The rhizomicrobiome of Sorghum ; impact on plant growth and stress tolerance

Schlemper, T.R.

Chapter 4

Impact of rhizoplane bacterial community on drought tolerance of *Sorghum bicolor* (L.) Moench

Thiago R. Schlemper, Andrielli S. Pulcrano, Francisco A. Souza, Paulo C. Magalhães, Jos M. Raaijmakers, Johannes A. van Veen, and Eiko E. Kuramae

(To be submitted)

Abstract

Drought is a major limitation of agricultural productivity worldwide. Several studies have suggested that plants exposed to drought can rely on root-associated microorganisms to overcome, to some extent, the negative impact of this abiotic stress factor on growth. Here we investigated if sorghum plants pre-cultivated in soils with different bacterial community composition (microbial seed bank) responded differently to water stress. To this end, drought susceptible (DS) and drought tolerant (DT) sorghum lineages were grown in five different soils for 21 days, transplanted with their rhizoplane microbial community to a standardized substrate and grown for an additional 38 days under water stress conditions or no stress (field capacity). The results showed that the DS lineage showed significant increase in shoot biomass at water deficient conditions when hosting rhizoplane bacterial community from Calcareous soil. This effect was not observed when the DS lineage was pre-cultivated in the other soils. Despite these phenotypic differences, we did not observe an apparent relation between the growth of sorghum and the rhizoplane bacterial community composition. However, we did find at water deficient conditions high abundances of the *Caulobacteraceae* family in the rhizosplane of the DS lineage planted in Cerrado and of the *Rhizobiaceae* family in the rhizoplane of the DT lineage planted in Sorghum field soil, two soils with a history of low rainfall regimes. These results suggest that pre-cultivation of sorghum in soils with a history of low rainfall regimes provided representatives of the *Alphaproteobacteria* a selective advantage in colonizing the rhizoplane of sorghum grown at water deficient conditions.

Introduction

Drought is one of the major limitations to agricultural productivity worldwide (Zolla *et al.*, 2013, Vurukonda *et al.*, 2016, Xu *et al.*, 2018). Currently, approximately 70 percent of total freshwater withdrawal in the world is used for agricultural activities (FAO, 2015). Although irrigation may ensure crop productivity in low rainfall areas, it cannot be applied everywhere due to freshwater shortage. Thus, alternatives to improve plant water use efficiency in agriculture without loss of productivity are needed. Breeding programs have made major steps in improving and developing drought-tolerant crops, but this effort alone is unlikely to be sufficient to meet the demand for food by an increasing world population (Hu & Xiong, 2014). Engineering beneficial root microbiomes has been proposed as a novel and sustainable approach that could complement plant breeding and other management practices to alleviate abiotic and biotic stress (Mendes *et al.*, 2013). Kavamura *et al.* (2013) suggested that plants are dependent on microorganisms capable to enhance their metabolic activity to resist stress.

Plants respond to drought stress by suppression of photosynthesis and stomatal closure to reduce water loss (Rizhsky *et al.*, 2004, Rahdari *et al.*, 2012). Root-associated bacteria may confer drought tolerance to plants by a variety of mechanisms that include uptake of nutrients, production of exopolysaccharides and phytohormones (Sandhya *et al.*, 2009, Rolli *et al.*, 2015, Vurukonda *et al.*, 2016). Bacterial exopolysaccharide are hydrated compounds that, besides protecting the producing bacteria from desiccation, can provide water stress resistance to the plant by contributing to biofilm formation on the roots and improving soil structure and aggregation (Naseem & Bano, 2014, Costa *et al.*, 2018). Therefore, several efforts are made to identify root-associated bacterial genera that assist their hosts to overcome drought events (Govindasamy *et al.*, 2017, Xu *et al.*, 2018). Whereas some studies focus on culturable bacterial species or strains, others include analyses of the total soil microbial community. Naveed *et al.* (2014) showed that the bacterial endophyte *Burkholderia phytofirmans* strain PsJN had positive influence on plant growth, photosynthetic rate and stomatal conductance of two maize cultivars grown under drought stress. Also Rolli *et al.* (2015) showed that isolates of *Pseudomonas* sp., *Acinetobacter* sp., *Sphingobacterium* sp. and a bacterial consortium composed of five other bacterial genera increased the root weight of pepper, particularly under drought conditions. In their study on the role of soil bacterial communities in drought tolerance of *Arabidopsis*, Zolla *et al.* (2013) found that the bacterial groups from a natural site with a history of *Arabidopsis* growth significantly increased plant biomass under drought conditions as compared with

a non-sympatric microbiome. This study suggests that selection and/or host specificity of members of the microbiome may contribute to the ability to confer stress tolerance.

A better understanding of the mechanisms by which root-associated microorganisms interact with sorghum under water stress conditions is of interest since sorghum is tolerant to drought and grown predominantly in arid regions. Only few studies have addressed the effect of bacteria on drought tolerance, growth, and yield of sorghum. Rashad *et al.* (2001) tested the influence of *Bradyrhizobium japonicum* (*Bradyrhizobium*) and *Rhizobium leguminosarum* (*Rhizobium*) strains on the growth and yield of two sorghum cultivars (Dorado and Shandawell) at different soil moisture conditions (100, 60 and 40% of field capacity). They found that under drought stress, the Dorado cultivar showed a larger growth response to *R. leguminosarum* than to *B. japonicum,* whereas the sorghum cultivar Shandaweel showed the opposite response. Khalili *et al.* (2008) evaluated the effect of chemical and biological phosphorus fertilizers (phosphorus solubilizing bacteria) on sorghum yield at different irrigation regimes and found that the application of the phosphorus-solubilizing bacteria *Bacillus lentus* and *Pseudomonas putida* together with 50% ammonium phosphate increased the soil buffer index resulting in higher water retention in soil at moisture stress conditions than the non-inoculated treatment. Grover *et al.* (2014) observed that *Bacillus* strains inoculated on the root surface of sorghum at moisture stress conditions promoted a significant increase in shoot length and root biomass. Xu *et al.* (2018), studying the root associated microbiome of different drought tolerant sorghum phenotypes (BTx642 and RTx430) at three different irrigation treatments, demonstrated that drought reduces root microbial diversity, alters the sorghum root microbiome and causes increased abundance and activity of monoderm bacteria, which lack outer membranes and contain thick cell walls.

These promising examples prompted us to investigate the effect of the bacterial soil community seed bank on sorghum growth under drought conditions with the ultimate goal to identify potential bacterial species/taxa recruited in the rhizoplane of sorghum that could alleviate drought stress. We used a transplantation approach that minimize soil physical-chemical characteristics in water stress alleviation. We hypothesize that soils differ significantly in the abundance of microbial genera/species that can alleviate drought stress. More specifically we hypothesize that (i) rhizoplane bacterial communities recruited by sorghum from soil with a history of sorghum cultivation and drought have higher potential to sustain sorghum growth under water deficiency than do rhizoplane bacterial community recruited from other soils, and (ii) sorghum plants select specific rhizoplane bacterial populations to alleviate water deficiency.

Material and Methods

Soil

Soil samples were collected in different locations in Minas Gerais State - Southeast of Brazil. The selection was based on different crop history backgrounds, soil characteristics, and precipitation regimes. The soil samples were named Calcareous (19º 26' 31.41" S 44º 10'23.15" W), Cerrado (19º 24' 58.01" S 44º 09' 08.10" W) and sorghum field (15º 45' 47.578" S 43º 17' 20.220" W) (Figure 1A). Calcareous was characterized as a highly fertile soil, Cerrado was characterized by its natural vegetation and low phosphorus availability and sorghum field characterized as an agricultural soil cropped with sorghum for more than 25 years. Furthermore, Calcareous and Cerrado areas have a mean annual precipitation of 1250 mm while the sorghum field region has a mean annual precipitation of 650 mm (Guimarães *et al.*, 2010). Each soil sample composed of six subsamples collected in a "W" format (0 – 20 cm topsoil layer). Besides the three soils, two other substrates were included in the experiment: a enriched microbial substrate (EM substrate) and the control constituted of sterilized sand and vermiculite (2:1 v/v) (Figure 1A). The EM substrate was produced using soil of a maize-soybean rotation field cropped with maize (cultivar BRS Caimbé) and *Crotalaria spectabilis* Roth and mixed with sand and vermiculite (1:10:10 v/v). Chemical and physical properties of the three soils and the control substrate are in Supplementary Table S1.

