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

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“Whoever wants to become what is not, should begin not being what is”

Carlos Bernardo González Pecotche

Contents

Chapter 1

General introduction

Sorghum is an economically important cereal crop used for animal feed and human food worldwide, in particular for subsistence farmers in Sub-Saharan Africa. Due to the high demand for its different uses, development of new sustainable strategies that improve or safeguard sorghum production is needed. These strategies not only encompass plant breeding and agricultural management practices, but also harnessing beneficial microbe-crop relations which are key to the development of sustainable crop production. Despite the large number of studies addressing plant-microbiome interactions, little is known about the sorghum microbiome and how it affects sorghum growth and tolerance to biotic and abiotic stresses. The **overall objectives** of my thesis are to investigate the dynamics of the sorghum root microbiome and to explore the beneficial effects of the root microbiome on sorghum growth and stress tolerance. In this general introduction I will give a brief description of sorghum, its uses, characteristics and importance of this cereal worldwide. Then, I will provide background information on the composition, spatial distribution and dynamics of the root microbiome and its importance for plant growth and health. Furthermore, I will present the role of root exudates in the recruitment of the rhizosphere microbiome and will discuss other drivers of rhizosphere microbial community assembly. Additionally, I will provide examples on how the root microbiome can provide tolerance to the host plant against abiotic disturbances, in particular drought. Finally, I will provide insights into how microbial inoculants can impact plant growth and nutrient acquisition.

1 - Sorghum

Sorghum bicolor (L.) Moench. is a C4 plant belonging to *Poaceae* family that, based on anthropological evidences, has been consumed as early as 8000 BC and domesticated in Ethiopia and neighbouring countries around 4000-3000 BC (Smith & Frederiksen, 2000, Dillon *et al.*, 2007). Sorghum is currently the 5th most cultivated cereal worldwide (Ramu *et al.*, 2013). It has a short growth period and is relatively drought tolerant, which makes sorghum a preferred cereal in arid and semi-arid regions (Farre & Faci, 2006, Wu *et al.*, 2010, Funnell-Harris *et al.*, 2013). Sorghum serves as a food crop and is used for biofuel production (Dutra *et al.*, 2013), soil coverage (Bean *et al.*, 2013), beer production (Smith & Frederiksen, 2000) and silage (Pinho *et al.*, 2015). As food-grade, special attention is given to sorghum because it is gluten-free and contains high levels of health-promoting phytochemicals (Asif *et al.*, 2010). Due to the nutritional similarity of sorghum and maize, the gain in weight and milk production of cattle fed with sorghum is comparable to that of cattle fed with maize (Aydin *et al.*, 1999, Oliver *et al.*, 2004, Sauvant, 2004). For ethanol production, sorghum has a preference over other plant biomass sources such as corn, sugarcane and sugar beet due to the

reduced water requirement (Farre & Faci, 2006, Walker, 2011, Dutra *et al.*, 2013). In general, sorghum only needs one-third of the water required for sugarcane cultivation and only half of the water required for corn production (Wu *et al.*, 2010). Additionally, sorghum has a short growth period of 3-5 month compared to 9-12 month for sugarcane (Davila-Gomez *et al.*, 2011). Given these favorable characteristics and its diverse usages, it is highly relevant to identify sustainable methods for disease prevention and tolerance against abiotic stress (Funnell-Harris *et al.*, 2013). The problems associated with sorghum production are to some extent geographically determined. In Brazil, sorghum producers are often faced with unfertile soils low in available phosphate and high in aluminium (Ribeiro *et al.*, 2001, Magalhaes *et al.*, 2007) and with many fungal diseases (Rodrigues *et al.*, 2009, Cota *et al.*, 2012, Cota *et al.*, 2013). In Africa, producers often face problems with infection by the parasitic weed *Striga hermonthica* (Del.) Benth. causing substantial yield losses (Hassan *et al.*, 2009).

Plant breeding programs make considerable progress by engineering sorghum varieties resistant to specific diseases and adverse environmental conditions and varieties with improved nutrient acquisition. Next to plant breeding, soil and plant-associated microbiomes are receiving increasing interest for their untapped potential to contribute to plant growth, development and health (Raaijmakers *et al.*, 2009, Bulgarelli *et al.*, 2015). In this context, combining plant breeding and microbiome-based crop production strategies is potentially a powerful strategy, realising that breeding programs typically do not consider the interaction with the soil and plant-associated microbiomes. Dangl *et al.* (2013) and Schlaeppi & Bulgarelli (2015) argued that selection and development of plants based on a combination of functional genes and plant responsiveness to beneficial soil microorganisms are expected to provide highly durable protection against diseases.

2 – The Soil Microbiome

Soil is a large reservoir of microorganisms interacting with plants in a variety of ways. For example, soil microorganisms play a crucial role in biogeochemical processes such as the decomposition of organic matter and the regulation of C and N cycles (Maul & Drinkwater, 2010, Nielsen *et al.*, 2011, Lavecchia *et al.*, 2015). They also play key roles in the growth of plants and strongly regulate plant nutrient uptake (Nielsen *et al.*, 2015). The interaction between plants and soil microorganisms can be positive, neutral or negative (Rasmussen *et al.*, 2013). Positive interactions include symbiotic associations of plants with arbuscular mycorrhizal fungi (AMF) and microbes that promote plant growth, whereas negative interactions include pathogenesis and competition for nutrients (Bais *et al.*,

2006). The result of the interaction depends on many factors such as plant species and genotype, soil type, soil microbial community diversity and abiotic factors (Philippot *et al.*, 2013, Van der Putten *et al.*, 2013). For example, the study by Govindasamy *et al.* (2017) indicated that the soil plays a crucial role in the rhizobacterial endophyte composition of four sorghum cultivars. Moreover, inorganic and organic fertilizers can influence the composition of soil bacterial communities (Marschner *et al.*, 2001) and in turn the plant rhizosphere microbiome assembly. In this sense, Lavecchia *et al.* (2015) found that the taxonomic composition of bacterial communities inhabiting the sorghum rhizosphere are more affected by organic fertilization with compost than by inorganic fertilization with urea.

3 – The Root Microbiome

Plant roots can be divided into three main compartments, i.e. the rhizosphere, rhizoplane and endosphere. The rhizosphere is defined as the small zone surrounding and influenced by the plant root via the release of root-derived compounds that select and activate members of the soil microbial community (Hiltner 1904). The rhizoplane is the surface of the plant roots, whereas the endosphere represents internal root tissue, including the vascular system. Nunan et al. (2015) suggested that the influence of plant root-derived compounds on the microbial community is likely to be greater in the rhizoplane than in the rhizosphere (Nunan *et al.*, 2005). In this regard, as the rhizosphere microbial community is considered to be a subset of the microbial community of the bulk soil (Mendes *et al.*, 2014, Lima *et al.*, 2015, Cipriano *et al.*, 2016, Yan *et al.*, 2016), the rhizoplane microbial community is a subset selected from the rhizosphere. A second level of selection from the root microbiome occurs when going from the rhizoplane into the endosphere (Edwards *et al.*, 2015). Studying the structure and assembly of root-associated microbes in rice, Edwards *et al.* (2015) found that the bacterial diversity decreased going from rhizosphere to endosphere. Once inside the host, endophyte communities change their metabolism and become adapted to the internal environment (Turner *et al.*, 2013, Mitter *et al.*, 2017). Microbial endophytes may accelerate seedling emergence, modify root morphology, help plants to remove contaminants, solubilize phosphorus, enhance uptake of other plant nutrients and promote plant growth (Dudeja *et al.*, 2012).

Within the root microbiome, plant growth-promoting rhizobacteria (PGPR) are functionally highly relevant microbial groups. PGPR are defined as rhizosphere microbiota that, in association with their host plants, directly or indirectly stimulate root and/or shoot growth (Bhattacharyya & Jha, 2012). PGPR can promote plant growth by facilitating resource acquisition or modulating plant hormone levels, decreasing the inhibitory effects of pathogenic agents on plant development,

increasing the availability of nutrients in the rhizosphere, increasing root surface area, and enhancing beneficial symbiosis (Bhattacharyya & Jha, 2012, Glick, 2012). For sorghum, recent studies focused on the mechanisms of sorghum root microbiome recruitment and composition (Lavecchia *et al.*, 2015, Mareque *et al.*, 2015), whereas other studies investigated the potential effects of PGPR on sorghum growth, yield, nutrient uptake and abiotic stress alleviation (Ali *et al.*, 2009, Cobb *et al.*, 2016, Dhawi *et al.*, 2016, Dos Santos *et al.*, 2017).

3.1. - Root Microbiome Assembly by Rhizodeposition

Through a variety of mechanisms such as exudation, secretion, mucilage production, and cell debris, roots provide a variety of compounds such as carbohydrates, amino acids, phenolic compounds, sugars and inorganic ions to their surrounding soil microbiome (Haas & Défago, 2005, Haichar *et al.*, 2008, Bever *et al.*, 2012). Also communication between plant and soil microorganisms often begins by root exudation with a subsequent recognition and response by microorganisms at community and individual levels (Singh *et al.*, 2008). The structure of the bacterial and fungal members of the root microbiome changes with the quantity and quality of rhizodeposition. For example, studying the spatial and temporal dynamics and composition of the rhizosphere microbiome of white lupin roots, Marschner *et al.* (2002) found that the fungal community composition correlated with citric acid exudation, whereas the bacterial community composition correlated with cis-aconitic, citric and malic acid exudation.

The outcome of the chemical interplay between the plant roots and the recruitment of specific members of the soil microbiome depends, in part, on the ability and efficiency of these microbiome members to utilize specific root deposits for growth and activity (Bais *et al.*, 2006). The same root compounds that attract beneficial microorganisms may also attract plant pathogens (Mendes *et al.*, 2013) or parasitic plants (Bouwmeester *et al.*, 2007). This is the case for strigolactones which play an essential role in the establishment of AMF symbiosis but are also (mis)used by parasitic plants of the genera *Striga*, *Orobanche* and *Phelipanche* (Cavar *et al.*, 2015). Moreover, the same root exudates that increase the abundance of a specific group of bacteria could decrease others. For example, Huang *et al.* (2017) recently observed that *Sorghum halepense* [L.] Pers. secretes the phenolic compounds p-hydroxybenzoic acid (p-HBA) and p-hydroxybenzaldehyde (p-HBAL). The addition of p-HBAL to soil significantly increased the abundance of members of the Acidobacteria, Chloroflexi, Verrucomicrobia and Cyanobacteria but decreased the relative abundance of members of the Proteobacteria.

3.2. Other Drivers of Root Microbiome Assembly

Various other biotic and abiotic factors determine root microbiome assembly, including root architecture (Berg & Smalla, 2009, Lindedam *et al.*, 2009, Pérez-Jaramillo *et al.*, 2017), soil factors (Smalla *et al.*, 2001, Girvan *et al.*, 2003, Kuramae *et al.*, 2012, Serna-Chavez *et al.*, 2013), land use (Wakelin *et al.*, 2013), plant genotype (Miethling *et al.*, 2000, Smalla *et al.*, 2001, Kowalchuk *et al.*, 2002), and plant growth stage (van Overbeek & van Elsas, 2008). As soil has a wide range of properties that may, independent or in combination, influence the growth and activities of microorganisms, soil is often reported as the major factor in shaping the rhizosphere microbiome (Singh *et al.*, 2007, Xu *et al.*, 2009, Kuramae *et al.*, 2012). Soil factors that influence the root microbiome composition include soil moisture, pH, organic matter content and nutrient availability (Kuramae *et al.*, 2012, Serna-Chavez *et al.*, 2013), soil type (Girvan *et al.*, 2003) and soil history (Smalla *et al.*, 2001).

The rhizosphere microbial community composition may vary during plant growth and development (Chaparro *et al.*, 2014). Different factors may be responsible for this temporal change, including seasonality. For example, in spring and summer due to the higher temperatures, the soil microbial community often increases its metabolic activity in conjunction with the accelerated mineralization of soil organic matter and accelerated root growth (Grayston *et al.*, 2001). During plant growth, rhizodeposition changes as well as root architecture (Marschner *et al.*, 2004). Chaparro *et al.* (2013) observed higher exudation of sugars and sugar alcohols at early stages of plant growth than at later growth stages, whereas the content of amino acids and phenolics increased with plant age. Micallef *et al.* (2009) found that with plant age, the bulk soil and rhizosphere community converged to a similar community, which coincides with the expected reduction in root exudation when plants are close to the end of their life cycle.

Also plant genotype is an important factor driving root microbiome assembly (Ettema & Wardle, 2002, Berg & Smalla, 2009). Several studies have shown that plant genotypes can recruit beneficial microorganisms to help plants against pathogenic attacks (Rudrappa *et al.*, 2008, Berendsen *et al.*, 2012, Yoon *et al.*, 2016). Therefore, plant genotype selection has been proposed as a means to stimulate the frequency and/or activities of PGPR (Cook, 2007, Picard & Bosco, 2008). Aiming to find bacterial isolates that significantly inhibited sorghum fungal pathogens, Funnell-Harris *et al.* (2013) found that the sorghum genotype affected the selection and persistence of *Pseudomonas* spp., which have the potential to ameliorate sorghum diseases. Yoon *et al.* (2016) found that the efficiency of *Gluconacetobacter diazotrophicus* in colonizing sorghum roots varied among

different genotypes, being higher in sweet sorghum genotypes than in grain genotypes. Dos Santos *et al.* (2017) further found that grain and forage sorghum genotypes exhibited superior nutritional and productivity responses to inoculation with a mixture of the PGPB bacteria *Herbaspirillum* and *Burkholderia* as compared with sweet sorghum.

