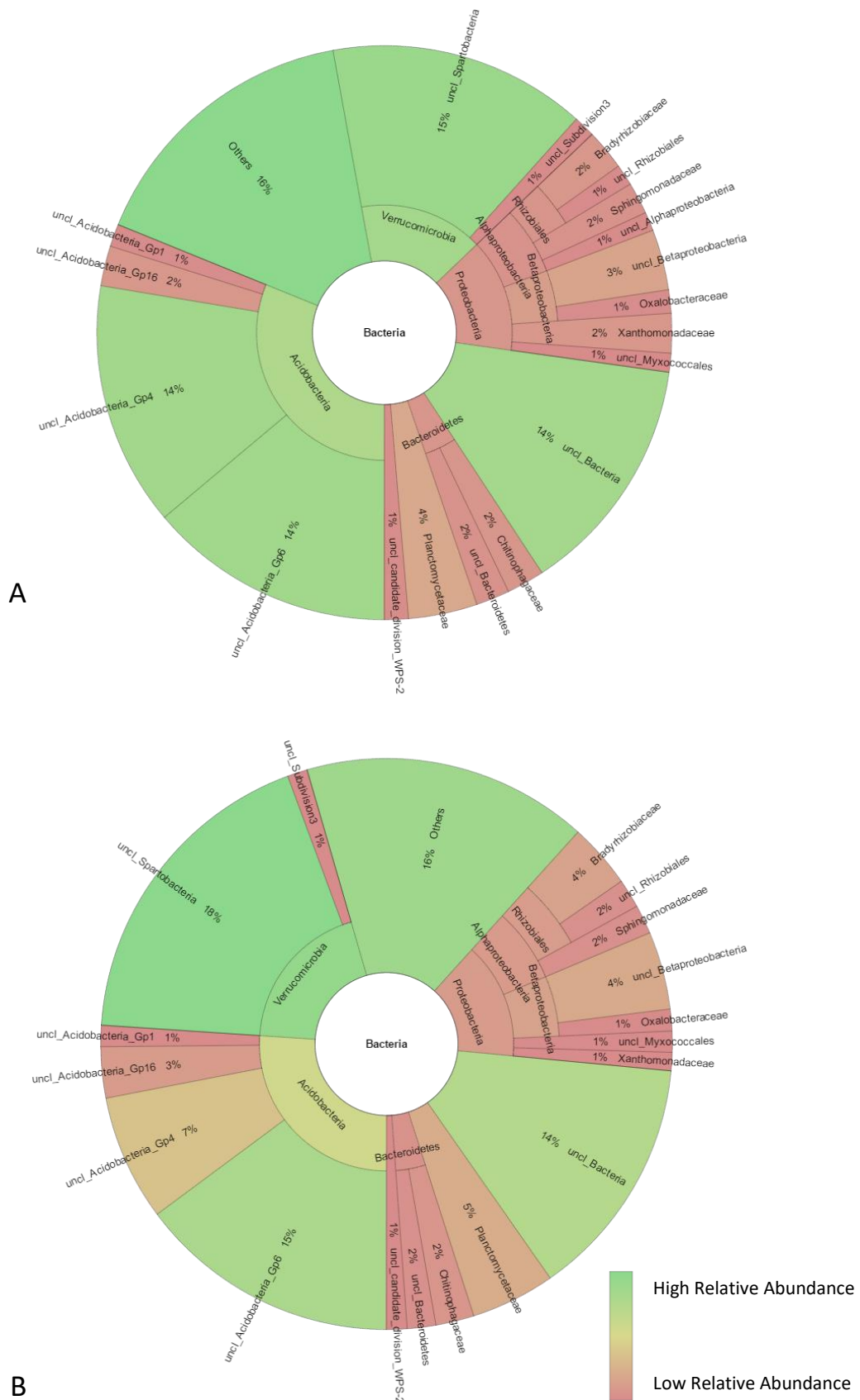


Figure S1. Relative abundance of bacteria in the bulk soil of (A) Clue Field and (B) Vredepeel.