Mesocosm experiment

The experiment was carried out in the greenhouse of the Brazilian Agriculture Research Corporation, Embrapa Milho e Sorgo, Sete Lagoas, Minas Gerais State, Brazil at controlled conditions of a photoperiod of 16/8h light/dark and average maximum and minimum temperature of 29 ºC and 23 ºC respectively. These conditions mimic to some extent the growth conditions of sorghum in that region. The experiment was conducted in two phases. The factorial experimental design of the first phase consisted of two sorghum lineages (drought susceptible - DS "9618158" and drought tolerant - DT "9910032") and five soil treatments (Calcareous, Cerrado, Sorghum field, EM substrate and Control; Figure 1A and 1B), in six replicates. Sorghum seeds were surface disinfected with ethanol (70%) for 1 min, followed by sodium hypochlorite (3%) treatment for 3 min, ethanol (70%) for 30 sec and finally 3 times washed with sterile distilled water. The last portion of rinsed water was plated on petri dishes containing 1% of water agar medium to check the seeds surface disinfection. Plastics containers (320 mL) for seedling production were filled up with 270 mL of the different soils types

and ten seeds of each Sorghum lineage were sowed per container with six replicates per soil type. Seedlings were grown for 21 days in the different soils for the recruitment of bacteria on the rhizoplane (Figure 1B). After 21 days, plants were carefully removed from the containers, and the roots were washed with sterile water to remove any visible soil particle from the root system (Figure 1C and 1D) leaving only the rhizoplane bacterial community attached to the root surface

Root washed plants were divided into two subsets. The first subset (Figures 1D and 1K) was used to assess the sorghum rhizoplane bacterial community selected by plants and to determine shoot biomass, whereas the second subset was transplanted to a standardized substrate comprehending the second phase of the experiment (Figure 1E). Five plants per treatment were transplanted to 6-liters plastic pots filled with sterilized substrate (2:1 v/v sand:vermiculite), with four replicates per treatment. Ten days after transplantation, the plants were thinned to two plants per pot (Figures 1E and 2F). The factorial experimental design of the second phase consisted of two sorghum lineages (drought susceptible - DS "9618158" and drought tolerant - DT "9910032") containing in its rhizoplane the bacterial community of five soil treatments (Calcareous, Cerrado, Sorghum field, EM substrate and Control) at two soil moisture contents (-18 and -138 Kpa) in four replicates. DT lineage was mainly used as a counterpoint for the results found to DS lineage. To assess the bacterial community from the rhizoplane compartment, a protocol of extraction containing sodium pyrophosphate was adapted, according to Salles *et al.* (2004). Briefly, 0.5 g of individualized and washed roots (without any soil) were submitted to 20 ml of $Na_4P_2O_7$ solution (sodium pyrophosphate 0.1%) containing 3g of metal spheres (3mm), sonicated for 30 sec by e ultrasonic cleaner (Branson 3210) and shaken for 30 min in an incubator shaker model SL 223 (Solab) at 180 rpm at room temperature (Figure 1K). Subsequently, the roots were removed from the tubes and rhizoplane bacterial cells suspended in sodium pyrophosphate solution were harvested by centrifugation at 1,664 xg for 30 min. The cells were stored at -80 ºC for DNA extraction. DNA was extracted from these cells using the Power Soil DNA Isolation Kit (Mo Bio Laboratories, Inc.) and DNA quality was checked by Agarose (1.0%) gel electrophoresis in Tris-Borate-EDTA (TBE) buffer. The DNA was quantified in a Spectrophotometer NanoDrop 2000 (Thermo scientific) and used as template for the amplification of the 16S ribosomal gene marker by PCR.

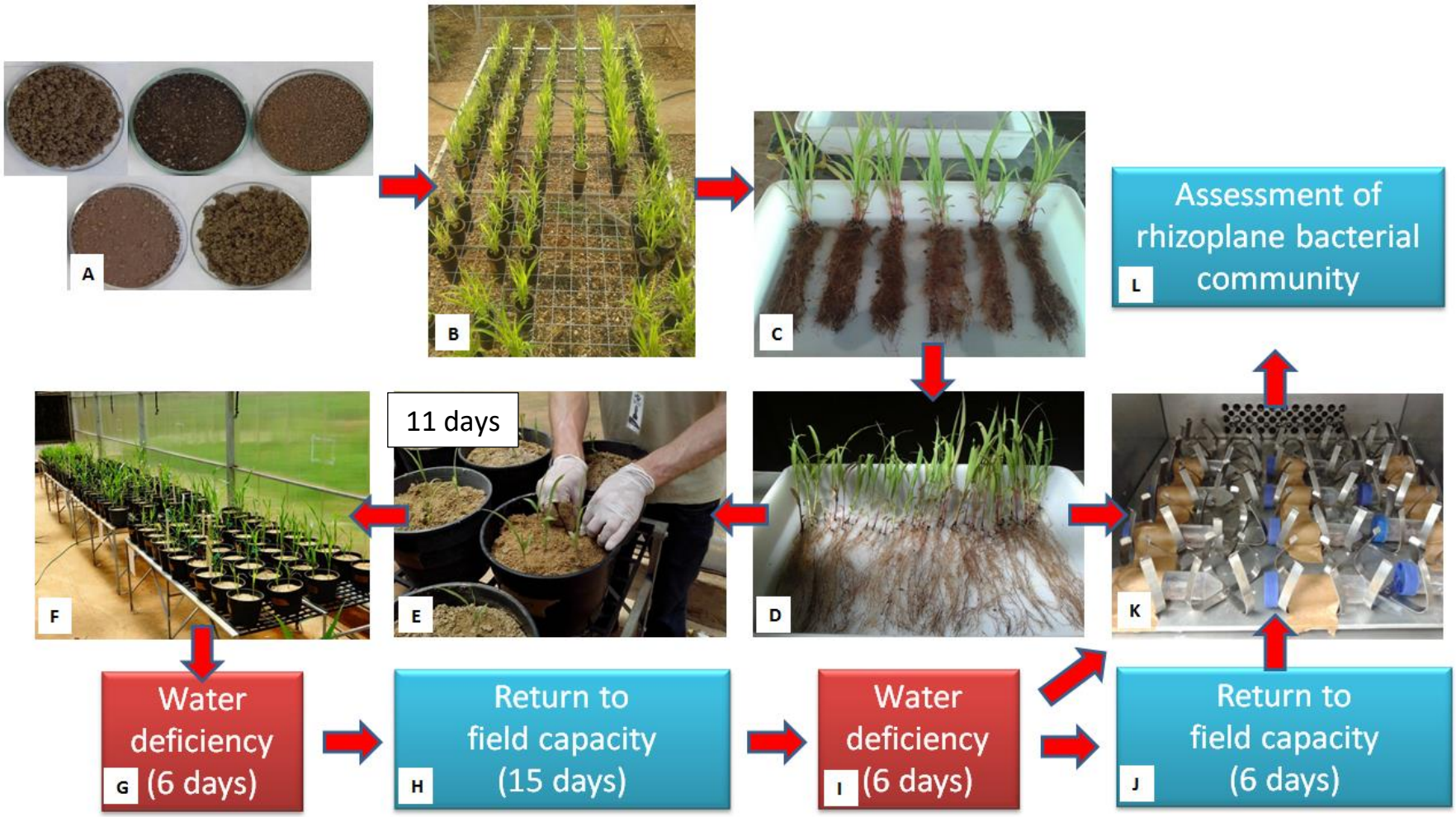

Figure 1. Experimental design. (A) different soils: i - Sterilized sand plus vermiculite (Control); ii - Calcareous; iii - Cerrado; iv - Sorghum field; and v – Enriched microbial substrate; (B) two sorghum linages (drought susceptible "9618158" and drought tolerant "9910032") planted in different soils; (C) process of soil particle removal in the sorghum roots; (D) plant individualization; (E) transfer of plants to the standardized substrate; (F) recovery of plants from post-transplant stress; (G) Half of plants were subjected to water deficiency (WD) and the other half remained at field capacity (FC); (H) plants under water deficiency return to field capacity (WD_R_FC); (I) second drought event application (WD); (J) plants grown under water deficiency return to field capacity (WD_R_FC); (K) rhizoplane bacterial community extraction; and (L) assessment of bacterial community.

Substrate moisture treatments

Eleven days after transplantation (Figure 1F), half of the plants of each treatment was submitted to water tension reduction of 50% of field capacity (-138Kpa) and another half remained at 100% of field capacity (18Kpa) as control (Figure 1G). All plants received Magnavaca nutrient solution (Magnavaca *et al.*, 1987). Soil water tension was daily controlled by Watermark Sensor (Irrometer Company Inc. – Riverside – California). On the sixth day at water deficiency, plants were rewetted and maintained at field capacity for 15 days (Figure 1H). Subsequently, plants were submitted to the second round of water deficiency of 50% of field capacity for 6 more days and rewetted to 100% of field capacity for 6 more days (Figure 1I and 1J) until harvest.

Harvest and plant parameter measurements

At the end of the second round of water depletion and 6 days in which plants were recovered to field capacity (Figure 1i and 1j), cells from the rhizoplane were collected for DNA extraction using the procedure described previously. At harvest time, plants were on pre-flowering stage and had their dry biomass and root architecture evaluated. Root architecture was evaluated by Scanner (EPSON Flatbed Scanner EPSON Expression 10000XL 1.8 V1.0 2.00) using the program WinRHIZO Pro 2007a (Régent Instr. Inc.) with the parameters for specific root area (SRA), specific root length (SRL), and specific root density (RDENS). Specific root area was calculated by dividing the surface area by the root dry biomass. Specific root length was calculated by the formula:

$$SRL = \frac{\frac{\text{root length}}{100}}{\text{root dry biomass}} \times 10$$

16S rRNA partial gene sequencing

DNA of each treatment was used as a template for 16S rRNA gene fragment amplification. The amplification of the *16S rRNA* V4 gene region was performed using the primer set 515F (forward) (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (reverse) (5′-GGACTACVSGGGTATCTAAT-3′) (Bergmann *et al.*, 2011). The primers were tagged with multiplex barcodes for sample identification. The PCR was carried out using 0.2 µl of 0.056 U fastStart Exp*Taq* Polymerase (Roche Applied Sciences, Indianapolis, IN, USA), 2.5µl of dNTP (2mM each), 0.25µl of each primer, 2.5 µl of Faststart high fidelity reaction buffer (10X concentration with 18mM of $MgCl_2$) and 1.0 µl of DNA template. The conditions of the thermocycling were: denaturing at 95 °C for 5 min followed by 35

cycles of denaturation at 95 °C for 45 s, annealing at 55 °C for 45s, extension at 72 °C for 60 s followed by a final extension at 72 °C for 5 min. As positive control isolated cells of *Escherichia coli* were used and as a negative control water instead of DNA was used.

The 16S rRNA PCR products were purified using Agencourt ® AMPure ® XP Reagent (Beckman Coulter, USA). The quality of PCR products was checked before and after the purification in agarose (1.5%) gel electrophoresis in TBE buffer. Furthermore, the final concentration and quality of the PCR products were checked using Fragment analyser™ - Automated CE system (Advanced Analytical Technologies, Inc). PCR products were equalized at the concentration of 20 ng/µl and pooled for sequencing at an Illumina Miseq platform at BGI Co., Ltd. (Shenzhen, China).