The mechanisms underlying compatibility between the plant genotype and the indigenous microbial community or introduced microbial inoculants are not well understood yet, but differences in rhizodeposition between different plant species and genotypes are most likely a key determining factor. For sorghum it is know that a variety of root derived products is genotype specific (Czarnota *et al.*, 2003). For example, Mohemed *et al.* (2016) showed that sorghum genotypes Korokollow, Fakimustahi and Wadfahel exuded the highest amounts of the strigolactone 5-deoxystrigol while the genotypes Wadbaco and SRN-39 produced the highest amount of orobanchol. Akiyama *et al.* (2010) suggested that both orobanchol and 5-deoxystrigol induce hyphal branching of the arbuscular mychorrizal fungi *Gigaspora margarita*. Moreover, Tesfamariam *et al.* (2014) found that different sorghum genotypes produced different amounts of sorgoleone that plays a predominant role in the inhibition of nitrification in the rhizosphere. Sorgoleone inhibited the activity of *Nitrosomonas*, which is one of the bacterial groups responsible for the nitrification process (Tesfamariam *et al.*, 2014). Despite these effects on specific root-associated microorganisms, however, little is known about the overall effect of strigolactones on the sorghum root microbiome.

Although the rhizosphere microbiome composition changes according to the plant species, plant genotype, soil type and developmental stage, there is also a group of microbiome members that remains stable for the aforementioned factors and is referred to as the core microbiome (Lundberg *et al.*, 2012, Yeoh *et al.*, 2016, Pfeiffer *et al.*, 2017). Yeoh *et al.* (2016) found that despite striking differences in the composition of two soil microbial community investigated, sugarcane root microbiome showed a bacterial core enriched by *Bradyrhizobium, Rhizobium, Burkholderia, Herbaspirillum, Bacillus* and *Streptomyces* relative to bulk soil. Pfeiffer *et al.* (2017) suggested that the bacterial taxa *Microvirga zambiensis*, *Bradyrhizobium* sp., *Sphingobium vermicomposti*, the genus SMB53 of the *Clostridiaceae* family and the actinobacterial species *Blastococcus* sp. were tightly associated with potato rhizosphere irrespective of site and vegetation stage. Lundberg *et al.* (2012) observed that from 256 OTUs identified in the root compartments rhizosphere and endosphere and in soil, 164 OTUs were defining the *Arabidopsis thaliana* endophytic compartment core microbiome. It should be emphasized, however, that core microbiome data reported to date are mostly based on taxonomy and not on functional traits of the microbiome.

3.3. Impact of Disturbances on Root Microbiome Assembly

Disturbances are defined here as events that alter environmental conditions such that a microbial community is impacted. Disturbances are generally classified as *pulses* or *presses*. While a pulse disturbance is short-term disturbance that rapidly diminishes, a press disturbance is characterized as a continuous event maintained over longer periods of time (Bender *et al.*, 1984, Lake, 2000). Many biotic and abiotic disturbances may alter the soil microbial community, which in turn influence the functioning of the soil ecosystem (Lavecchia *et al.*, 2015, Suleiman *et al.*, 2016). Because of its sensitivity to disturbances, soil and root microbial communities are considered as bioindicators of soil quality (Mendes *et al.*, 2013). Under the influence of an abiotic disturbance, microbial communities can be resilient, tolerant, resistant or susceptible (Shade *et al.*, 2012). Microbes that can cope with abiotic disturbances might be beneficial to plants by alleviating stress conditions through diverse mechanisms like enhanced water and nutrient uptake, stimulation of plant growth by hormones such as indole acetic acid (IAA) and by triggering the plants' defense systems to biotic and abiotic stresses (Kavamura *et al.*, 2013, Rolli *et al.*, 2015).

Some bacterial genera are able to withstand drought better than others. To overcome stress effects, microbes rely on different physiological and morphological strategies such as dormancy, spore formation, growth rate changes and exopolysaccharide production (Sandhya *et al.*, 2009, Vurukonda *et al.*, 2016, Naylor *et al.*, 2017). Under moisture stress conditions, Actinobacteria have been reported to enrich in soil (Bouskill *et al.*, 2013), rhizosphere (Taketani *et al.*, 2017) and endosphere (Naylor *et al.*, 2017). In soils of the Brazilian semi-arid region, Taketani *et al.* (2017) determined the rhizosphere bacterial community composition of two different leguminous tree species: *Mimosa tenuiflora* and *Piptadenia stipulacea* during the dry and rainy season. They found that during the dry season the abundance of Actinobacteria increased in the rhizosphere of the two tree species whereas their abundance decreased during the rainy season. Barnard *et al.* (2013), studying the responses of soil bacterial communities to extreme desiccation and rewetting, showed that Actinobacteria (Actinomycetales order) strongly increased in relative abundance when exposed to water stress which was reversed again after rewetting. Actinobacteria have the capability to produces spores in response to drought stress which allows them to remain in a dormant state for a long period of time (Fang *et al.*, 2017).

The composition of the root microbiome of plants growing under drought conditions can be different according to the plant genotype or growth stage. For example, Naylor et al. (2017) found that bacterial communities associated with the rhizosphere of 18 plant species, including two sorghum

varieties, exposed to drought can change bacterial community composition at later stages of plant growth. Furthermore, bacterial species like *Pseudomonas* and *Rhizobium,* often found in the sorghum rhizosphere (Matiru & Dakora, 2004, Funnell-Harris *et al.*, 2013), appeared to be well adapted to stress conditions possibly due to the production of exopolysaccharides (EPS) (Sandhya *et al.*, 2009, Alves *et al.*, 2014). Casanovas *et al.* (2002) and Marasco *et al.* (2012) further showed that representatives of the bacterial genera *Azospirillum, Achromobacter, Klebsiella and Citrobacter* have the potential as PGPR to alleviate plant drought stress. Yandigeri *et al.* (2012) showed that the drought-tolerant endophytic actinobacteria promote growth of wheat under water stress conditions. Similarly, Sandhya *et al.* (2009) showed that *Pseudomonas putida* strain GAP-P45 inoculated onto sunflower seedlings relieved drought stress, increased plant survival and plant biomass through the production of exopolysaccharides. Also sorghum inoculated with *Rhizobium* showed increased yields under drought stress, although these effects were genotype dependent (Rashad *et al.*, 2001). Govindasamy *et al.* (2017) studied the functional and phylogenetic diversity of culturable rhizobacterial endophytes of sorghum growing at different moisture conditions and found a dominance of *Bacillus* species among the isolates identified to present at least one PGPR trait that could alleviate water stress. Interestingly, sorghum inoculated by four *Bacillus sp.* strains isolated from sorghum rhizosphere cropped at semi-arid locations, showed a higher relative water content of leaves and soil moisture content compared to the non-inoculated control treatment (Grover *et al.*, 2014). In this context, the authors proposed that microorganisms isolated from stressed ecosystems may be ideal candidates to be applied as bio-inoculants in crops susceptible to the respective stress condition (Grover *et al.*, 2014).

4 –Microbial Inoculants

Following detailed plant microbiome analyses, numerous bacterial and fungal genera have been isolated from rhizosphere, rhizoplane and endosphere and tested for their beneficial effects on plant growth and health (Berendsen *et al.*, 2012, Funnell-Harris & Sattler, 2014, Vasanthakumari & Shivanna, 2014). Indeed, application of microbial inoculants to plants has been shown to be a promising practice to increase plant growth, crop yield, and resistance to plant pathogen (Dutta *et al.*, 2014, da Silveira *et al.*, 2016). Microbial inoculants have also been employed as part of integrated nutrient management systems (Richardson *et al.*, 2011). To date, PGPR and AMF are the most common microorganisms used for plant inoculation. PGPR can be applied to seeds or seedlings prior to be transferred to their growth substrates (e.g. rockwool, soil) (Cipriano *et al.*, 2016) or applied to

the substrate after seeds or seedlings have been transferred (Malusa *et al.*, 2012, Dos Santos *et al.*, 2017). For sorghum, several studies over the past five years have indicated that PGPR treatment reduced diseases caused by fungal pathogens, increased plant biomass, nutrient uptake and yield (Funnell-Harris *et al.*, 2013, Yoon *et al.*, 2016, Dos Santos *et al.*, 2017)

While most studies to date focused on microbial inoculants with one single microbial strain, there is an increased interest in designing consortia of microorganisms with different synergetic modes of action (Rajasekar & Elango, 2011, Dos Santos *et al.*, 2017). Consortia containing different microorganisms with supplementary or synergistic characteristics are presumed to be more effective or more consistent than single microbial inoculants (Mendes *et al.*, 2013). For example, Artursson *et al.*, (2006) and Bonfante & Anca (2009) showed a beneficial effect of PGPR and AMF co-inoculation on AMF symbiosis. Hameeda *et al.* (2007) found that application of bacterial isolates together with AMF provided in 45 days the same or greater plant and root growth and mycorrhizal colonization than provided by the AMF inoculum alone in 90 days. Also Dhawi *et al.* (2016) found that the combination of PGPR with AMF increased sorghum biomass more than the treatment with AMF alone. Similarly, Duponnois *et al.* (2006) observed that strains of fluorescent pseudomonads in combination with AMF, increased heavy metal tolerance, mycorrhizal colonization and shoot length of sorghum.

Although these examples indicated additive and synergistic effects of the interaction of PGPR and AMF, it remains a challenge to establish compatibility and enhanced activity within a microbial consortium. Furthermore, the costs and technical complexity involved in the creation of single and combined microbial inoculants, together with legislative and regulatory obstacles, is a major impediment in the development of this microbial technology. Hence, alternative techniques to large-scale microbial inoculant production and registration are needed. An alternative that contemplates inoculum production in a broad perspective is microbiome transplantations (Gopal *et al.*, 2013). In this sense, mixing small amounts of naturally disease suppressive soil into a disease-conducive soil has been shown to be a successful alternative pathogen abatement (Weller *et al.*, 2002, Mendes *et al.*, 2011). Understanding the keystone microbial taxa involved in the transferability and predictability of these microbiome-associated plant phenotypes (Oyserman *et al.*, 2018) is an essential element of future research to construct microbial inoculants that provide effective and consistent effects under diverse field conditions.

Outstanding Questions in this thesis

What is the relative importance of plant genotype, plant growth stage and soil type on the composition of the sorghum rhizobacterial community?

Are fungal-bacterial interactions in the sorghum rhizosphere modulated by plant genotype, plant growth stage and/or soil type?

Can rhizobacterial communities contribute to drought tolerance of sorghum?

Are bacterial communities recruited from soil with a history of sorghum cultivation and drought more effective in conferring drought tolerance?

Are endophytic strains, characterized as PGPB in sugarcane, able to provide beneficial effects on sorghum performance?

Thesis outline

In **Chapter 2,** I describe the differences in rhizobacterial community composition of seven different sorghum cultivars grown in the greenhouse in two different soil types at four different plant growth stages. The aim of this work was to evaluate the relative impact of each factor (soil type, cultivar, plant growth stage) on the sorghum rhizobacterial community composition. The rhizobacterial taxonomic composition was assessed by high-throughput 16S rRNA amplicon sequencing. Also, the profile of strigolactones exuded by roots of the different sorghum cultivars was assessed and correlated with rhizobacterial community composition.

The goal of the work described in **Chapter 3** was to study the co-occurrence of bacterial and fungal communities in the rhizosphere of different sorghum cultivars. For this purpose, I selected a subset of the DNA samples from the rhizosphere of two sorghum cultivars, two soils and three plant growth stages from the initial mesocosm experiment described in Chapter 2. The taxonomic composition of rhizobacterial and fungal communities was assessed by high-throughput 16S and 18S rRNA amplicon sequencing, respectively. Subsequently, I investigated if fungal-bacterial interactions in the sorghum rhizosphere are modulated by soil type, plant genotype and plant growth stage.

Chapter 4 addresses the effects of different rhizobacterial community compositions on growth and drought tolerance of sorghum. I aimed to pinpoint possible bacterial taxa associated with plant water stress alleviation. For that, we used a microbiome transplantation approach to minimize the effects of abiotic characteristics on plant growth and plant stress alleviation. I analysed the

diversity and relative abundance of rhizobacterial communities from two sorghum cultivars (drought susceptible, drought tolerant) that were pre-cropped in five microbiologically and physico-chemically different soils and subsequently transplanted to a standardized soil and exposed to drought stress.

In **Chapter 5**, the effects of five endophytic bacterial strains on the growth of four sorghum cultivars are described **.** These bacterial strains were originally selected as PGPB of sugarcane and were tested here for their beneficial effects on sorghum growth. Dry biomass and root architecture were evaluated as indicators of plant growth. Furthermore, I checked if their PGPB effects could be linked to the plant genotype and bacterial isolate identity.