Data analysis

PANDASeq (Masella *et al.*, 2012) was used to align the Illumina reads. Paired-end reads were assembled and filtered for quality criteria by a minimum sequence length of 200 bp and with a Phred score of 25. Primers were removed and the sequences were converted to FASTA format and concatenated into a single file. All procedure of OTU table construction and subsequent BIOM format conversion was performed according to the Brazilian Microbiome Project (Pylro *et al.*, 2014).

Statistical analysis

Average reads per sample were 27913 (Table S2) and the number of Operational Taxonomic Units (OTUs) was 4604. BIOM file was rarefied to the library size of 3300 reads in order to equalize the sampling depth (Weiss *et al.* (2017) because the library sizes differed in at least 10 fold magnitude. Thus, Shannon index was performed and ANOVA and Tukey as post hoc statistical tests ($p<0.05$) were applied to determine the bacterial community Alpha diversity. Principal Coordinate Analysis (PCoA) was performed using Bray-Curtis as dissimilarity distance and permutation analysis PERMANOVA as statistical test ($p<0.05$) to determine the similarities and dissimilarities among the bacterial communities. Both Alpha diversity index and PCoA analysis were performed on MicrobiomeAnalyst (Dhariwal *et al.*, 2017). Dissimilarities in rhizoplane bacterial communities were tested by Welch's test ($P<0.05$) by Statistical Analysis of Metagenomics Profiles (STAMP) v2.1.3 software (Parks *et al.*, 2014). False Discovery Rates (FDR) Benjamini-Hochberg (Benjamini & Hochberg, 1995) was applied to correct P values. To infer the differences in plant biomass and root morphology ANOVA and Tukey as post hoc statistical tests ($p<0.05$) were performed. To evaluate the rate of sorghum biomass increase after transplantation we used the mixed linear model with time

as random factor using the package lme4 (Bates *et al.*, 2014). Both, biomass and morphology statistical analysis were performed in R version 3.1.3 (R Development Core Team, 2017).

Results

Soil characteristics

The different soils had distinct physical and chemical properties (Table S1). Calcareous soil had the highest N, P, K, Organic matter (OM), C, Ca, Mg, Sum of bases (SB), exchange cationic capacity (CEC), the degree of base saturation (V%), pH, and silt. Cerrado had the lowest pH and P availability and the highest H+Al, Al, Aluminum saturation, Fe, and clay. 'Sorghum field' soil showed the highest Mn, Zn and fine sand.

Plant biomass and root architecture

Following pre-cultivation of sorghum in the different soils for 21 days, shoot biomass of the drought susceptible (DS) lineage was significantly higher in Calcareous soil than in the control and Cerrado soils (Table 1). After transplantation to a standardized substrate and growth for an additional 38 days under field capacity, however, no statistically significant differences in shoot or root biomass was evidenced between the plants pre-cultivated in the different soils (Table 2). Likewise, no significant difference was found on the increment of shoot dry biomass of the DS lineage originally cropped in different soils and transplanted to standardized substrate at field capacity (Table 3). Concerning root architecture, the DS lineage pre-cultivated in Cerrado soil showed significant smaller specific root length (SRL) and specific root area (SRA) compared to the control at field capacity conditions (Table 4).

Table 1. Shoot biomass of sorghum lineages drought susceptible (DS) and drought tolerant (DT) cultivated for 21 days in different soils.

Soils	Sorghum lineage	
	DS	DT
Control	0.07 ± 0.00 B a	0.05 ± 0.00 C b
Calcareous	0.18 ± 0.02 A b	0.26 ± 0.01 A a
Cerrado	0.06 ± 0.02 B a	0.03 ± 0.00 C a
Sorghum field	0.13 ± 0.00 AB b	0.21 ± 0.00 B a

The values are means of replicates (n=4) ± (SE). Capital letters compare (on column) the means of shoot dry biomass within the same sorghum lineage and between the soil bacterial sources. Lowercase letters compare (on row) the means of shoot dry biomass within the same soil source and between sorghum lineages. Means followed by the same letter are not statistically different by Tukey test ($P<0.05$).

Table 2. Sorghum shoot and root dry biomass of two sorghum lineages (drought susceptible "9618158" **(DS)** and drought tolerant "9910032" **(DT)**) originally cropped in different soils (Control, Calcareous, Cerrado, Sorghum field and Enriched microbial - EM substrate) and later transplanted to a standardized mixed substrate of sterilized sand and vermiculite (2:1 v/v). Plants were harvested after grown for 38 days at standardized substrate at the last day of water disturbance. Water treatments were **FC** – Field Capacity; **WD** – Water Deficiency.

Plant system	Sorghum cultivar	Rhizoplane soil source	Water treatment	
			Field Capacity	Water deficiency
Shoot	DS	Control	1.64 ± 0.68 A a	0.26 ± 0.09 C a
		Calcareous	2.00 ± 0.38 A b	3.55 ± 0.40 AB a
		Cerrado	6.63 ± 2.05 A a	1.52 ± 0.44 BC a
		Sorghum field	5.23 ± 2.01 A a	1.47 ± 0.95 BC a
		EM substrate	6.87 ± 2.28 A a	5.30 ± 0.88 A a
	DT	Control	0.44 ± 0.06 B a	0.62 ± 0.20 B a
		Calcareous	9.44 ± 0.56 A a	1.26 ± 0.26 B b
		Cerrado	1.97 ± 0.81 B a	0.96 ± 0.36 B a
		Sorghum field	3.08 ± 1.33 B a	1.01 ± 0.31 B a
		EM substrate	9.53 ± 1.93 A a	4.31 ± 0.74 A b
Root	DS	Control	0.49 ± 0.19 A a	0.09 ± 0.03 B a
		Calcareous	0.63 ± 0.33 A a	1.14 ± 0.20 A a
		Cerrado	1.82 ± 0.46 A a	0.35 ± 0.12 AB b
		Sorghum field	1.32 ± 0.63 A a	0.52 ± 0.28 AB a
		EM substrate	1.87 ± 0.63 A a	1.04 ± 0.20 A a
	DT	Control	0.14 ± 0.03 A a	0.20 ± 0.07 A a
		Calcareous	2.45 ± 0.68 A a	0.46 ± 0.14 A b
		Cerrado	0.65 ± 0.24 A a	0.4 ± 0.11 A a
		Sorghum field	1.36 ± 0.89 A a	0.34 ± 0.07 A a
		EM substrate	2.64 ± 0.73 A a	0.72 ± 0.1 A b

The values are means of replicates (n=4) ± (SE) expressed in grams. For each cultivar and plant system Capital letter compares (on column) the means of dry biomass within each water treatment and between the rhizoplane soil sources. Lowercase letters compare (on row) the means of dry biomass within the same rhizoplane soil source and between the water treatments. Means followed by the same letter are not statistically different by Tukey test ($P<0.05$).

Table 3. Ratio of increment of sorghum shoot dry biomass from plants originally cropped in different soils for 21 days and plants transplanted to a standardized soil grown for 38 days at different moisture conditions (Field capacity and water deficiency).

Soils	Ratio of increment of shoot dry biomass (g/day)			
	Field Capacity		Water deficiency	
	DS	DT	DS	DT
Control	0.041	0.010	0.005	0.015
Calcareous	0.047	0.241*	0.088*	0.026
Cerrado	0.172	0.051	0.038	0.024
Sorghum field	0.139	0.075	0.035	0.021

Ratio of biomass increment represents the regression coefficient from a linear mixed model that expresses, in grams per day, the changes in plant growth as affected by each treatment. Asterisk (*) represents the significant statistical differences between regression coefficient values of different soils within the same lineage (DS - drought susceptible lineage "9618158" and DT - drought tolerant lineage "9910032") and moisture conditions (ANOVA $p<0.05$).

Table 4. Specific root length **(SRL)**, Specific root area **(SRA)** and Specific root density (**RDENS)** of two sorghum lineages (drought susceptible "9618158" **(DS)** and drought tolerant "9910032" **(DT)**) originally cropped in different soils (Control, Calcareous, Cerrado, Sorghum field and Enriched microbial - EM substrate), and later transplanted to a standardized mixed substrate of sterilized sand and vermiculite (2:1 v/v). Plants were harvested after grown for 38 days at standardized substrate at the last day of water disturbance.