In **Chapter 6,** I provide a general discussion of the main findings of this thesis and highlight the importance of sorghum-microbiome interactions. I discuss the approaches used in this thesis to give future directions and perspectives for fundamental research as well as for practical application of the knowledge obtained.

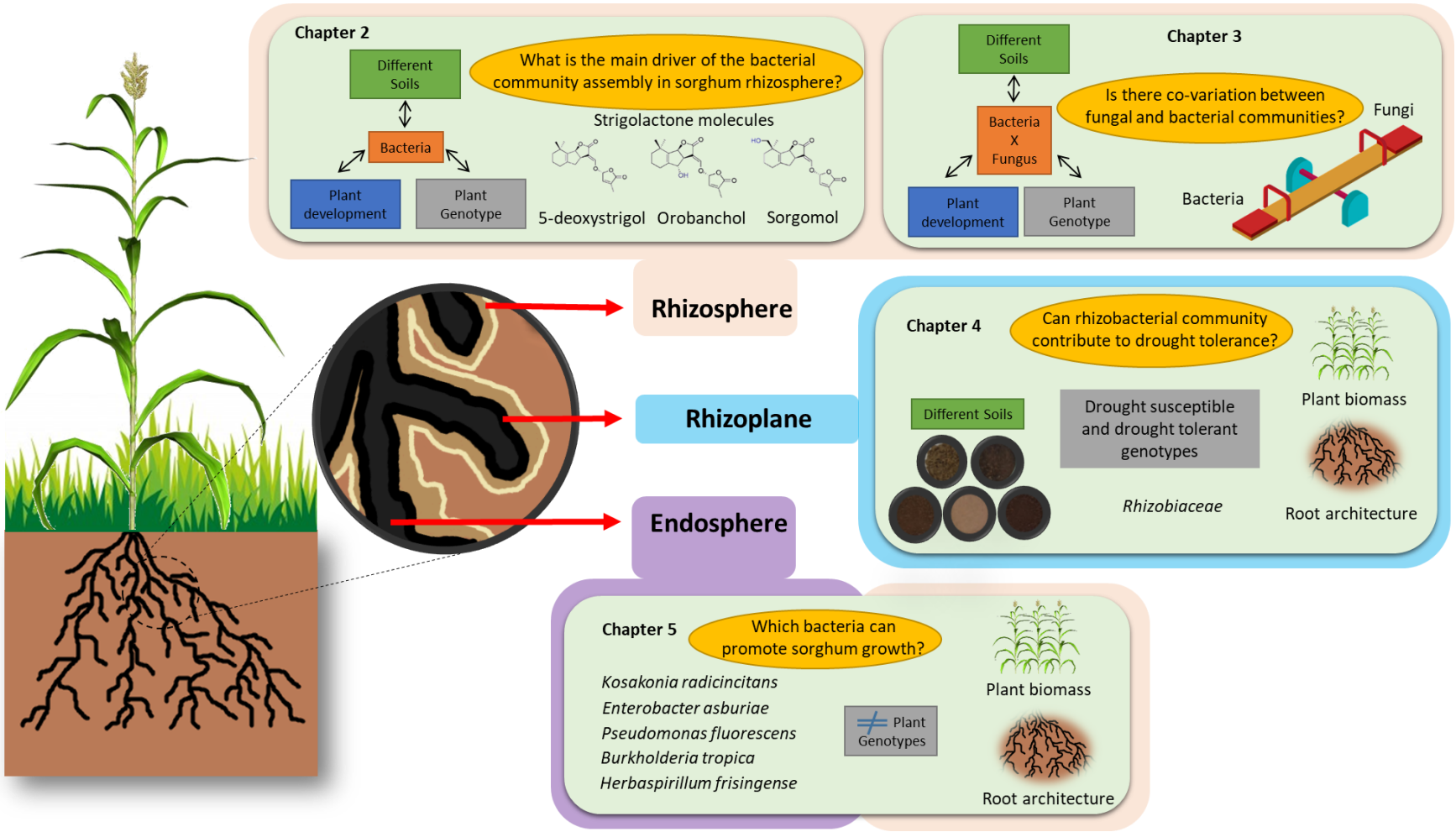

Figure 1. Schematic overview of the chapters presented in this thesis.

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, Schlaeppi & 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 ºC/17 ºC 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 ºC 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^{TM} 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 □L of 25:75 acetonitrile:water and filtered through a 0.45-μm 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).

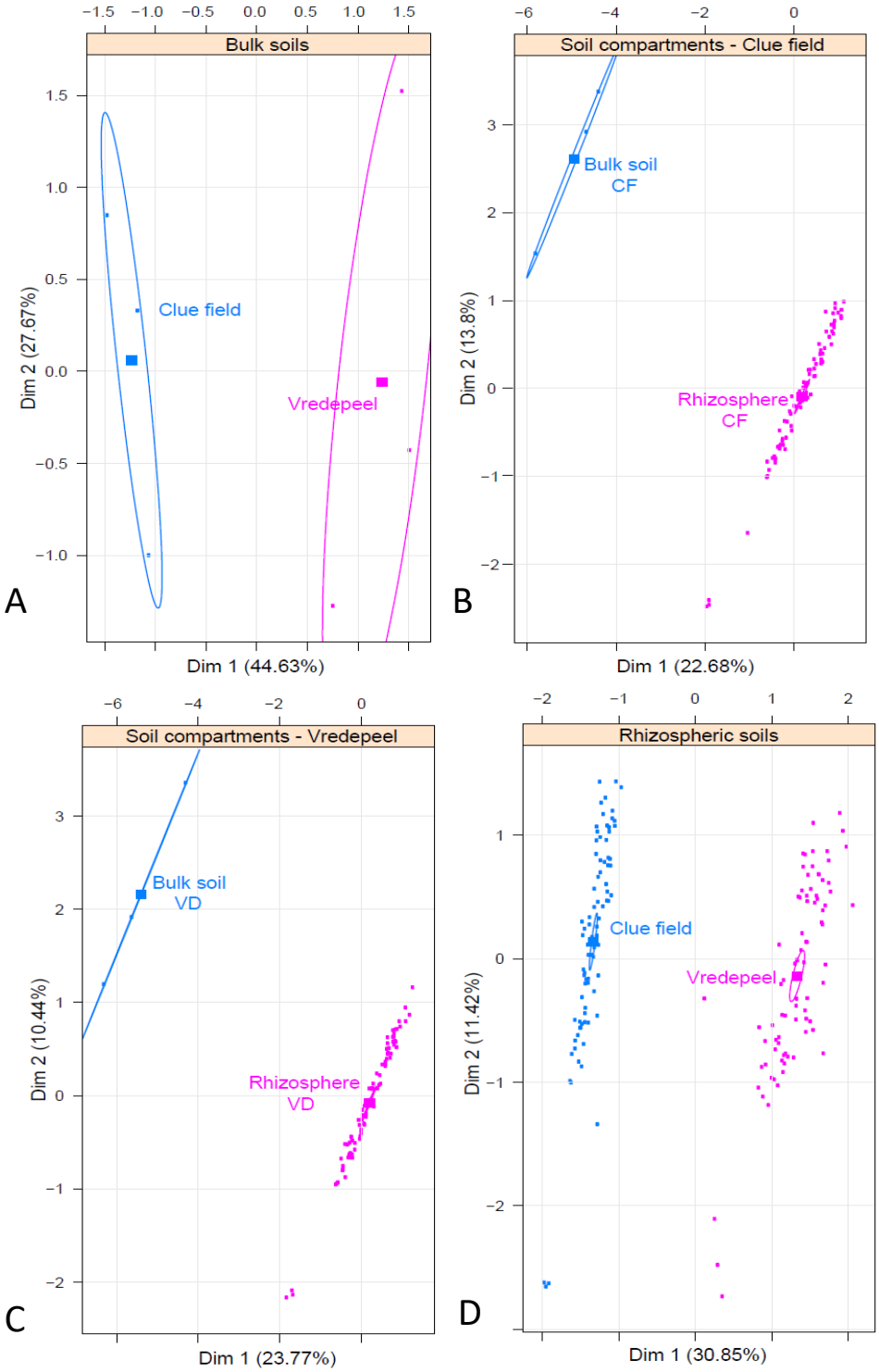

Figure 1. Multiple factor analysis (MFA) with supplementary variables emphasizing the factor soil and showing the bacterial community dissimilarity between (A) bulk soils from Clue Field (CF) and Vredepeel (VD); (B) bulk soil and rhizosphere soil from CF; (C) bulk soil and rhizosphere soil from VD; (D)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

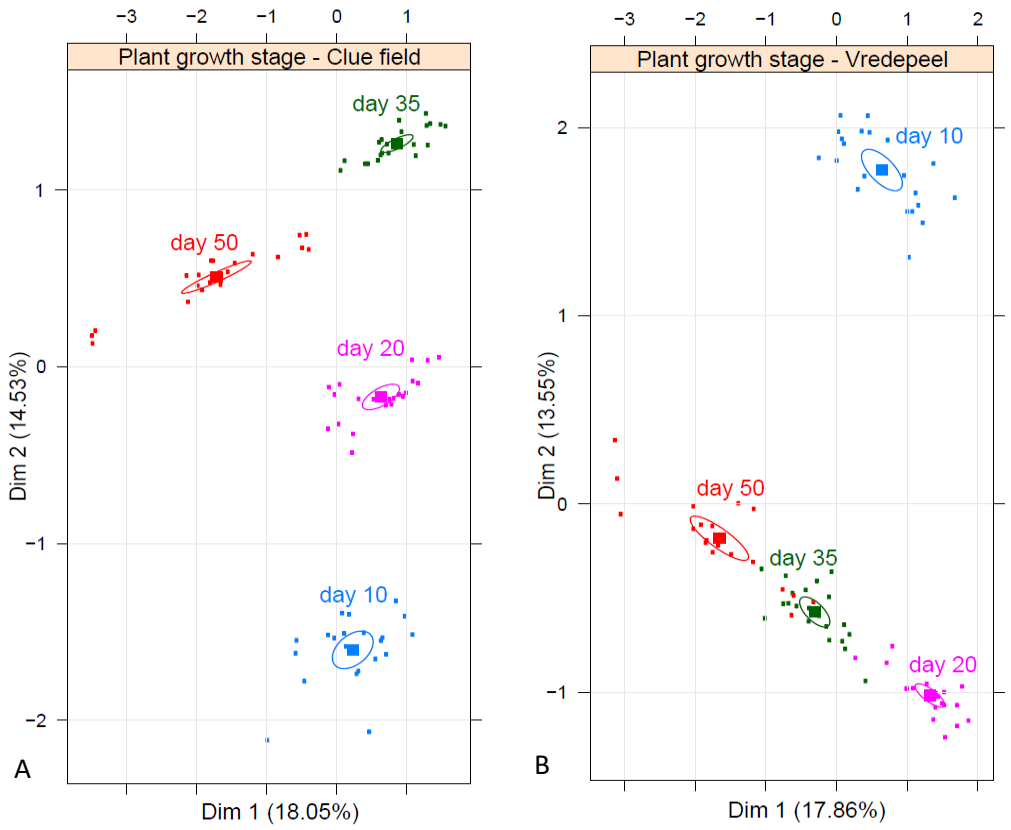

Figure 2. Multiple factor analysis (MFA) with supplementary variables emphasizing the factor plant growth stage and showing the rhizosphere bacterial community dissimilarity in (A) Clue Field (CF) soil and (B) Vredepeel (VD) soil.

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).