Parameter	Sorghum cultivar	Rhizoplane soil source	Water treatment	
			Field Capacity	Water Deficiency
SRL (cm) x 10^3	DS	Control	85.75 ± 26.43 A a	179.18 ± 53.09 A a
		Calcareous	53.92 ± 10.58 AB a	39.82 ± 6.41 B a
		Cerrado	26.25± 4.27 B b	64.77 ± 8.63 AB a
		Sorghum field	49.49 ± 6.85 AB a	83.52 ± 20.49 AB a
		EM substrate	39.78 ± 4.12 AB a	46.58 ± 16.71 B a
	DT	Control	103.62 ± 33.60 A a	92.20 ± 20.97 A a
		Calcareous	59.88 ± 15.68 A a	59.07 ± 5.83 A a
		Cerrado	63.51 ± 13.37 A a	65.65 ± 11.53 A a
		Sorghum field	48.03 ± 15.87 A a	51.51 ± 7.05 A a
		EM substrate	32.81 ± 4.00 A a	43.78 ± 2.92 A a
SRA (cm^2) x 10^2	DS	Control	10.27 ± 1.49 A a	21.47± 6.51 A a
		Calcareous	8.92 ± 0.47 AB a	7.60 ± 0.65 A a
		Cerrado	6.42 ± 0.42 B b	8.79 ± 0.49 A a
		Sorghum field	7.54 ± 0.32 AB a	10.97 ± 3.53 A a
		EM substrate	6.88 ± 0.42 B a	9.53 ± 3.02 A a
	DT	Control	10.58 ± 2.38 A a	10.82 ± 1.82 A a
		Calcareous	10.73 ± 2.79 A a	7.81 ± 0.63 A a
		Cerrado	10.45 ± 0.80 A a	9.07 ± 1.12 A a
		Sorghum field	7.51 ± 1.29 A a	7.23 ± 0.56 A a
		EM substrate	6.82 ± 0.57 A a	7.54 ± 0.30 A a
RDENS (g/cm^3)	DS	Control	0.09 ± 0.01 A a	0.1 ± 0.06 A a
		Calcareous	0.08 ± 0.01 A a	0.08 ± 0.01 A a
		Cerrado	0.07 ± 0.00 A a	0.1 ± 0.00 A b
		Sorghum field	0.1 ± 0.00 A a	0.11 ± 0.03 A a
		EM substrate	0.1 ± 0.01 A a	0.07 ± 0.01 A a
	DT	Control	0.12 ± 0.02 A a	0.1 ± 0.01 A a
		Calcareous	0.07 ± 0.01 A a	0.12 ± 0.02 A a
		Cerrado	0.07 ± 0.00 A a	0.1 ± 0.01 A a
		Sorghum field	0.1 ± 0.00 A a	0.12 ± 0.01 A a
		EM substrate	0.09 ± 0.01 A a	0.09 ± 0.00 A a

The values are means of replicates (n=4) ± (SE). For each cultivar and root architecture parameter Capital letters compare (on column) the means within each water treatment and between the rhizoplane soil sources. Lowercase letters compare (on row) the means within the same rhizoplane soil source and between the water treatments. Means followed by the same letter are not statistically different by Tukey test ($P<0.05$).

At water deficiency, the DS lineage pre-cultivated in the Calcareous soil or EM substrate had significant higher shoot and root dry biomass than the control (Table 2). Sorghum plants grown in Calcareous and EM substrate had a significantly smaller SRL than in the control, indicating a root architecture modification of DS plants pre-cultivated in different soils (Table 4). We observed that the DS lineage from Calcareous soil showed significantly higher shoot biomass at water deficiency than when grown at field capacity. We also observed that the DS lineage coming from Cerrado had a significantly higher SRL and SRA at water deficiency compared with plants under field capacity (Table 4). Furthermore, the DS lineage from Cerrado soil showed significant higher root dry biomass under field capacity than under water deficient conditions (Table 2).

For the drought tolerant (DT) sorghum lineage, we observed that shoot biomass of plants pre-grown in Calcareous soil for 21 days was higher than for plants pre-cultivated in other soils (Table 1). After transplantation to a standardized substrate and growth for an additional 38 days under field capacity, significant higher shoot biomass was evidenced for plants pre-cultivated in Calcareous soil and EM substrate as compared to plants pre-cultivated in the other soils (Table 2). Under water deficient conditions, the DT lineage pre-cultivated in EM substrate showed significant higher shoot biomass compared with the other soils. At field capacity, the highest increment of shoot dry biomass of the DT lineage was for those pre-cultivated in Calcareous soil (Table 3). No significant difference in root dry biomass or root architecture was observed for the DT lineage between different soils (Tables 2 and 4). Due the accidental loss of material, it was not possible to collect dry biomass data from plants that grew in EM substrate for 21 days.

Sorghum rhizoplane bacterial community structure

In general, except for the control treatment, the number of OTUs and Shannon diversity indices of the rhizoplane communities just before transplantation were significantly higher than after transplantation to the standardized substrate regardless of water regime condition (Table S3). No difference in number of OTUs or in Shannon index of diversity was found for plants growing steadily at field capacity as compared to plants subjected to moisture stress.

The bacterial taxa detected in the rhizoplane of sorghum grown in the different soils before and after transplantation were assigned to the Proteobacteria (71.4%), Bacteroidetes (8.1%), Actinobacteria (6.7%), Verrucomicrobia (3.3%) and Acidobacteria (3.2%). Within these phyla, the most abundant bacterial families were *Burkholderiaceae* (21.7%), *Oxalobacteraceae* (10.1%), *Rhizobiaceae* (7.4%), *Xanthomonadaceae* (7.1%), and *Chitinophagaceae* (5.2%). PCoA plots and PERMANOVA analysis ($P<0.05$) showed that the differences in rhizoplane bacterial communities of

the pre-cultivated, 21-day-old DS plants was explained by the soil type (Figure 2A). The bacterial families driving this dissimilarity ($P < 0.05$) were *Oxalobacteraceae* (45.3%) and *Burkholderiaceae* (24.4%) for the control soil, and *Comamonadaceae* (8.1%), *Chitinophagaceae* (6.3%), and *Rhizobiaceae* (4.8%) for the Sorghum field soil (Table 5). Due to the accidental loss of material, it was not possible to assess the rhizoplane bacterial community for sorghum plants pre-cultivated for 21 days in EM substrate.

Table 5. Relative abundance of rhizoplane bacterial families of two sorghum lineages (drought susceptible "9618158" (DS) and drought tolerant "9910032" (DT)) planted on different soils (Control, Calcareous, Cerrado and Sorghum field) for 21 days.

Sorghum lineages	Bacterial families	Soils			
		Control	Calcareous	Cerrado	Sorghum field
DS	*Oxalobacteraceae*	**45.29 ± 0.77 a**	1.94 ± 0.55 c	0.92 ± 0.15 c	7.39 ± 0.33 b
	Burkholderiaceae	**24.41 ± 0.44 a**	0.61 ± 0.11 c	1.11 ± 0.28 c	3.2 ± 0.26 b
	Pseudomonadaceae	0.03 ± 0.02 c	0.44 ± 0.11 bc	**19.73 ± 2.81 a**	0.29 ± 0.03 b
	Sphingomonadaceae	1.09 ± 0.19 c	4.41 ± 0.46 a	0.78 ± 0.25 b	**11.09 ± 1.75 a**
	Comamonadaceae	0.98 ± 0.15 b	0.81 ± 0.22 b	0.57 ± 0.13 b	**8.07 ± 0.44 a**
	Chitinophagaceae	0.27 ± 0.08 c	1.64 ± 0.17 b	0.32 ± 0.01 c	**6.26 ± 0.74 a**
	Rhizobiaceae	1.23 ± 0.32 b	1.4 ± 0.17 b	0.18 ± 0.08 c	**4.85 ± 0.66 a**
	Hyphomicrobiaceae	0.18 ± 0.02 c	**5.47 ± 0.25 a**	0.95 ± 0.08 d	1.57 ± 0.22 b
	Sphingobacteriaceae	**4.28 ± 0.21 a**	0.4 ± 0.1 b	0.21 ± 0.04 b	0.42 ± 0.09 b
	Planctomycetaceae	0 ± 0 b	**6.07 ± 0.66 a**	0.06 ± 0.04 b	0.08 ± 0.04 b
DT	*Enterobacteriaceae*	**50.73 ± 5.69 a**	0.76 ± 0.12 b	3.49 ± 3.07 b	0.74 ± 0.06 b
	Oxalobacteraceae	**19.28 ± 2.72 a**	1.42 ± 0.16 c	3.78 ± 1.47 b	7.67 ± 0.33 b
	Burkholderiaceae	**10.71 ± 1.08 a**	0.34 ± 0.06 c	1.9 ± 0.76 b	2.49 ± 0.23 b
	Comamonadaceae	0.52 ± 0.07 c	1.53 ± 0.17 b	1.82 ± 1.09 bc	**12.19 ± 0.27 a**
	Chitinophagaceae	1.77 ± 0.24 b	1.55 ± 0.31 b	0.76 ± 0.53 b	**6.18 ± 0.39 a**
	Gaiellaceae	0 ± 0 c	**4.18 ± 0.52 a**	1.91 ± 0.34 b	1.16 ± 0.24 b
	Pseudonocardiaceae	0.01 ± 0.01 c	**4.37 ± 0.29 a**	1.44 ± 0.19 b	1.25 ± 0.06 b
	Rhizobiaceae	0.64 ± 0.12 b	1.26 ± 0.27 b	0.22 ± 0.12 c	**4.2 ± 0.57 a**

Values are means of replicates (n=3) ± (SE). Values in bold highlight the highest relative abundance of a family group among different treatments. Lowercase letters compare (on row) the original data of bacterial family dissimilarities. Means followed by the same letter are not statistically different by Welch's test ($P<0.05$) followed by Bonferroni correction.