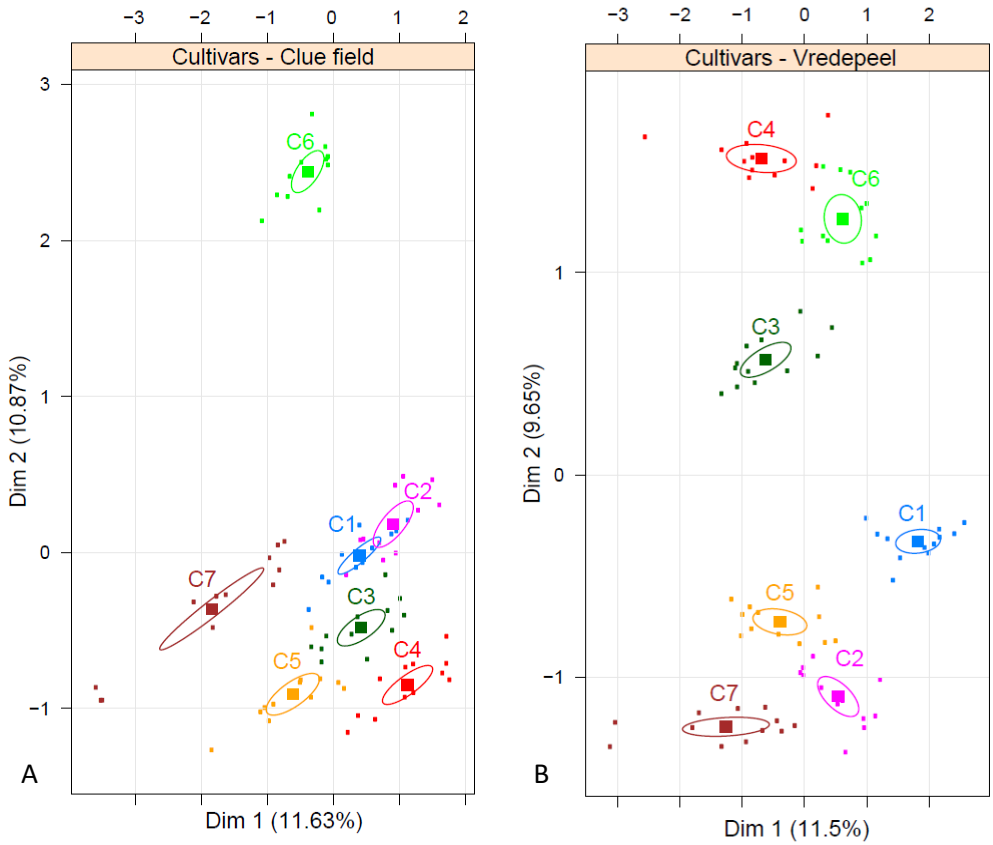

Figure 3. Multiple factor analysis (MFA) with supplementary variables emphasizing the factor cultivar and showing the rhizosphere bacterial community dissimilarity among cultivars: C1 = Hybrid grain (BRS330), C2 = Sweet hybrid (BRS509), C3 = Hybrid silage (BRS655), C4 = Hybrid grazing (BRS802), C5 = *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).

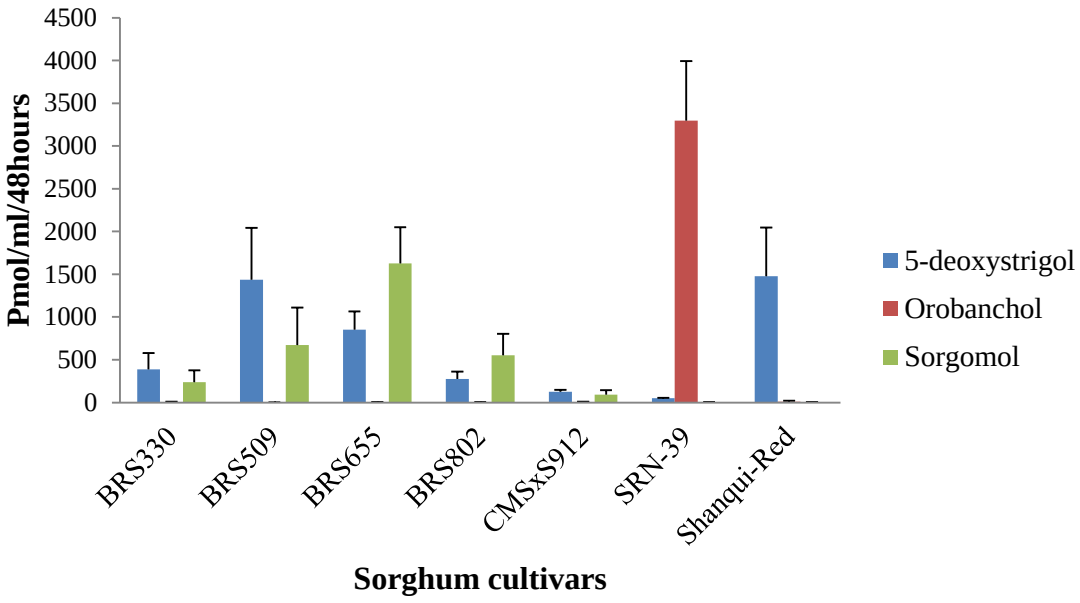

Figure 4. Strigolactone profile in different sorghum cultivars. The bars represent the mean values of biological replicates (n=3) ± (SE).

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 (Czarnota *et al.*, 2003, Mohemed *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 (Mohemed *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	*Chitinophagaceae*	1.75	1.35	2.2	4.0
	uncl_Bacteroidetes	uncl_Bacteroidetes	1.20	1.13	1.6	2.3
Planctomycetes	Planctomycetia	*Planctomycetaceae*	1.86	1.35	4.8	2.6
Proteobacteria	Alphaproteobacteria	*Bradyrhizobiaceae*	1.42	1.29	3.7	6.0
		Sphingomonadaceae	1.13	1.50	1.6	2.2
		uncl_*Rhizobiales*	1.55	1.19	1.6	2.7
	Betaproteobacteria	*Oxalobacteraceae*	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	*Xanthomonadaceae*	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	*Planctomycetaceae*	0.77	2.54	3.9	2.0
Proteobacteria	Alphaproteobacteria	*Bradyrhizobiaceae*	1.43	1.24	2.3	3.9
		Sphingomonadaceae	1.41	1.69	1.8	3.1
		uncl_*Rhizobiales*	1.07	0.57	1.2	2.7
	Betaproteobacteria	*Burkholderiaceae*	1.17	0.41	0.6	1.1
		Oxalobacteraceae	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	*Xanthomonadaceae*	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	*Planctomycetaceae*	**2.6 ± 0.002**	**2 ± 0.001**
Proteobacteria	Alphaproteobacteria	*Bradyrhizobiaceae*	**6 ± 0.002**	**3.9 ± 0.001**
		Sphingomonadaceae	**2.2 ± 0.001**	**3.1 ± 0.001**
		uncl_Alphaproteobacteria	**1.6 ± 0**	**2.7 ± 0.001**
	Betaproteobacteria	*Burkholderiaceae*	**1.7 ± 0.002**	**1.1 ± 0**
		Comamonadaceae	**2.2 ± 0.001**	**2.4 ± 0.001**
		Oxalobacteraceae	**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	*Xanthomonadaceae*	**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	*Chitinophagaceae*	4.3 ± 0.003	4.2 ± 0.003	4.2 ± 0.002	**3.2 ± 0.003**
		Sphingobacteriaceae	**1.3 ± 0.002**	1 ± 0.002	0.4 ± 0.001	0.2 ± 0
Planctomycetes	Planctomycetia	*Planctomycetaceae*	2.2 ± 0.002	2.3 ± 0.002	2.3 ± 0.003	**3.7 ± 0.004**
Proteobacteria	Alphaproteobacteria	*Bradyrhizobiaceae*	6.2 ± 0.005	6.6 ± 0.004	6.5 ± 0.004	**4.6 ± 0.005**
		Sphingomonadaceae	2.3 ± 0.001	2.3 ± 0.002	2.5 ± 0.001	**1.8 ± 0.002**
	Betaproteobacteria	*Comamonadaceae*	2.5 ± 0.003	2.5 ± 0.002	2.1 ± 0.002	**1.7 ± 0.002**
		Oxalobacteraceae	**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	*Xanthomonadaceae*	1.9 ± 0.001	1.9 ± 0.001	1.7 ± 0.001	**1.3 ± 0.001**
	uncl_Proteobacteria	uncl_Proteobacteria	1.5 ± 0.001	1.7 ± 0.001	1.6 ± 0.001	**1.3 ± 0.001**
Verrucomicrobia	Opitutae	*Opitutaceae*	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	*Sphingobacteriaceae*	**1.2 ± 0.001**	0.9 ± 0.001	0.6 ± 0	**0.6 ± 0.001**
Planctomycetes	Planctomycetia	*Planctomycetaceae*	2.2 ± 0.003	**1.2 ± 0.002**	2 ± 0.002	**2.8 ± 0.003**
Proteobacteria	Alphaproteobacteria	*Sphingomonadaceae*	**3.8 ± 0.002**	3.5 ± 0.002	2.8 ± 0.002	**2.3 ± 0.001**
		Caulobacteraceae	1 ± 0.001	**1.3 ± 0.001**	0.8 ± 0.001	**0.6 ± 0.001**
	Betaproteobacteria	*Comamonadaceae*	2.5 ± 0.002	**2.9 ± 0.002**	2.2 ± 0.001	2.1 ± 0.002
		Oxalobacteraceae	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	*Xanthomonadaceae*	5 ± 0.003	**5.5 ± 0.006**	3.5 ± 0.002	**2.7 ± 0.002**
		Polyangiaceae	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	**1.5 ± 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	*Burkholderiaceae*	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
	Comamonadaceae	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	*Burkholderia*	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
	Cupriavidus	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	*Acidovorax*	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
	Albidiferax	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
	Polaromonas	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
	Variovorax	**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_*Comamonadaceae*	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** g/kg	**P** g/kg	**K** g/kg	**Ca** g/kg	**Mg** g/kg	**S** g/kg	**Fe** mg/kg	**Mn** mg/kg	**Cu** mg/kg	**Zn** mg/kg	**Mo** mg/kg	**Al** mg/kg	**Cl** mg/kg	**Dry biomass** (g)
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.

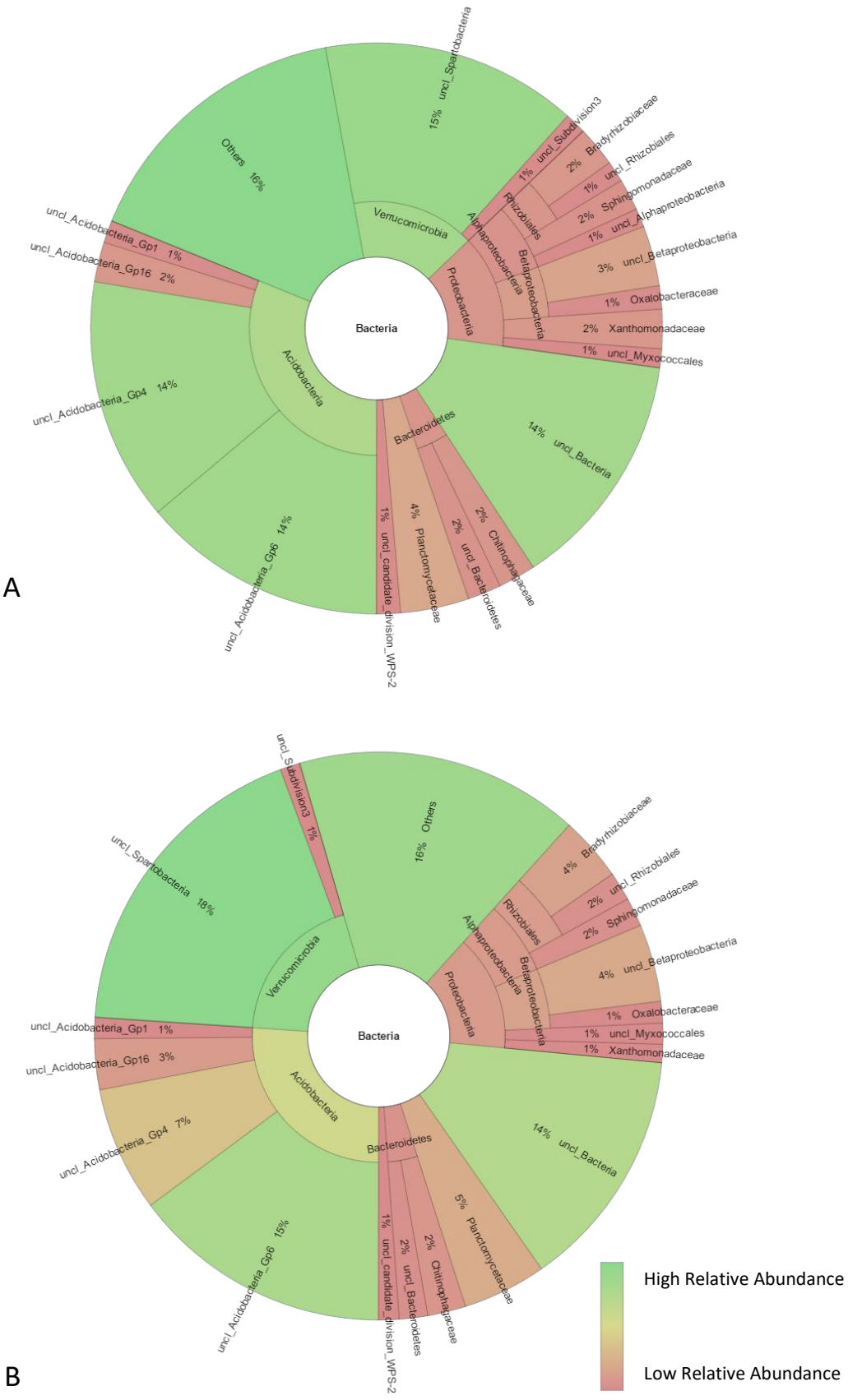

Figure S1. Relative abundance of bacteria in the bulk soil of (A) Clue Field and (B) Vredepeel.