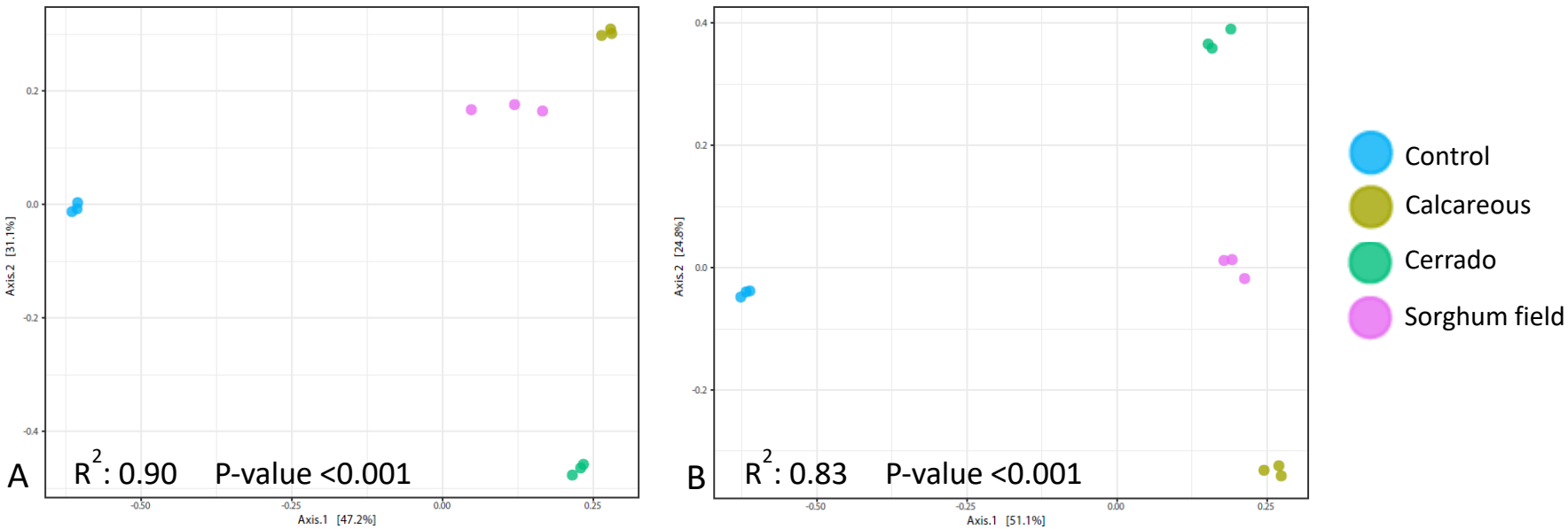

Figure 2. Principal Coordinate Analysis (PCoA) representing the dissimilarities in rhizoplane bacterial communities of two sorghum lineages (A) drought susceptible "9618158" and (B) drought tolerant "9910032" grown on different soils (Control, Calcareous, Cerrado and Sorghum field) for 21 days.

The rhizobacterial community of the DS lineage transplanted to and subsequently grown in standardized soil for 38 days under field capacity showed significant dissimilarity (PERMANOVA) between the soils (Figure 3A). The bacterial family with highest relative abundance ($P < 0.05$) was *Burkholderiaceae* (63.4%) for the Control soil (Table 6). When the DS lineage was grown under water deficient conditions, significant highest relative abundance of *Caulobacteraceae* (6.2%) was observed from plants pre-cultivated in the Cerrado soil (Table 7).

When DT was exposed to the water deficient conditions, the rhizoplane bacterial community of plants pre-cultivated in Sorghum field soil showed a significantly higher abundance of *Rhizobiaceae* (26.2%) than plants pre-cultivated in the other soils (Table 7). *Rhizobiaceae* of DT grown in Sorghum field and Cerrado soils were significantly more abundant under water deficiency compared with plants at field capacity or under conditions where water deficiency was recovered to field capacity (Figure 6).

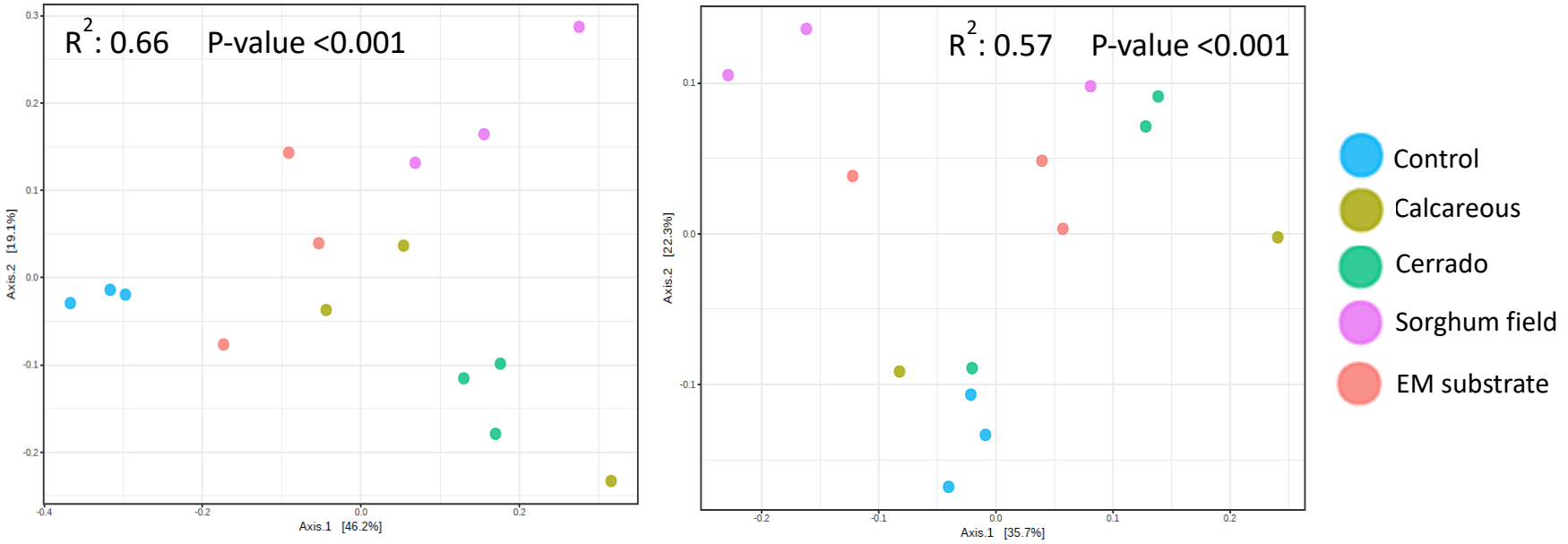

Figure 3. Principal Coordinate Analysis (PCoA) representing the dissimilarities of bacterial communities of two sorghum lineages (A) drought susceptible "9618158" and (B) drought tolerant "9910032" grown on different soils (Control, Calcareous, Cerrado, Sorghum field, and Enriched microbial – EM substrate) for 21 days and later transplanted to a standardized mixed substrate of sterilized sand and vermiculite (2:1 v/v). The rhizoplane bacterial community was sampled after grown for 38 days at standardized substrate at field capacity moisture conditions.

Table 6. Relative abundance of rhizoplane bacterial families of two sorghum lineages (drought susceptible "9618158" (DS) and drought tolerant "9910032" (DT)) grown on different soils (Control, Calcareous, Cerrado, Sorghum field, and Enriched microbial – EM substrate) for 21 days and later transplanted to a standardized mixed substrate of sterilized sand and vermiculite (2:1 v/v). Rhizoplane bacterial community was sampled after grown for 38 days at standardized substrate at field capacity moisture conditions.

Sorghum Lineages	Family	Soils				
		Control	Calcareous	Cerrado	Sorghum	EM substrate
DS	*Burkholderiaceae*	**63.41 ± 0.62 a**	23.11 ± 8.24 c	14.16 ± 1.91 c	15.66 ± 6.35 bc	31.77 ± 3.76 b
DT	*Hyphomicrobiaceae*	2.74 ± 0.41 b	0.91 ± 0.34 c	2.21 ± 0.17 b	0.99 ± 0.08 c	**4.85 ± 0.57 a**

Values are means of replicates (n=3) ± (SE). Values in bold highlight the highest relative abundance of a family group among different treatments. Lowercase letters compare (on row) the original data of bacterial family dissimilarities. Means followed by the same letter are not statistically different by Welch's test (P<0.05) followed by Bonferroni correction.

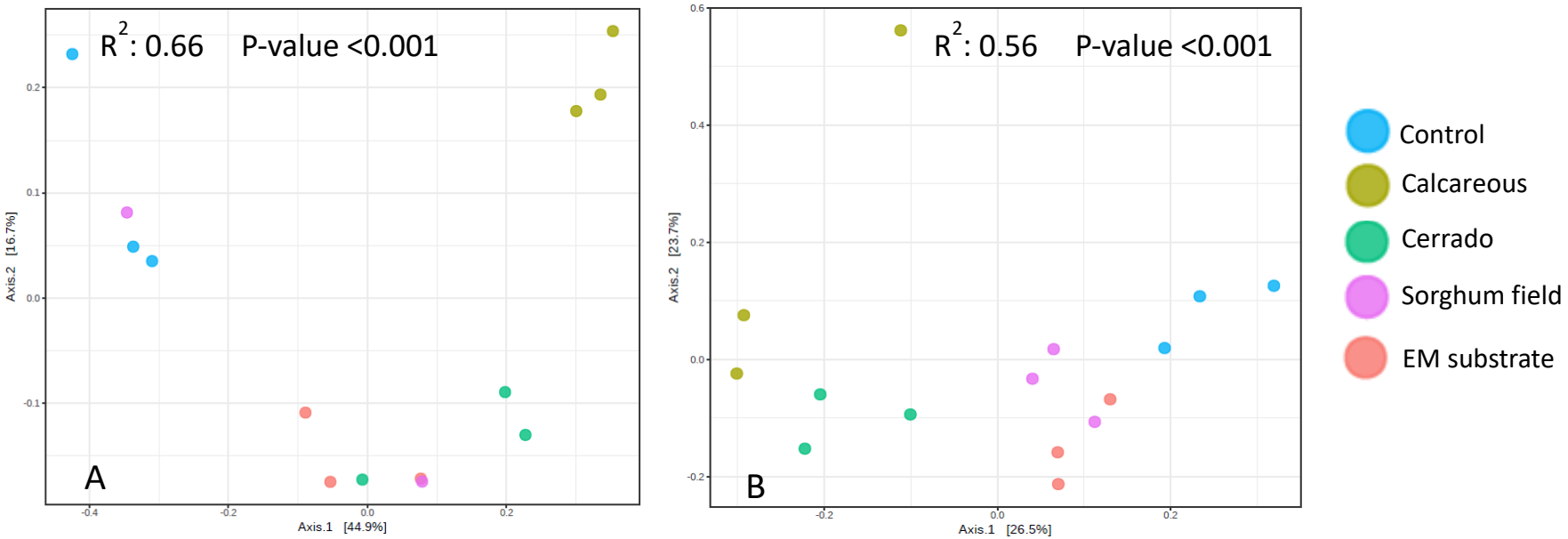

Figure 4. Principal Coordinate Analysis (PCoA) representing the dissimilarities of bacterial communities of two sorghum lineages (A) drought susceptible "9618158" and (B) drought tolerant "9910032" grown on different soils (Control, Calcareous, Cerrado, Sorghum field, and Enriched microbial – EM substrate) for 21 days and later transplanted to a standardized mixed substrate of sterilized sand and vermiculite (2:1 v/v). The rhizoplane bacterial community was sampled after grown for 38 days at standardized substrate at water deficiency moisture conditions.