Chapter 3

Co-Variation of Bacterial and Fungal Communities in Different Sorghum Cultivars and Growth Stages is Soil Dependent

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Abstract

Rhizosphere microbial community composition can be influenced by different biotic and abiotic factors. We investigated the composition and co-variation of rhizosphere bacterial and fungal communities from two sorghum genotypes (BRS330 and SRN-39) in three different plant growth stages (emergence of the second leaf, (day10), vegetative to reproductive differentiation point (day 35) and at the last visible emerged leaf (day 50) in two different soil types, Clue field (CF) and Vredepeel (VD). We observed that either bacterial or fungal community had its composition stronger influenced by soil followed by plant growth stage and cultivar. However, the influence of plant growth stage was higher on fungal community composition than on the bacterial community composition. Furthermore, we showed that sorghum rhizosphere bacterial and fungal communities can affect each other's composition and structure. In this sense, the decrease in relative abundance of the fungus genus *Gibberella* over plant growth stages was followed by decrease of the bacterial families *Oxalobacteracea* and *Sphingobacteriacea*. Although cultivar effect was not the major responsible for bacterial and fungal community composition, cultivar SRN-39 showed to promote a stronger co-variance between bacterial and fungal communities.

Introduction

The rhizosphere harbors a wide range of microorganisms, which have been shown to influence significantly plant growth, root architecture and nutrient uptake (Bonfante & Anca, 2009, Berendsen *et al.*, 2012, Vacheron *et al.*, 2013, Mendes *et al.*, 2014). Conversely, the composition of microbial rhizosphere communities is influenced by biotic and abiotic factors including plant species (or genotypes) and soil management (Inceoglu *et al.*, 2010, Navarrete *et al.*, 2013, Lima *et al.*, 2015).

Studies on the impact of different soil fertilization managements on the composition of the bacterial community in the rhizosphere of sorghum have shown that the bacterial community is more affected by compost than by inorganic fertilizers (Lavecchia *et al.*, 2015). In addition, geographic location and soil characteristics are the main factors explaining the variability in the structure of the bacterial community in the rhizosphere of sorghum (Ramond *et al.*, 2013). Moreover, in an earlier study we found soil to be the most important factor on sorghum rhizosphere bacterial community assembly followed by plant growth stage and plant genotype (Schlemper *et al.*, 2017). Furthermore, we found that along plant growth stage, the impact of soil on the bacterial community assembly reduced and, instead, the impact of plant genotype increased.

Most of rhizosphere community studies focused on either bacterial or fungal communities. However, the dynamics of both communities combined in different plant species are rather uncommon, but are of great relevance. Marschner *et al.* (2001) showed that arbuscular mycorrhizal fungi (AMF) infection changes the bacterial community composition in the rhizosphere of maize with time. While studying the impact of elevated atmospheric CO_2 on the carbon flow in the rhizosphere in *Festuca rubra*, Drigo *et al.* (2013) found that the allocation of labile photosynthates from AMF to soil promoted shifts on fungal and bacterial rhizosphere microbial communities. Vázquez *et al.* (2000) showed that the interaction between AMF and the microbial inoculants *Azospirillum*, *Pseudomonas* and *Trichoderma* induced changes in the microbial population in the rhizosphere of maize. Additionally, through the taxonomic assignment of the annotated rRNA and mRNA reads Chapelle *et al.* (2016) found that *Sphingobacteriaceae* and *Oxalobacteraceae* were more abundant in rhizosphere of sugar beet inoculated with *Rhizoctonia solani* than in non-fungal inoculated plant cultivated in suppressive soil. However these studies are focused in a single group or single species of fungi effect on bacterial community.

Although studies of combined fungal and bacterial diversity and community composition have been performed in rhizosphere, very few studies have directly correlated the composition of one community to another (Bell *et al.*, 2014, Cassman *et al.*, 2016). Particularly in sorghum, as far as we

know, there are no studies on mutual effects on the composition and diversity of bacteria and fungi in the rhizosphere. *Sorghum bicolor* (L.) Moench is the fifth cereal most produced worldwide and is a staple food for more than 500 million people in 30 countries (Rao *et al.*, 2014). Sorghum is considered to be drought and salinity tolerant and its adaptation to low fertility soils allow the cultivation of this cereal in tropical areas under adverse climate conditions (Pinho *et al.*, 2015). Here, we aimed to evaluate the variation of fungal and bacterial communities and the relationship of both communities in rhizosphere of different sorghum genotypes in different soils. We tested the hypothesis that (i) fungal-bacterial interaction in the sorghum rhizosphere is modulated by the tripartite factors: plant genotype, soil type, and plant growth stage and (ii) fungal and bacterial rhizosphere communities composition are modulated by changes in each other's abundances.

Material and Methods

Soil sampling

The soils were collected from two locations in The Netherlands: Clue Field (CF) (52° 03' 37.91"N and 5° 45'7.074"E) characterized as Arenosol soil (natural soil on former but abandoned field) and Vredepeel (VD) (51° 32' 25,8"N and 5° 51'15,1" E) characterized as Gleyic Podzol soil (agriculture field). From each area, the soil samples were collected (0 – 20 cm topsoil layer) from five points equidistant at 50 meters from each other. Once collected, the soil was sieved (4 mm mesh size) and homogenized. The physical and chemical characteristics of each soil are described in (Table S1).

Sorghum bicolor Cultivars and Mesocosm Experiment

Two different cultivars from different origins were chosen to assess the bacterial and fungal communities composition in the rhizosphere of *S. bicolor*: BRS330 cultivar - a hybrid grain resistant to anthracnose, leaf blight, leaf rust and sooty stripe (Cota *et al.*, 2012, Cota *et al.*, 2013), and cultivar SRN-39 (grain) - a high producer of orobanchol (strigolactone molecule) root exudate (Schlemper *et al.*, 2017) and resistant against the root parasitic weed *Striga hermonthica* (Del.) Benth (Gobena *et al.*, 2017). The seeds of cultivar BRS330 were from 'Embrapa Milho e Sorgo' (Brazil) and the seeds of cultivar SRN-39 originally released in Niger and Sudan (Africa) by International Crops Research Institute for the Semi-Arid Tropics - ICRISAT (Ejeta, 2005, Olembo *et al.*, 2010) were provided by the Laboratory of Plant Physiology – Wageningen University (Netherlands). The experimental design and sampling consisted of three replicates of two soil types, two sorghum cultivars and three plant

growth stages, in total 36 experimental units randomly distributed in a greenhouse. Fifteen seeds of each sorghum cultivar were sown in soils in plastic pots (6.5 L). The pots were kept under controlled temperature and photoperiod conditions (22 ºC/17 ºC day/night and photoperiod 16/8 h light/dark). After five days, plantlets were trimmed to five seedlings per pot. Rhizosphere soil was sampled after in three different plant growth stages: at the emergence of the second leaf (day10), at the emergency of the fifth leaf when the plants migrate from vegetative to reproductive differentiation point (day 35) and at the last visible emerged leaf (day 50) before the plant flowering. At the first stage of plant growth (day 10) rhizosphere soil was sampled removing the whole plant and brushing the soil adhered to the seminal roots, and for the last stages of plant growth (Days 35 and 50), rhizosphere soil was sampled with a cylindrical auger (6 × 150 mm). Bulk soil samples were taken from pots without plants. Rhizosphere and bulk soil samples for DNA extraction were kept at -80 ºC.

DNA extraction and 16S rRNA partial gene sequencing

DNA was extracted from 0.25 g of soil of each sample using DNA Power soil DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). DNA integrity was checked by agarose gel (1.5%) electrophoresis in TBE (Tris-borate-EDTA) buffer. DNA from each treatment was used as template for 16S rRNA and 18S rRNA partial genes fragments amplification. The amplification of the 16S rRNA partial gene was performed using the primer set 515F and 806R (Bergmann *et al.,* 2011). Primers contained multiplex tags for sample identification. PCR was carried out using 0.2 µl of 0.056 U fast StartExp*Taq* Polymerase (Roche Applied Sciences, Indianapolis, IN, USA), 2.5µl dNTP (2mM each), 0.25µl of each primer and 1.0 µl of DNA template. Thermocycling conditions were: denaturing at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30s, extension at 72 °C for 60 s followed by a final extension at 72 °C for 10 min. As negative control, water was used instead of DNA, and as positive control DNA of *Escherichia coli* was used. For the 18S rRNA partial gene amplification, a fungal-specific primer set FR1 and FF390.1 (Verbruggen *et al.*, 2012) was used to amplify a 350 bp region of the 18S rRNA gene. Primers contained multiplex tags for sample identification. PCR reactions were carried out using 2.5 µl of 2mM dNTP, 0.5 µl of each primer, 1.0 µl of DNA template, and 0.2 µl of 0.056 U of Fast StartExp-Polymerase (Roche Applied Sciences, Indianapolis, IN, USA). The PCR reaction had an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 60 s, and the final extension at 72 °C for 10 min. As negative control, water was used instead of DNA. The PCR products were purified using QIAquick PCR Purification Kit (Qiagen

Technologies) and their quality were checked before and after the purification in agarose gel electrophoresis in TBE buffer. The PCR amplicons were quantified using Fragment analyser™ - Automated CE system (Advanced Analytical Technologies, Inc) and equimolar pooled. The samples were sequenced in PGM machine on Ion Torrent (Life technology) in Korea (Macrogen Inc. Company, South Korea).

Data Analyses

16S and 18S rRNA sequences processing

Forward and reverse primer sequences in the library FASTQ file of each sample were removed using Flexbar version 2.5 (Dodt *et al.*, 2012). Sequences were filtered for quality criteria with a Phred quality score of 25 and with minimum sequence length of 150bp by running the FASTQ-MCF (Aronesty, 2011). After filtering, FASTQ files were converted to FASTA format and concatenated into a single file. Chimera sequences were detected using the UCHIME algorithm implemented in VSEARCH (Edgar *et al.*, 2011). The reads were clustered into Operational Taxonomic Units (OTU), within evolutionary distance of 97% using the UPARSE (Edgar, 2010) performed with VSEARCH version 1.0.10 (Flouri *et al.*). The OTU table was converted to biological observation matrix (BIOM) format 1.3.1 (McDonald *et al.*, 2012) and using the RDP Classifier version 2.10 (Cole *et al.*, 2014), taxonomic information for each OTU was added to the BIOM file. All procedures were implemented in a Snakemake workflow (Köster & Rahmann, 2012). The number of sequences in each library was rarefied (*alpha_rarefaction.py*) to 2.000 sequences for bacteria and to 550 sequences for fungi prior to diversity analyses in QIIME 1.8.0 (Caporaso *et al.*, 2010). The 16S rRNA and 18S rRNA sequence data are available at the European Nucleotide Archive (ENA) (https://www.ebi.ac.uk/ena/) under the study accession number PRJEB21895 (ERP024198).

Statistical analyses

To check the treatment effects on sorghum rhizosphere bacterial and fungal communities composition, Between-Classes Analysis (BCA) and Co-inertia analysis (COIA) were performed in R v3.3.3 (R Development Core Team, 2017) using the package “ade4” (Dray & Dufour, 2007). To explore the dissimilarities of treatments within each community, a Principal Component Analysis (PCA) was used to create BCA tables using the function “bca”. In order to find the similarity of bacterial and fungal community within treatments, BCA tables were used to conduct Co-inertia

analysis for the two soils using the function “coinertia”. Monte-Carlo test was applied for BCA and COIA using 999 random permutations. For co-inertia “RV.test” R function was used to perform Monte-Carlo test. As a result of COIA, plots with arrows are formed. The back of the arrow represents the location of bacterial community organisms and the tip of arrow represents the location of fungal community organisms. The strength of the relationship between both communities is inversely related to the length of the arrow. Arrows projected to the same direction showed strong association between the treatments with respect to the microbial composition (Culhane *et al.*, 2003). Bacterial and fungal community structure co-variance scores were given by COIA analysis. Family groups responsible for such co-variance were those had higher score than the 95% of sample normal distribution. This was calculated by the standard deviation multiplied by 1.96, what is the range that corresponds to 95% of normal distribution of the standard deviation.

To infer how the rhizosphere bacterial community co-varied with the factors soil, cultivar and plant growth stage, the bacterial and fungal abundance data were transformed by Hellinger transformation (Legendre & Gallagher, 2001) using the package Vegan” version 2.4.0 (Oksanen *et al.*, 2016) and the co-variance was measured by the coefficient RV-Value by Multiple Factor Analysis (MFA) using the package “FactoMineR” (Lê *et al.*, 2008) in R v3.1.3 program. Moreover, using the same R package, we applied Permutational Multivariate Analysis of Variance (PERMANOVA) using Bray-Curtis distance matrix with 999 permutations to test the influence of the factors soil, plant growth stage and cultivar in the rhizosphere bacterial and fungal community.

In order to check for dissimilarities within the microbial communities, treatments were divided into subsets and Principal Coordinate Analysis (PCoA) were performed in QIIME 1.9.1 using the script *beta_diversity_through_plots.py* with Bray-Curtis distance matrices. Distance matrices generated by PCoA were used to perform PERMANOVA analysis with 9999 random permutations ($p<0.05$). For the PCoAs where the treatment effects were significant, microbial community family groups responsible for the dissimilarities were checked. Differences in mean proportion was tested through Welch’s test ($P<0.05$) using the Statistical Analysis of Metagenomics Profiles (STAMP) v2.1.3 program (Parks *et al.*, 2014). To avoid False Discover Rates (FDR), Benjamini-Hochberg (Benjamini & Hochberg, 1995) was applied.