Table 7. Relative abundance of rhizoplane bacterial families of two sorghum lineages (drought susceptible "9618158" (DS) and drought tolerant "9910032" (DT)) grown on different soils (Control, Calcareous, Cerrado, Sorghum field, and Enriched microbial – EM substrate) for 21 days and later transplanted to a standardized mixed substrate of sterilized sand and vermiculite (2:1 v/v). The rhizoplane bacterial community was sampled after grown for 38 days at standardized substrate at water deficiency moisture conditions.

Sorghum lineages	Family	Soils				
		Control	Calcareous	Cerrado	Sorghum	EM substrate
DS	*Xanthomonadaceae*	4.34 ± 0.27 ab	3.23 ± 0.56 b	8.6 ± 1.22 a	3.4 ± 1.78 b	**9.73 ± 1.73 ab**
	Caulobacteraceae	1.61 ± 0.28 b	1.68 ± 0.18 b	**6.21 ± 0.41 a**	2.6 ± 1.21 b	3.7 ± 1.06 a
DT	*Rhizobiaceae*	8.62 ± 4.15 b	6.12 ± 3.15 b	12.82 ± 0.37 ab	**26.21 ± 3.86 a**	11.3 ± 2.19 b

Values are means of replicates (n=3) ± (SE). Values in bold highlight the highest relative abundance of a family group among different treatments. Lowercase letters compare (on row) the original data of bacterial family dissimilarities. Means followed by the same letter are not statistically different by Welch's test (P<0.05) followed by Bonferroni correction.

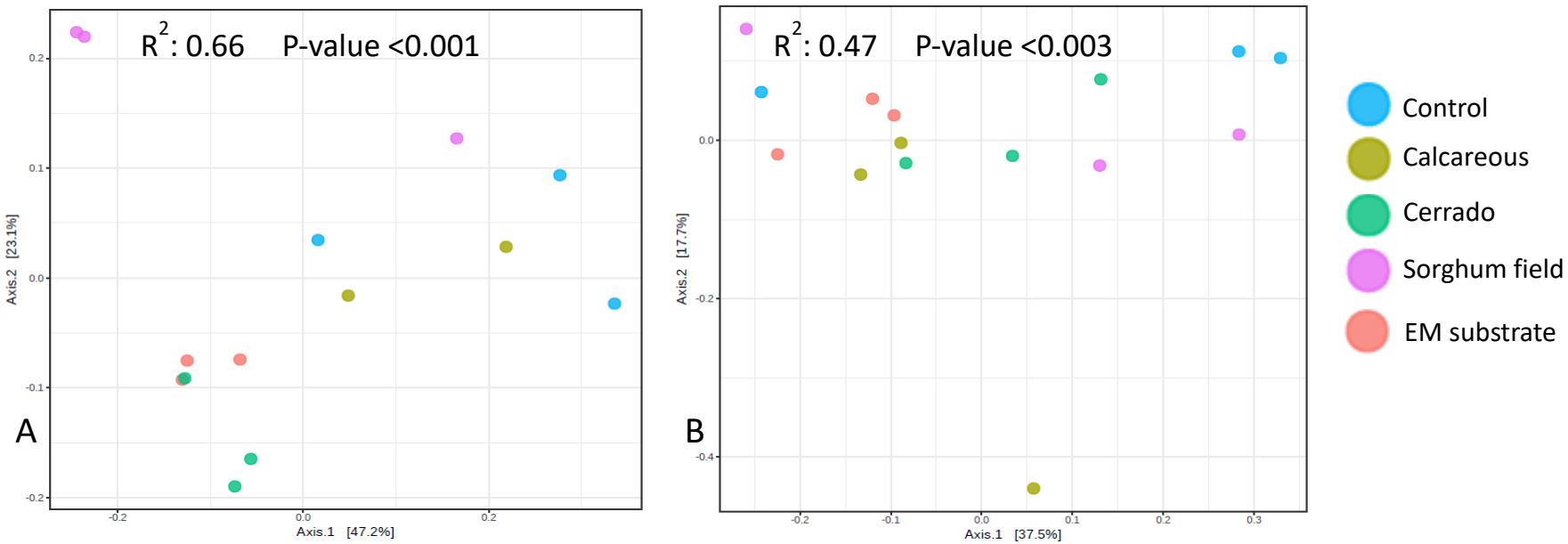

Figure 5. Principal Coordinate Analysis (PCoA) representing the dissimilarities of bacterial communities of two sorghum lineages (A) drought susceptible "9618158" and (B) drought tolerant "9910032" planted on different soils (Control, Calcareous, Cerrado, Sorghum field, and Enriched microbial – EM substrate) for 21 days and later transplanted to a standardized mixed substrate of sterilized sand and vermiculite (2:1 v/v). The rhizoplane bacterial community was sampled after grown for 44 days when plants were recovered from water deficiency conditions to field capacity.

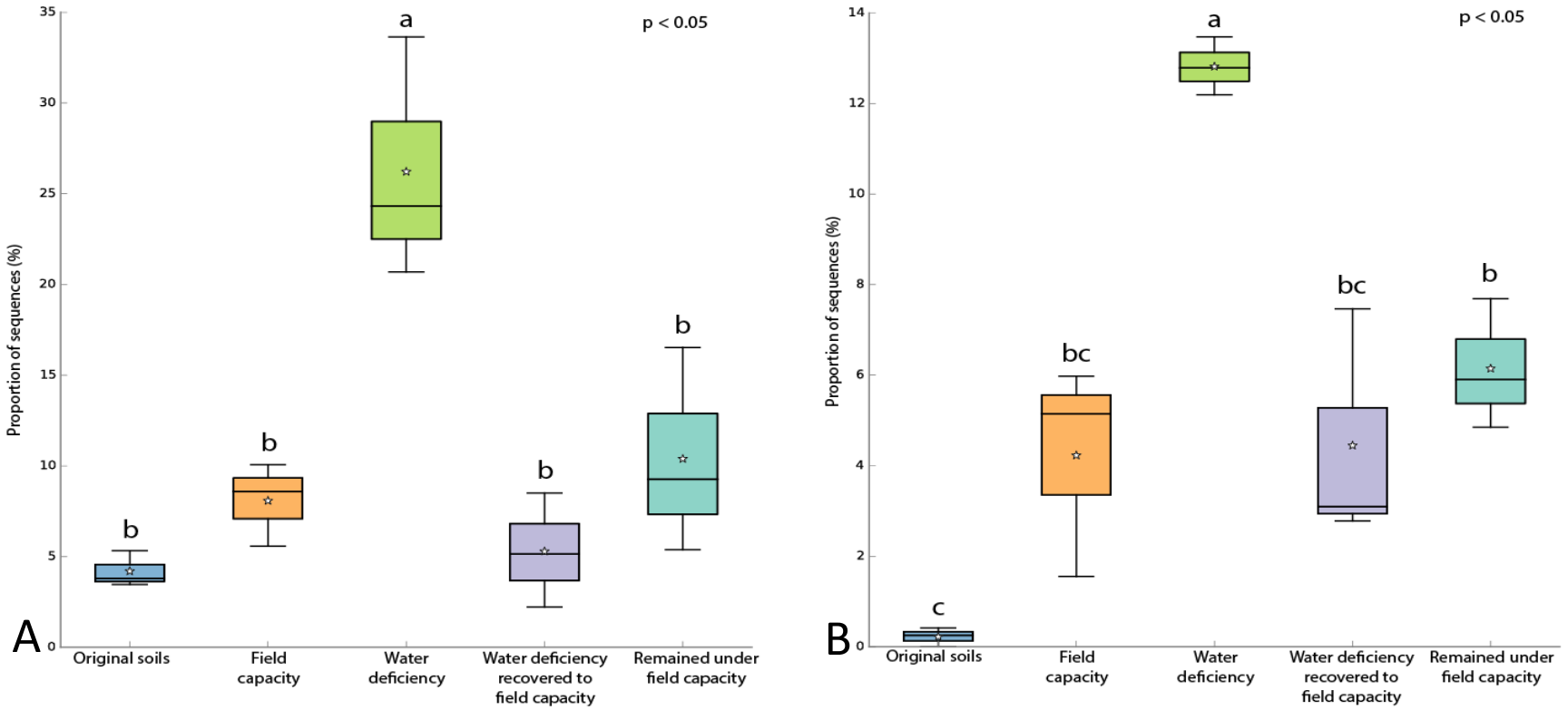

Figure 6. Box plots represent the mean of the relative abundance (n=3) of *Rhizobiaceae* in the rhizoplane of drought tolerant sorghum lineage "9910032" originally cropped at (A) Sorghum field and (B) Cerrado soils. The bacteria were assessed in different conditions: from the rhizoplane of plants planted on original soils; and after plants been transplanted to standardized substrate under different water regimes (under field capacity, water deficiency; water deficiency recovered to field capacity and remained in Field Capacity . The error bar indicates the confidence interval of the mean. Lowercase letters compare the original data of the bacterial dissimilarities among the water regimes. Means followed by the same letter are not statistically different by Welch's test (P<0.05).

Discussion

It is well established that sorghum is highly drought tolerant (Farre & Faci, 2006), but this tolerance depends on the severity of the drought (Berenguer & Faci, 2001). Drought not only causes losses of yield and biomass, but also affects root architecture (De Micco & Aronne, 2012). In our study, the differences in biomass between the drought sensitive (DS) and drought tolerant (DT) sorghum lineages were soil dependent. Furthermore, the DS lineage at water deficiency and the DT lineage at field capacity coming from Calcareous soil and transferred to the standardized substrate showed a significantly larger increment in shoot dry biomass compared with plants pre-cultivated in the other soils. Since, no relation between the increases of sorghum biomass and the rhizoplane bacterial community composition was found, we suggest that this increase is explained by higher nutrient availability in this soil than in the other soils. Concerning root architecture modification, our results showed that, at water deficiency, plants of the DS lineage coming from the control substrate had a significantly higher root length compared with plants in the Calcareous and EM substrate. Since plants increase their root length and surface area to improve water uptake at soil moisture stress (De Micco & Aronne, 2012), we suggest that plants of the control treatment were stressed at the water deficiency treatment.

The community of the plant rhizoplane of both sorghum lineages grown for 21 days in the sterilized substrate (control) did harbour bacterial communities with a significant higher relative abundance of *Oxalobacteraceae* and *Burkholderiaceae* (both from the order *Burkholderiales*) than those grown in the other soils. It is possible that these bacteria were endophytic already present in sorghum seeds and migrated into the root compartment of the sorghum seedlings. However, we cannot rule out air and water-borne contamination. In both cases, the reduction in bacterial diversity in the control substrate by the autoclaving process may have benefited the prevalence of the fast growing bacteria (*Oxalobacteraceae* and *Burkholderiaceae*) by the lack of competition (Marshner and Rumberger 2004). The Sorghum field soil provided a significantly higher abundance of *Comamonadaceae* (*Burkholderiales*), *Chitinophagaceae* (*Chitinophagales*), and *Rhizobiaceae* (*Rhizobiales*), on roots of both sorghum lineages. Since the Sorghum field soil was cropped with different sorghum genotypes for more than 25 years, we hypothesize/speculate that these three bacterial families are well adaptated to sorghum. The proposition of Bakker *et al.* (2012) that the potential of beneficial soil bacteria to enter the rhizosphere of a new crop may depend on the selective effect of the previous crop, points to the importance of plant host specificity for the assemblage of rhizosphere/rhizoplane microbial communities.

The rhizoplane of the DS lineage at water deficiency conditions showed the highest abundance of *Caulobacteraceae* on roots of plants coming from Cerrado soil compared with the other soils. The Cerrado soil was collected from a savannah-like vegetation characterized by elevated temperatures, constant fires and low water availability (Borghetti *et al.*, 2005). Thus, the higher abundance of *Caulobacteraceae* on roots of the DS lineage at water deficiency could be due to its thermotolerance characteristic (Nunes *et al.*, 2018), and due to their ability to form biofilm on the surface of eukaryotes (Abraham *et al.*, 2014). Bacterial biofilm helps to adhere to a given surface and protect the bacterial colony from desiccation contributing to its survival in low-water-content habitats (Penaloza-Vazquez *et al.*, 2009). Similarly, the highest abundance of *Rhizobiaceae* in the rhizoplane of the DT lineage at water deficiency conditions was observed particularly for plants coming from Sorghum field soil. In line with our findings, Bouskill *et al.* (2013) also found an increase of *Rhizobiales* abundance in tropical forest soil under drought conditions. Furthermore, members of the *Rhizobiaceae* family were reported to be associated with an increased synthesis of plant growth regulators in sorghum under drought stress (Rashad *et al.*, 2001). Furthermore, members of *Rhizobiaceae* family produce exopolysaccharides that have an important role in the host plant cell recognition, adhesion, as well as on the maintenance of water content (Alves *et al.*, 2014).

Although high abundance of *Caulobacteraceae* and *Rhizobiaceae* on roots of the DS and DT lineages was not correlated with an increase in plant biomass, we suggest that pre-exposition to low rainfall regimes may have provided some form of adaptation to these *Alphaproteobacteria* representatives to inhabit the sorghum rhizoplane compartment under water deficient conditions. As an example of Cerrado soil, Sorghum field in Janaúba is also a semi-arid region with the aggravation of having half of the average precipitation as compared to the other locations, where we sampled soils. Interestingly, the abundance of the *Rhizobiaceae* family on roots of the DT lineage previously cultivated in Cerrado and Sorghum field soils significantly decreased when plants were recovered from water deficiency to field capacity (Figure 6). Under sufficient soil moisture conditions, nutrients are carried to the roots by water. However low soil moisture conditions influences the availability and transport of soil nutrients such Ca, Mg and Si (Selvakumar *et al.*, 2012, Vurukonda *et al.*, 2016). As a reduced availability of nutrients may influence the plant to interact with beneficial microorganisms (van der Heijden *et al.*, 2008), the interaction of Sorghum with *Rhizobiaceae* does not seem to be relevant anymore, when water content returns to field capacity. Similarly, Xu *et al.* (2018) observed a significant enrichment for *Actinobacteria* core gene transcripts in sorghum rhizosphere under drought stress and a significant decrease in its transcripts upon rewetting. The aforementioned authors suggested that the resilience of the *Actinobacteria* in return to a low gene

transcripts when recovered from water stress may possibly be due to the enrichment of the fast-growing bacterial groups that recovered from the given stress. Likewise, we cannot rule out this possibility for our results.

No differences in total number of OTUs or diversity were found between water regime treatments. Corroborating with our findings, Bachar *et al.* (2010) showed that bacterial diversity in soils collected from Mediterranean, semi-arid, and arid sites was independent of the precipitation gradient. However, other studies show that drought can lead to microbial diversity reduction in plants adapted to arid and semi-arid regions. Xu *et al.* (2018) observed that within the root and the rhizosphere of sorghum, the overall microbial diversity decreased with drought and led to an increase in the abundance of monoderm bacterial groups. Similarly, Taketani *et al.* (2017) found that dry season constrains bacterial phylogenetic diversity in the rhizosphere of the semi-arid plants *Mimosa tenuiflora* and *Piptadenia stipulacea* (Benth.) Ducke.

In conclusion, our results showed that at water deficiency, *Caulobacteraceae* and *Rhizobiaceae* bacterial families were highly abundant in the rhizoplane of the DS and DT lineages, respectively, and that this composition was determined by the combination of soil and plant genotype. Although the high abundance of these families was not correlated with plant biomass, future experimental validation is required to investigate their contribution to sorghum drought tolerance. Considering that our study focused on changes in relative abundances of bacterial families, quantitative analysis of these families will be needed as many microbe-mediated plant phenotypes are density dependent. Taking into account the promising findings of our study, we suggest that plant pre-cultivation can be used as a whole bacterial community transplant to transfer beneficial microbes as a generic approach to confer enhanced stress tolerance to plants.

Supplementary Materials

Table S1. Soil physical and chemical analysis of different soils Control (mixed substrate of sterilized sand and vermiculite; 2:1 v/v), Calcareous, Cerrado and Sorghum field.

Parametes	Soils			
	Control	Calcareous	Cerrado	Sorghum field
N (%)	0 ± 0 d	1.2 ± 0.02 a	0.3 ± 0.01 b	0.1 ± 0.01 c
pH (H2O)	6.2 ± 0.07 b	7.6 ± 0.06 a	5.5 ± 0 c	6.3 ± 0 b
H+Al (cmolc/dm3)	0.7 ± 0.06 c	0 ± 0 c	8.5 ± 0.31 a	2.5 ± 0.5 b
P(mg/dm3)	30.3 ± 4.14 b	860.2 ± 85.5 a	6.1 ± 0.18 b	60.9 ± 0.38 b
OM (dag/kg)	0.2 ± 0.01 d	26.1 ± 0.86 a	6.4 ± 0.31 b	2.3 ± 0.09 c
C (%)	0.1 ± 0 d	15.2 ± 0.5 a	3.7 ± 0.18 b	1.3 ± 0.05 c
Al (cmolc/dm3)	0 ± 0 b	0 ± 0 b	0.5 ± 0.01 a	0 ± 0 b
Ca (cmolc/dm3)	0.5 ± 0.04 d	23.8 ± 0.17 a	2 ± 0.09 c	3.2 ± 0.19 b
Mg (cmolc/dm3)	1.3 ± 0.03 b	2.1 ± 0.01 a	1.3 ± 0.06 b	1.2 ± 0.06 b
K (mg/dm3)	73.4 ± 0.57 c	1246.7 ± 47 a	263 ± 6.23 b	311 ± 4.18 b
SB (cmolc/dm3)	2 ± 0.07 d	29 ± 0.26 a	4 ± 0.16 c	5.2 ± 0.26 b
CEC (cmolc/dm3)	2.7 ± 0.14 d	29 ± 0.26 a	12.5 ± 0.22 b	7.7 ± 0.64 c
V (%)	74.8 ± 1.12 b	100 ± 0 a	31.9 ± 1.51 c	67.8 ± 3.82 b
Sat. Al (%)	1.3 ± 0.2 b	0 ± 0.01 c	10.7 ± 0.32 a	0 ± 0 c
Cu (mg/dm3)	0.6 ± 0.18 b	0.1 ± 0.01 c	1 ± 0.03 a	1.3 ± 0.03 a
Fe (mg/dm3)	44.6 ± 0.58 b	0.7 ± 0.06 c	107.3 ± 8.27 a	39.1 ± 2.61 b
Mn (mg/dm3)	19.5 ± 0.24 c	7.9 ± 0.72 c	53.4 ± 4.79 b	103.3 ± 4.17 a
Zn (mg/dm3)	0.5 ± 0.04 c	0.2 ± 0.03 c	1 ± 0.04 b	5 ± 0.12 a
Coarse sand (dag/kg)	78.7 ± 1.45 a	18 ± 0 c	23 ± 1 b	22.3 ± 0.33 b
Fine sand (dag/kg)	12.3 ± 0.88 b	12 ± 0 b	7 ± 0.58 c	34 ± 1 a
Total sand (dag/kg)	91 ± 0.57 a	30 ± 0 c	30 ± 0.57 c	56.33 ± 0.66 b
Silt (dag/kg)	5 ± 0.58 c	34.3 ± 0.33 a	25 ± 0 b	24.3 ± 0.33 b
Clay (dag/kg)	4.3 ± 0.67 d	35.7 ± 0.33 b	45.3 ± 0.33 a	19.3 ± 0.33 c

The values are means of replicates (n=3) ± (SD). For each physical and chemical parameter lowercase letters compare (on row) the means between different soils. Means followed by the same letter are not statistically different by Tukey test (P<0.05). *Due the loss of material, it was not possible to collect physical and chemical properties data from Enriched microbial - EM substrate.