Alpha diversity index (Shannon), species richness (Chao1), as well as the total number of OTUs were calculated in QIIME 1.9.1 using the command *alpha_diversity.py*. In order to check for significant differences among samples, analysis of variance ANOVA and Tukey test ($p<0.05$) was performed in R for each Alpha diversity index.

Results

Analysis of co-inertia (RV-coefficient) at family level revealed that soil type, plant growth stage and cultivar explained 52.62%, 22.70% and 12.73% of the rhizosphere bacterial community variation, respectively (Table S2). For the fungal community, soil type, plant growth stage and cultivars explained 42.83%, 26.02% and 14.99%, of the variation, respectively (Table S3). We tested the statistical significance of the factors soil, plant growth stage and cultivar on the rhizosphere bacterial and fungal community structures by PERMANOVA using Bray-Curtis as distance matrix. The results showed that soil had significant effects on both the bacterial ($F=6.87$; $p<0.001$) and fungal ($F=7.89$; $p<0.001$) communities; plant growth stage had a significant effect only on the fungal community ($F=2.68$; $p<0.001$) and cultivar had no significant effect on both communities (Table S4).

Differences in Bacterial Community Structure

PERMANOVA test showed that the bacterial communities from the bulk soils of CF and VD were not significantly different (Pseudo-F: 1.40; P=0.40) (Figure S1). However, the same analysis, showed that the bacterial community was significant different in the rhizosphere soils of CF and VD (Pseudo-F: 6.9; P< 0.05) (Figure S2A). Through Welch's test we found that among the bacteria families driving this dissimilarity, *Bradyrhizobiaceae* was more abundant in rhizosphere soil of CF than VD, whereas *Caulobactereaceae*, *Phyllobacteriaceae* and *Xanthomonadaceae* were more abundant in VD (Figure S2B). Welch's test revealed a significant difference in rhizosphere bacterial composition between both CF (Pseudo-F: 2.3; P<0.05) and VD (Pseudo-F: 2.55; P<0.05) soils (Figures S3A and S3C). At CF soil, this difference was mainly caused by unclassified *Spartobacteria* family with high abundance in bulk soil, and *Comamonadaceae*, *Oxalobacteraceae* families and unclassified Alphaproteobacteria with higher abundances in the rhizosphere than in the bulk soil (Figure S3B). At VD soil, *Oxalobacteraceae* as well as organisms that could not be classified at family taxonomic level belonging to *Acidobacteria* Gp1, *Myxococcales* (Gammaproteobacteria) and Proteobacteria were significantly more abundant in rhizosphere than in bulk soil (Welch's test; P<0.05) (Figure S3D). PERMANOVA analysis comparing cultivars in the CF soil showed that the rhizosphere bacterial community of cultivar BRS330 significantly differed from that of cultivar SRN-39 (Pseudo-F: 1.14; P< 0.05) (Figure 1A). Performing Welch's test we found *Bradyrhizobiaceae* and *Sphingomonadaceae* with mean proportion significant highest in rhizosphere of BRS330, whereas *Comamonadaceae* and unclassified *Acidobacteria* Gp1 were significant highest in SRN-39 rhizosphere (P< 0.05) (Figure 1B).

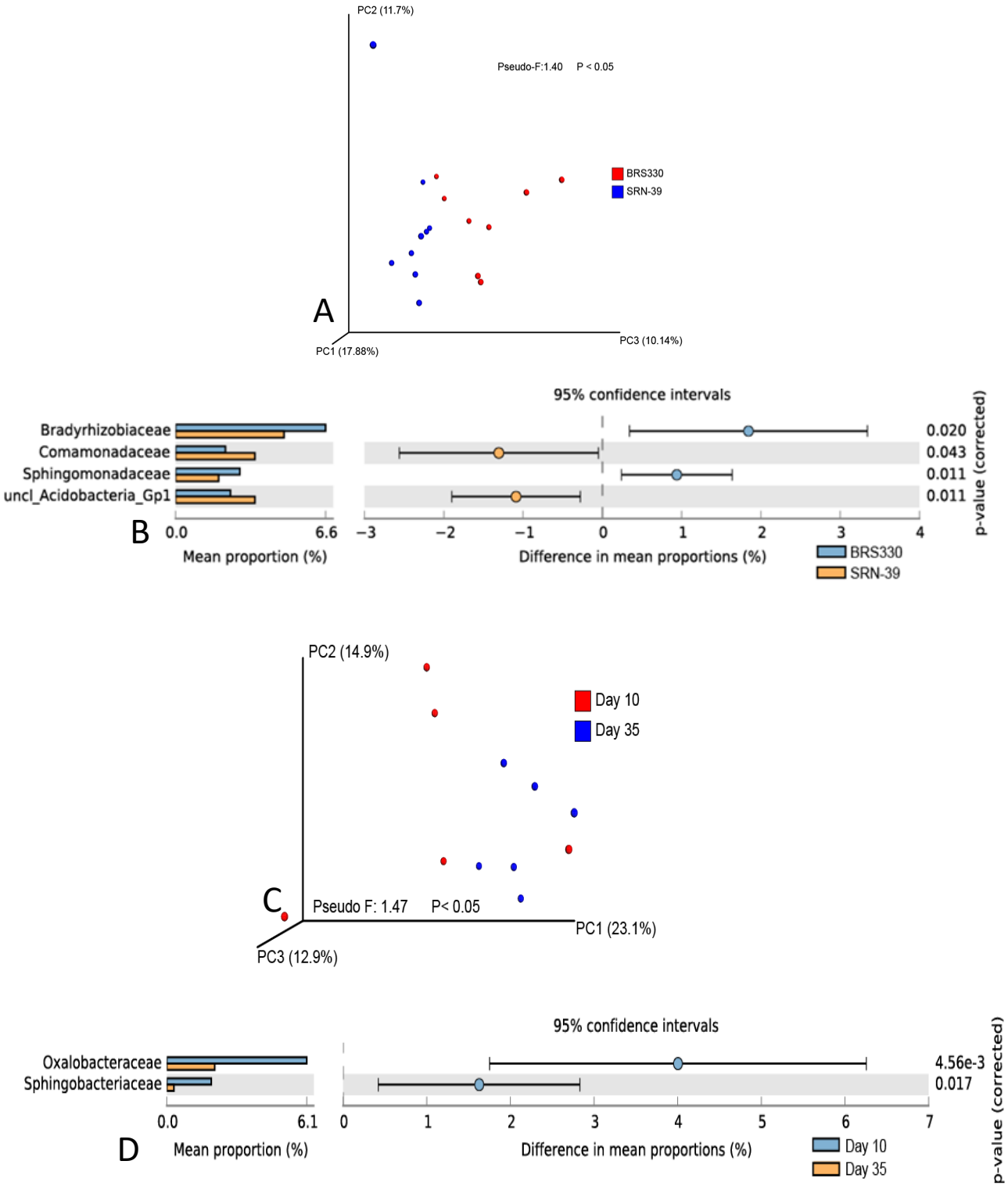

Figure 1: (A) Principal Coordinate Analysis (PCoA) and (B) differences in relative abundance of bacterial families between cultivars BRS330 and SRN-39, and (C) PCoA and (D) differences in relative abundance of bacterial families between days 10 and 35 at Clue field soil (Welch's test; P<0.05).

The only significant difference in the bacterial community composition found over growth stages was in CF soil between the day 10 and 35 of plant growth (Pseudo-F: 1.47; P<0.05) (Figure 1C). The two families responsible for this dissimilarity were *Oxalobacteraceae* and *Sphingobacteriaceae* with significant highest abundance at day 10 and not at day 35 (P<0.05) (Figure 1D).

Differences in Fungal Community Structure

The fungal community in both CF and VD bulk soils did not significantly differ (Pseudo-F: 2.00; P=0.20) (Figure S4). However, the fungal rhizosphere community in CF soil was significantly different from that in VD soil (Pseudo-F: 7.9; P< 0.05) (Figure S5A). *Hypocreaceae* and unclassified *Mortierellales* were more abundant in the sorghum rhizosphere in CF soil than in VD soil. In contrast, the organisms that could not be classified at the family level belonging to the groups of *Saccharomycetales, Sordariales, Sordariomycetes* were significantly more abundant in the rhizosphere community in VD than in CF soil (Figure S5B). PCoA showed a clear distinction in the rhizosphere fungal communities at day 10 as compared to day 35 (Pseudo-F: 2.75; P< 0.05) and 50 (Pseudo-F: 2.24; P< 0.05) in CF soil (Figures 2A and 2C). *Nectriaceae* was found to be the major group responsible for these dissimilarities with higher abundance at day 10 than at days 35 and 50. On the other hand, the abundances of unclassified *Chaetothyriales* and unclassified *Leotiomycetes* were lower at day 10 than at days 35 and 50 (Figures 2B and 2D). Overall, *Nectriaceae* was the most abundant fungal family in the Clue field rhizosphere soil (Figure S6).

In VD soil, the rhizosphere fungal community also showed to be different between early (day 10) and late (day 50) plant growth stages (Figure 3A). Despite the difference in rhizosphere fungal community presented by PCoA plot and PERMANOVA analysis, only one fungal group could be assigned to be responsible for this dissimilarity; unclassified *Hypocreales* showed higher abundance at day 10 than at day 50 of plant growth (Figure 3B).

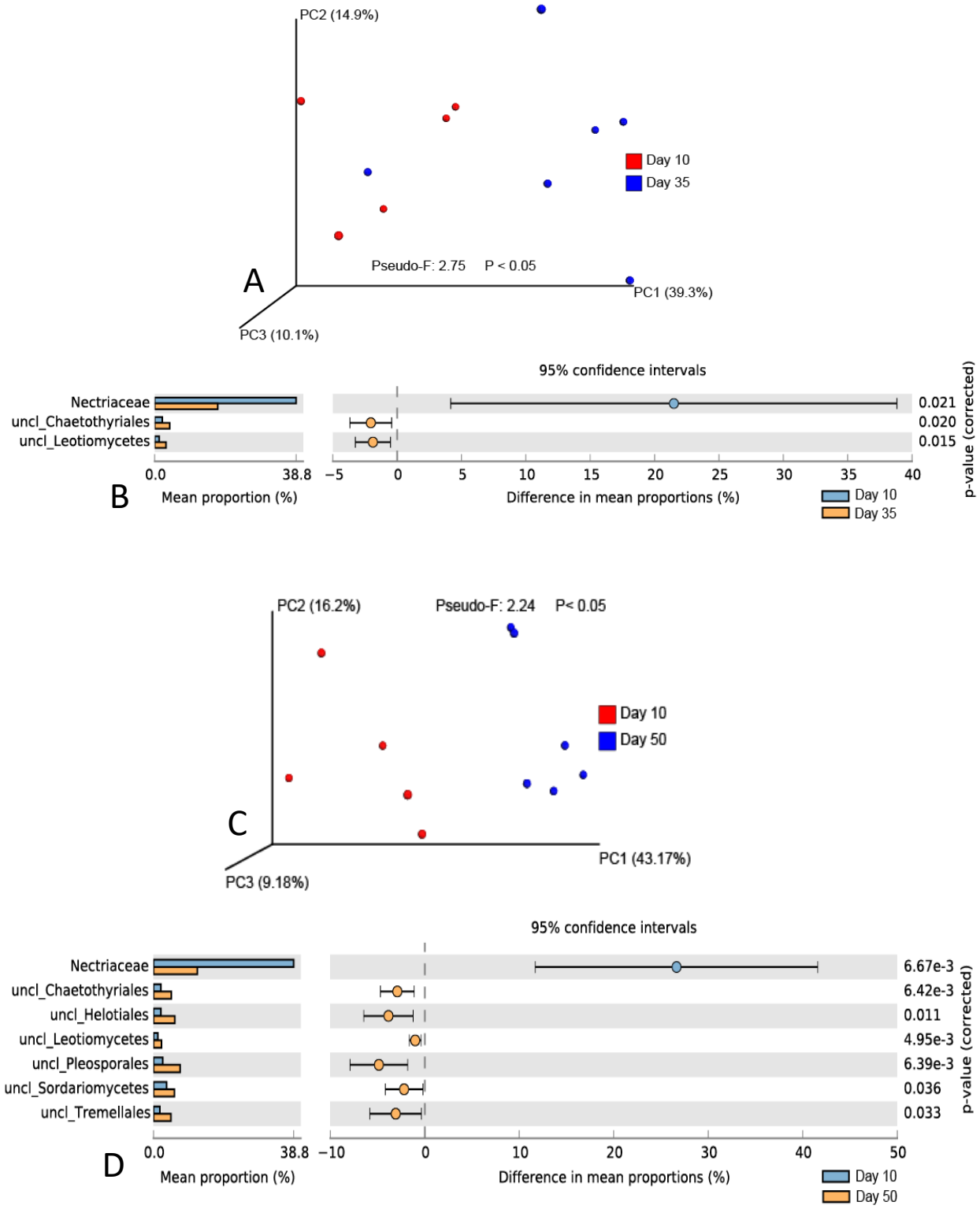

Figure 2. (A) Principal Coordinate Analysis (PCoA) and (B) differences in the relative abundance of fungi between days 10 and 35, and (C) PCoA and (D) differences in relative abundance of fungi between days 10 and 50 in Clue field rhizosphere samples (Welch's test; P<0.05).

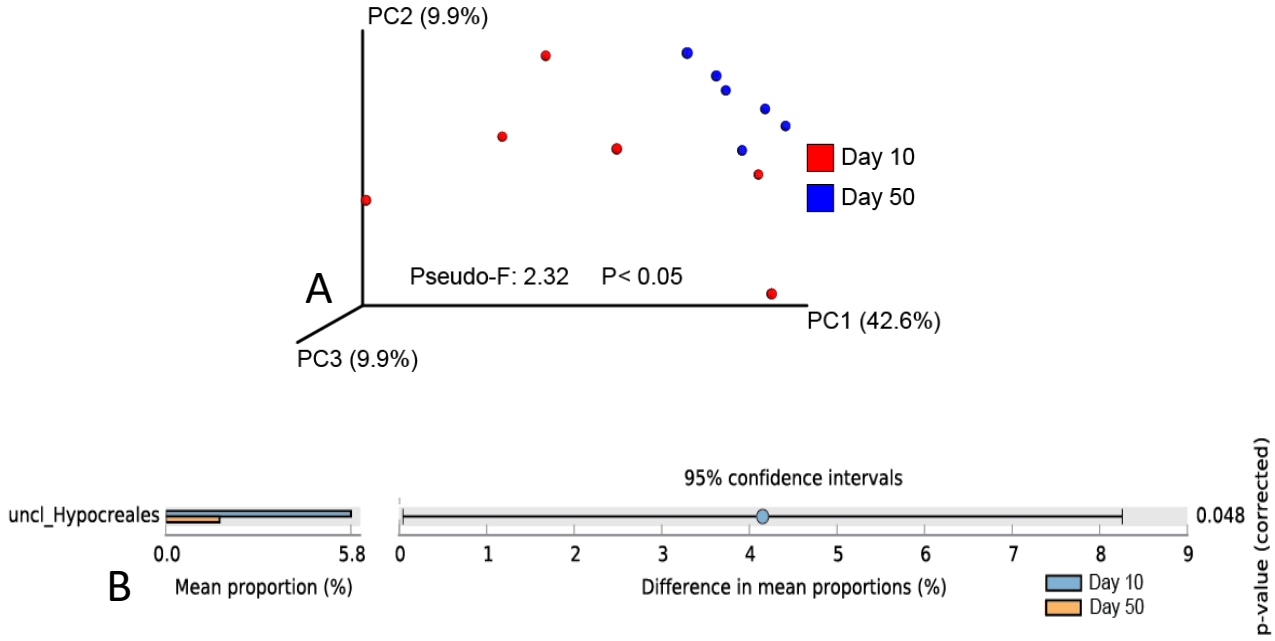

Figure 3. (A) Principal Coordinate Analysis (PCoA) and (B) differences in the relative abundance of fungi between days 10 and 50 in Vredepeel rhizosphere samples (Welch's test; P<0.05).

Between-Class and Co-inertia Analyses

Between classes analysis (BCA) was performed to check for dissimilarities in the total microbial rhizosphere community. At CF soil, bacterial and fungal communities composition were significantly different across sorghum treatments explaining 38% (P=0.03) and 37% (P=0.04) of total variation, respectively. Ellipses representing bacterial community composition of the cultivars BRS330 and SRN-39 at early sampling showed a clear separation from the ellipses of the two later samplings (Figure 4A). For the fungal community, although this separation remained consistent for cultivar SRN-39, for cultivar BRS330 the ellipse separation was more evident in the last sampling (day 50) than the early sampling points (days 10 and 35) (Figure 4B). At VD soil the rhizosphere bacterial community composition was significantly different among sorghum treatments, explaining 36.8% (P=0.001) of the total variation. Ellipses dispositions representing the bacterial community of cultivar BRS330 showed a clear separation between the composition of days 10 and 35 to the day 50 of plant growth. Conversely, bacterial community present in rhizosphere cultivar SRN-39 showed similarity between the latest two stages of plant growth (days 35 and 50) with dissimilarity to the day 10 of plant growth (Figure 5A). No significant difference was found for rhizosphere fungal community at VD soil (Monte-Carlo test) (Figure 5B).

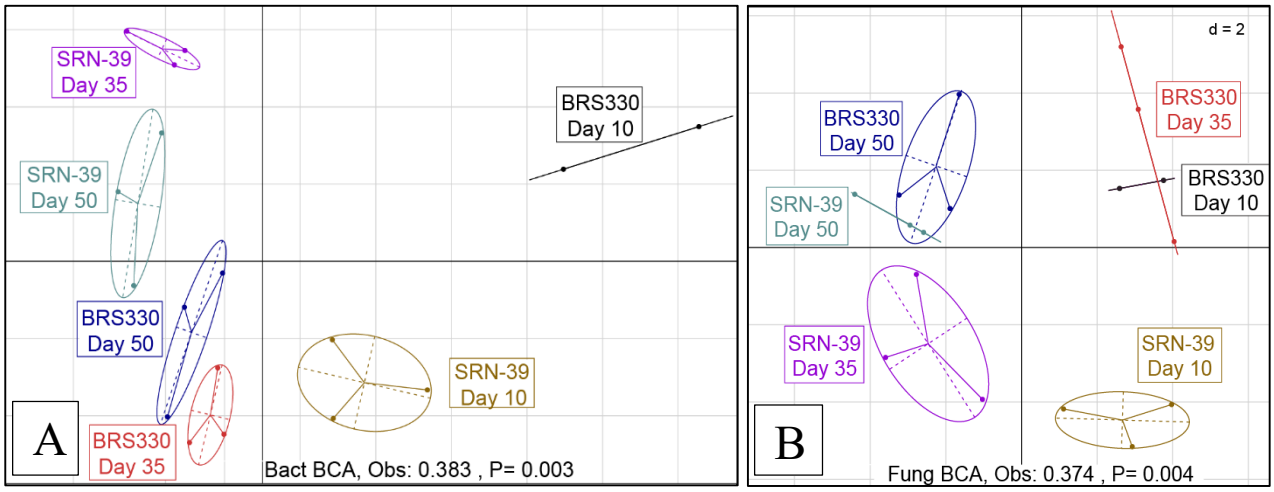

Figure 4. Between-Class Analysis (BCA) of (A) bacterial and (B) fungal communities in the rhizosphere of sorghum cultivars BRS 330 and SRN-39 at days 10, 35 and 50 of plant growth stage in Clue field soil.

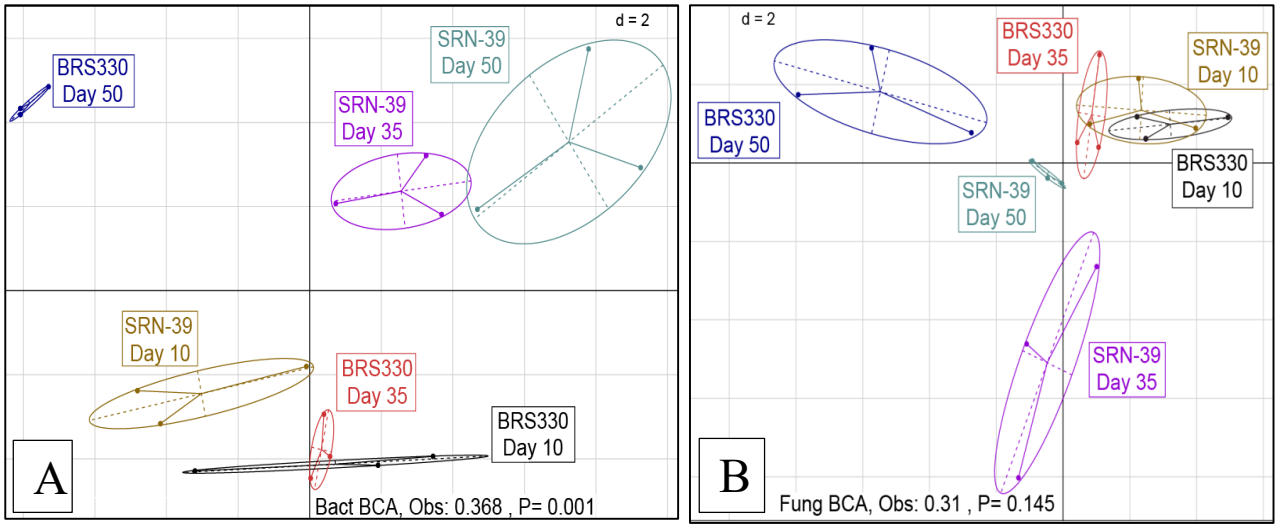

Figure 5. Between-Class Analysis (BCA) of (A) bacterial and (B) fungal communities in the rhizosphere of sorghum cultivars BRS 330 and SRN-39 at days 10, 35 and 50 of plant growth stage in Vredepeel soil.

Co-variance between rhizosphere bacterial and fungal community structures was determined using co-inertia analysis (COIA). Plotting bacterial and fungal community's ordination together resulted in a new ordination plot where an arrow links bacterial to fungal community positions. We observed that treatments in CF and VD soils explained 94% and 91% of the rhizosphere microbial community variation, respectively (Figures 6A and 6B). The variation between the bacterial and fungal communities was significantly different in CF soil (P=0.02). Shorter arrows in cultivar SRN-39 than in cultivar BRS330, in each growth stage, indicates stronger relationship between bacterial and fungal communities in the SRN-39 rhizosphere than in the BRS330 rhizosphere. For cultivar

SRN-39, the projection of arrows by day 10 in the opposite direction of days 35 and 50 of plant growth showed that day10 had a weak similarity on the variation of bacterial-fungal communities compared with days 35 and 50 of plant growth stage. No significant difference was found for VD soil (P=0.22) (Monte-Carlo test) (Figure 6B). For each soil, we assessed the representatives of rhizosphere bacterial and fungal communities responsible for the co-variance of each co-inertia axis (Tables S5 and S6).

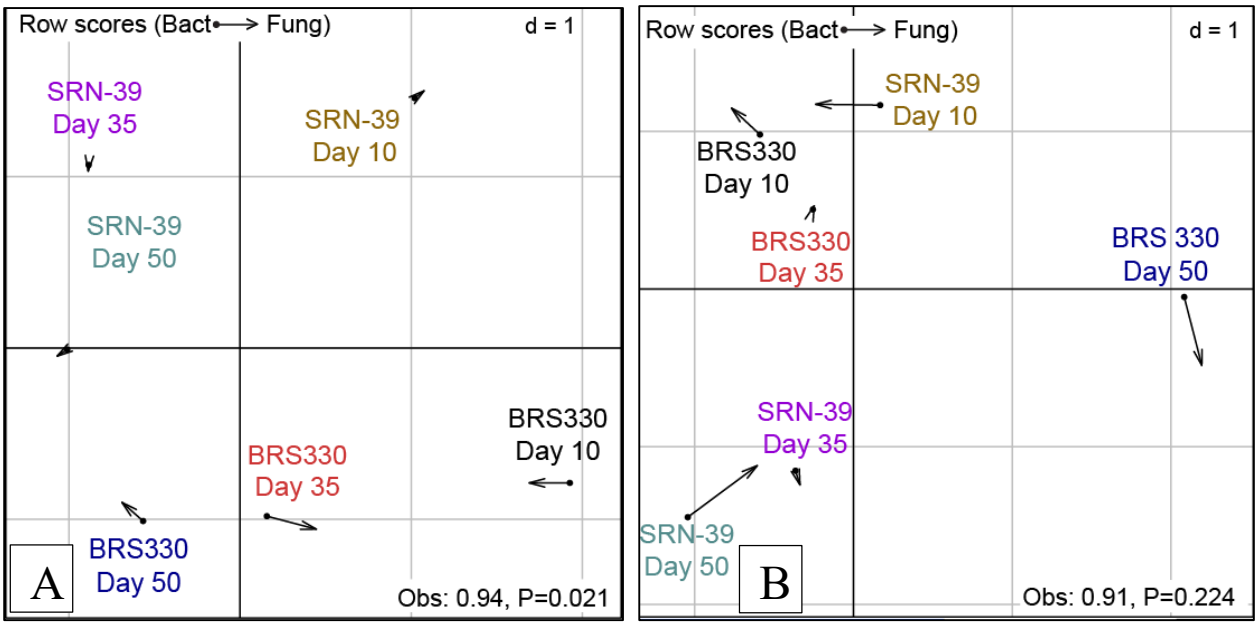

Figure 6. Co-inertia Analysis (COIA) of bacterial and fungal communities in (A) Clue field and (B) Vredepeel soils. Arrows represent the co-variation of both communities within the treatments: cultivar BRS 330 and SRN-39 at days 10, 35 and 50 of plant growth stage.