Table S2. Number of 16S rRNA sequencing reads per DNA sample extracted from the rhizoplane of two sorghum lineages (drought susceptible "9618158", **DS** and drought tolerant "9910032", **DT**) originally cropped on different soils (**SFS** – Selected from soil) (Control (mixed substrate of sterilized sand and vermiculite; 2:1 v/v), Calcareous, Cerrado, Sorghum field and Enriched microbial substrate, and later transplanted to a substrate mixed of sterilized sand and vermiculite (2:1 v/v) under different water treatments: **FC** – Field Capacity; **WD** – Water Deficiency; **WD_R_FC** – Water Deficiency Recovered to Field Capacity and **M_FC** – Maintained in Field Capacity

	water treatments									
Rhizosphere source	**SFS**		**FC**		**WD**		**WD_R_FC**		**M_FC**	
	Samples	**Reads**	**Samples**	**Reads**	**Samples**	**Reads**	**Samples**	**Reads**	**Samples**	**Reads**
Control_DS	**19r1**	24854	**145A**	77054	**149A**	18451	**151A**	19810	**147A**	42442
	19r2	38407	**145C**	86812	**149C**	16299	**151C**	6968	**147C**	45279
	19r3	30604	**146A**	28510	**150A**	15846	**152A**	7721	**148A**	NA
Control_DT	**20r1**	13668	**153A**	26632	**157A**	4844	**159A**	8107	**155A**	4409
	20r2	11548	**153C**	20117	**157C**	40703	**159C**	18089	**155C**	86500
	20r3	41233	**154A**	73482	**158A**	11669	**160A**	31824	**156A**	42190
Calcareous _DS	**5r1**	13692	**33A**	12359	**37A**	5050	**39A**	2	**35C**	12575
	5r2	5463	**33C**	23079	**37C**	3974	**39C**	12366	**36A**	17266
	5r3	8479	**34A**	3302	**38A**	3974	**40A**	45359	**36C**	NA
Calcareous _DT	**6r1**	4139	**41A**	8203	**45A**	4141	**47A**	6519	**43A**	109689
	6r2	11564	**41C**	2965	**45C**	8527	**47C**	19270	**43C**	18202
	6r3	10936	**42A**	50529	**46A**	12383	**48A**	5700	**44A**	11869
Cerrado_DS	**3r1**	15832	**17A**	51933	**21A**	4644	**23A**	20730	**19A**	47951
	3r2	7509	**17C**	9023	**21C**	10998	**23C**	168182	**19C**	65565
	3r3	6278	**18A**	14229	**22A**	28110	**24A**	18150	**20A**	2
Cerrado_DT	**4r1**	12960	**25A**	40462	**29A**	9090	**31A**	11472	**27A**	134424
	4r2	59270	**25C**	3693	**29C**	33228	**31C**	23900	**27C**	42260
	4r3	16397	**26A**	6115	**30A**	10594	**32A**	16254	**28A**	61080
Sorghum field_DS	**9r1**	6479	**65A**	10327	**69A**	31798	**71A**	49053	**67A**	7373
	9r2	3591	**65C**	16281	**69C**	4	**71C**	43654	**67C**	58869
	9r3	8160	**66A**	8984	**70A**	26274	**72A**	18272	**68A**	30481
Sorghum field_DT	**10r1**	15364	**73A**	132421	**77A**	4162	**79A**	37807	**75A**	97055
	10r2	10960	**73C**	54441	**77C**	28985	**79C**	26601	**75C**	60514
	10r3	26997	**74A**	19634	**78A**	26254	**80A**	53220	**76A**	66552

EM substrate_DS	**1r1**	NA	**1A**	41059	**5A**	4465	**7A**	34252	**3A**	56252
	1r2	NA	**1C**	13836	**5C**	7997	**7C**	36370	**3C**	71791
	1r3	NA	**2A**	58612	**6A**	34630	**8A**	41827	**4A**	32610
EM substrate _DT	**2r1**	NA	**9A**	7123	**13A**	41521	**15A**	44651	**11A**	12051
	2r2	NA	**9C**	9162	**13C**	22645	**15C**	53884	**11C**	3003
	2r3	NA	**10A**	11766	**14A**	10935	**16A**	29793	**12A**	6874

Table S3. Number of Operational Taxonomic Units (OTU's) and Shannon index of diversity based on extracted DNA for two sorghum lineages (drought susceptible "9618158" **(DS)** and drought tolerant "9910032"**(DT)**) originally cropped in different soils (**SFS** – Selected from soil) (Control, Calcareous, Cerrado, Sorghum field and Enriched microbial - EM substrate and later transplanted to a substrate mixed of sterilized sand and vermiculite (2:1 v/v) under different water treatments: **FC** – Field Capacity; **WD** – Water Deficiency; **WD_R_FC** – Water Deficiency Recovered to Field Capacity and **M_FC** – Maintained in Field Capacity

α-diversity index	**Rhizospheric soil source**	**Sorghum lineage**	**water treatments**				
			SFS	**FC**	**WD**	**WD_R_FC**	**M_FC**
Number of OTU	Control	DS	150.3 ± 8.6 b	247.6 ± 2.5 a	203 ± 20.5 ab	248.6 ± 43.0 a	194 ± 5.6 ab
		DT	131.3 ± 19.8b	286.6 ± 20.7a	268 ± 32.7a	225.3 ± 38.2 ab	268 ± 69.2 a
	Calcareous	DS	160 ± 11.7 a	72 ± 11.1 b	62.6 ± 6.1 b	70 ± 15.5 b	73 ± 14.1 b
		DT	814.6 ± 70.5 a	243 ± 2.8 b	272.3 ± 31.8 b	329.6 ± 142.4 b	282 ± 39.7 b
	Cerrado	DS	524 ± 67.1 a	372 ± 25.2 b	278.6 ± 41.6 b	274.3 ± 67.6 b	279.5 ± 43.1 b
		DT	620.3 ± 79.9 a	234 ± 19.3 b	216 ± 20.7 b	277 ± 44.1 b	224.3 ± 6.5 b
	Sorghum field	DS	614.6 ± 24.3 a	230.6 ± 16.7 b	203.5 ± 44.5 b	271.3 ± 47.0 b	238 ± 8 b
		DT	783 ± 28.0 a	198.3 ± 11.2 b	202.3 ± 41.0 b	301 ± 26.4 b	239 ± 23.0 b
	EM substrate	DS	NA	265 ± 22.1 a	266.3 ± 34.9 a	267.6 ± 2.5 a	283 ± 29.5 a
		DT	NA	246.3 ± 4.0 a	224.6 ± 27.0 a	266.6 ± 43.9 a	321.3 ± 179.9 a
Shannon	Control	DS	2.4 ± 0.0 ab	2.3 ± 0.0 b	2.3 ± 0.6 ab	3.3 ± 0.5 a	1.7 ± 0.0 ab
		DT	2.5 ± 0.2 b	4.1 ± 0.3 a	4.2 ± 0.2 a	3.1 ± 0.7 ab	3.4 ± 0.6 ab
	Calcareous	DS	3.9 ± 0.1 a	2.8 ± 0.1 b	2.7 ± 0.1 b	2.5 ± 0.0 b	2.9 ± 0.1 b
		DT	6.0 ± 0.1 a	4.0 ± 0.0 b	4.1 ± 0.2 b	4.3 ± 0.3 b	4.0 ± 0.1 b
	Cerrado	DS	4.3 ± 0.2 a	4.5 ± 0.1 a	4.1 ± 0.3 a	4.0 ± 0.2 a	4.1 ± 0.2 a
		DT	5.2 ± 0.3 a	3.5 ± 0.0 bc	3.8 ± 0.1 bc	4.0 ± 0.1 b	3.3 ± 0.1 c
	Sorghum field	DS	5.5 ± 0.0 a	3.8 ± 0.0 ab	3.2 ± 1.2 b	4.0 ± 0.9 ab	3.6 ± 0.4 b
		DT	5.7 ± 0.0 a	3.1 ± 0.2 b	3.5 ± 0.1 b	3.6 ± 0.5 b	3.5 ± 0.1 b
	EM substrate	DS	NA	3.5 ± 0.3 a	3.8 ± 0.2 a	3.9 ± 0.0 a	4.0 ± 0.3 a
		DT	NA	4.1 ± 0.0 a	4.0 ± 0.1 a	4.0 ± 0.3 a	4.4 ± 0.7 a

The values are means of replicates (n=3) ± (SE). For each α-diversity index lowercase letters compare (on row) the means of water treatments within the same cultivar and soil bacterial source. Means followed by the same letter are not statistically different by Tukey test ($P<0.05$). The sequences were rarefied by 3.000 reads prior the analysis