Alpha diversity

For bacteria community, Tukey tests applied to all alpha diversity indices (number of OTUs, Chao1 and Shannon (H')) showed no significant differences between VD and CF bulk soils ($p>0.05$). The rhizosphere bacterial community of cultivar SRN-39 at day 10 had significant lower number of OTUs and lower diversity (Shannon H') in CF than in VD soil. No significant difference in Shannon diversity, Chao1 or number of OTUs was found comparing bulk soil and rhizosphere in CF soil. The rhizosphere community of both cultivars grown in VD soil, at each growth stage, showed higher bacterial diversity and number of OTUs than in bulk soil. However, for both cultivars planted in VD soil, no difference was found among the OTUs and diversity of rhizosphere bacterial community throughout sampling time. For both cultivars planted in CF soil, the richness of the rhizosphere

bacterial community was not different from that of bulk soil. In VD soil, the richness (Chao1) in the rhizospheres of cultivars BRS330, at day 10 and SRN-39 at day 50 was significantly higher than the bulk soil, whereas no significant difference was evidenced among rhizosphere treatments (Table S7). For fungal community, no difference in alpha diversity was found (Table S8).

Discussion

Our first hypothesis that fungal-bacterial interaction in the sorghum rhizosphere is modulated by the tripartite factors: plant genotype, soil type, and plant growth stage is accepted. Our results showed that for both bacterial and fungal communities, soil plays the major role in their assembly in sorghum rhizosphere. Although bacterial and fungal community structures showed the same trend regarding to the influence of soil, growth stage and sorghum cultivar, fungal communities showed to be more influenced by plant growth stage than bacterial communities. Similarly, Han *et al.* (2017) found plant growth stage a dominant factor determining the structure of the fungal community as compared to edaphic factors in the soybean rhizosphere. We suggest that the fungal community composition was more affected by plant growth stage than the bacterial community composition as the result of the versatility that fungi can interact with plants in different stages of plant development, acting as pathogens, symbionts and saprotrophs (Pasqualini *et al.*, 2007, van der Wal *et al.*, 2015, Haack *et al.*, 2016). Moreover, plants release different exudates of different chemical structure complexities during different growth stages (Berg & Smalla, 2009), which may have larger effects on fungi in the rhizosphere than on bacteria.

The influence of plant growth stage on the fungal rhizosphere community is evidenced by the significant higher relative abundance of *Nectriaceae* at day 10 (38.8%) compared with day 35 (18%) and 50 (12%) in the CF soil. *Nectriaceae* showed to have the highest relative abundance (21%) among fungal families, all belonging to the *Gibberella* genus (Figure S6). Similar results were found by Grudzinska-Sterno *et al.* (2016) in wheat growth stages that *Gibberella avenacea* significantly decreased, at least 4 times fold, from young to mature plants. All *Gibberella* species are sexual stages of *Fusarium* species (Desjardins, 2003), which genus contains many plant pathogens and mycotoxin producers, being of great agricultural and economical importance (Karlsson *et al.*, 2016).

At CF soil, the bacterial families of *Sphingobacteriaceae* and *Oxalobacteraceae* decreased significantly in time. Corroborating with our findings, Green *et al.* (2006) studying the bacterial community composition of cucumber root observed a decrease in abundance of *Oxalobacteraceae* from early to late plant growth stage. The second hypothesis that fungal and bacterial rhizosphere

communities composition are modulated by changes in each other's abundances is also accepted. Although the relationship in the observed abundances of *Sphingobacteriaceae* and *Oxalobacteraceae* bacteria and *Gibberella* fungi was not experimentally assessed, we suggest that there may be some link between these organisms, as both bacterial families are known to be antagonist to fungal activity. *Oxalobacteraceae* were reported to have antifungal, chitinolytic and mycophagous characteristics, being suppressive toward fungi plant pathogens including *Fusarium* species, (de Boer *et al.*, 2004, Cretoiu *et al.*, 2013, Haack *et al.*, 2016). Moreover, *Fusarium* species are known to produce oxalic acid (Amaral *et al.*, 2017), that may have attracted members of *Oxalobacteraceae* that are characterized for their ability to degrade oxalate (Sahin *et al.*, 2009, Miller & Dearing, 2013).

Although the effect of plant growth on the dissimilarity of fungal community was evidenced for both soils, this effect was stronger in CF than VD soil. Furthermore, bacterial and fungal communities showed significant variation between each other at CF soil, whereas no difference was found in VD soil. We hypothesise that influence of CF soil on microbial community variation is linked with low soil fertility. The fertility of CF measured by the sum of bases, was less than half of that of VD soil (Schlemper *et al.*, 2017). Additionally, at CF soil the co-variance of bacterial and fungal communities of the rhizosphere of cultivar SRN-39 was higher than at cultivar BRS330 for all plant growth stages. Although cultivar had smaller effects on the selection of bacterial and fungal communities, it may play an important role in the interaction of both microbial communities. However, given the relative small effects of cultivars and growth stages on rhizosphere microbial community composition we conclude that the effects of growth stage and cultivar differences on microbial community composition were soil dependent.

The initial community (bulk soil) either for bacterial or fungal community did not differ between both soils regarding α and β-diversity. However, soils showed to have different microbial community β-diversity composition at the rhizosphere compartment. We speculate that this difference may be linked with the variation on carbon inputs released by plants to the rhizosphere depending on soil characteristics (Baudoin *et al.*, 2003, Badri & Vivanco, 2009). The fungal diversity did not differ among treatments for the both soils.

The results revealed in this work lead us to the conclusion that fungal and bacterial communities varied with each other in sorghum rhizosphere. The strength of this co-variance is dependent of soil, plant growth stage, plant genotype, and microbial composition. Although cultivar effect was not the major responsible for bacterial and fungal community composition, cultivar SRN-39 showed to promote a stronger co-variation between bacterial and fungal communities.

Acknowledgments

The authors acknowledge Agata Pijl for laboratory assistance, Mattias de Hollander and Marcio Leite for bioinformatics and statistics support, and Dr. Francisco de Souza and Prof. Harro Bouwmeester for providing sorghum seeds. This work was supported by Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES: 1549-13-8) and The Netherlands Organization for Scientific Research (NWO, 729.004.003). Publication number 6412 of the NIOO-KNAW, Netherlands Institute of Ecology.

Supplementary Materials

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

Parameter	Unit	Soils	
		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. *Data were derived from * Schlemper et al. [10].

Table S2. Inertia co-variance between the factors soil type, growth stage and cultivar for the rhizosphere bacterial community

	Soil type	Growth stage	Cultivar	Bacteria
Soil type	100.00%			
Growth stage	0.00%	100.00%		
Cultivar	0.00%	0.00%	100.00%	
Bacteria	**52.62%**	**22.70%**	**12.73%**	100.00%

Table S3. Inertia co-variance between the factors soil type, growth stage and cultivar for the rhizosphere fungal community

	Soil type	Growth stage	Cultivar	Fungi
Soil type	100.00%			
Growth stage	0.00%	100.00%		
Cultivar	0.00%	0.00%	100.00%	
Fungi	**42.83%**	**26.02%**	**14.99%**	100.00%

Table S4. Permutational Multivariate Analysis of Variance (Adonis) using Bray-Curtis distance matrix for testing the factors soil, plant growth stage and cultivar in rhizosphere bacterial and fungal community

Organism	**Factor**	**Df**	**Sum of Squares**	**Mean of Squares**	**F**	**R2**	**P**
Bacteria	Soil	**1**	**1.16**	**1.16**	**6.87**	**0.17**	**0.001**
	Growth stage	2	0.45	0.23	1.15	0.07	0.18
	Cultivar	1	0.23	0.23	1.16	0.03	0.197
Fungi	Soil	**1**	**0.92**	**0.92**	**7.89**	**0.19**	**0.001**
	Growth stage	**2**	**0.69**	**0.34**	**2.68**	**0.14**	**0.003**
	Cultivar	1	0.16	0.16	1.16	0.03	0.302

Table S5. List of taxonomic groups of bacteria and fungi in rhizosphere that contributed to co-variation in Clue field soil

Community	**Taxonomical group**			
	Phylum	**Class**	**Order**	**Family**
Bacteria	Bacteroidetes	Sphingobacteriia	*Sphingobacteriales*	*Sphingobacteriaceae*
	Proteobacteria	Alphaproteobacteria	*Rhodospirillales*	*Acetobacteraceae*
		Betaproteobacteria	*Methylophilales*	*Methylophilaceae*
			Burkholderiales	*Oxalobacteraceae*
				Alcaligenaceae
	Firmicutes	Bacilli	*Bacillales*	*Planococcaceae*
			Lactobacillales	*Lactobacillaceae*
Fungi	Ascomycota	Dothideomycetes	*Capnodiales*	unc_Capnodiales
		uncl_Pezizomycotina	uncl_Pezizomycotina	unc_Pezizomycotina
		Sordariomycetes	*Hypocreales*	*Nectriaceae*
				Hypocreaceae
	Basidiomycota	Tremellomycetes	*Tremellales*	unc_*Tremellales*
	Cryptomycota	uncl_LKM11	uncl_LKM11	unc_LKM11
	Glomeromycota	Glomeromycetes	*Glomerales*	unc_*Glomerales*

Table S6. List of taxonomic groups of rhizosphere bacteria and fungi that contributed to co-variation in Vredepeel soil

Community	Taxonomical group			
	Phylum	**Class**	**Order**	**Family**
Bacteria	Acidobacteria	Acidobacteria_Gp4	unc_Acidobacteria_Gp4	unc_Acidobacteria_Gp4
		Acidobacteria_Gp6	unc_Acidobacteria_Gp6	unc_Acidobacteria_Gp6
	Actinobacteria	Actinobacteria	*Actinomycetales*	*Microbacteriaceae*
	Bacteroidetes	Sphingobacteriia	*Sphingobacteriales*	*Chitinophagaceae*
	Proteobacteria	Alphaproteobacteria	unc_Alphaproteobacteria	unc_Alphaproteobacteria
			Rhizobiales	*Bradyrhizobiaceae*
				unc_*Rhizobiales*
		Betaproteobacteria	*Burkholderiales*	*Burkholderiaceae*
		Deltaproteobacteria	*Myxococcales*	*Polyangiaceae*
				unc_*Myxococcales*
		Gammaproteobacteria	*Xanthomonadales*	*Xanthomonadaceae*
		unc_Proteobacteria	unc_Proteobacteria	unc_Proteobacteria
	Firmicutes	Bacilli	*Bacillales*	*Alicyclobacillaceae*
	Verrucomicrobia	Opitutae	*Opitutales*	*Opitutaceae*
Fungi	Ascomycota	Dothideomycetes	*Pleosporales*	*Pleosporaceae*
		Saccharomycetes	*Saccharomycetales*	Saccharomycetales I.S.
		Pezizomycetes	*Pezizales*	unc_*Pezizales*
		Sordariomycetes	*Diaporthales*	unc_*Diaporthales*
			Hypocreales	unc_*Hypocreales*
	Basidiomycota	Agaricomycetes	*Auriculariales*	*Auriculariaceae*
	Chytridiomycota	Chytridiomycetes	*Spizellomycetales*	unc_*Spizellomycetales*
	Glomeromycota	Glomeromycetes	*Paraglomerales*	*Paraglomeraceae*
			Glomerales	*Glomeracea*
				unc_*Glomerales*
	Zygomycota	Mucoromycotina_I.S.	*Mucorales*	unc_*Mucorales*

Table S7. Diversity of soil bacteria samples comprising estimators of diversity (Shannon), Number of Operation Taxonomic Units (OTU's) and estimators of richness (Chao1) given by environmental DNA in two cultivars (BRS330 andSRN-39); in three different plant growth stages (days 10, 35 and 50) in bulk and rhizosphere compartments of Clue Field (CF) and Vredepeel (VD) soil

α-diversity index	Soil	Soil compartments				
		Bulk soil	Rhizosphere			
			Cultivars	Day 10	Day 35	Day 50
Shannon						
	CF	8.5 ± 0.1 Aab	BRS330	8.8 ± 0.1 Aab	8.9 ± 0 Aa	8.9 ± 0.1 Aa
			SRN-39	8.1 ± 0.5 Ab	9 ± 0.1 Aa	9 ± 0.1 Aa
	VD	8.6 ± 0.3 Ab	BRS330	9.3 ± 0.1 Aa	9.2 ± 0.1 Aa	9.2 ± 0 Aa
			SRN-39	9.3 ± 0.1 Ba	9.2 ± 0.1 Aa	9.4 ± 0 Aa
OTUs						
	CF	776.7 ± 31.6 Aab	BRS330	815.8 ± 11.7 Aab	867.3 ± 11.1 Aa	869.8 ± 27.6 Aa
			SRN-39	716 ± 74.4 Ab	861.8 ± 16.8 Aa	869.8 ± 16.5 Aa
	VD	770.6 ± 82.4 Ab	BRS330	957 ± 19.7 Aa	936.1 ± 13 Aa	916.9 ± 16.2 Aa
			SRN-39	942.6 ± 49.3 Ba	923 ± 33.6 Aa	966.5 ± 2.3 Aa
Chao1						
	CF	1637.5 ± 100 Aa	BRS330	1712.9 ± 18.5 Aa	1807.2 ± 31.1 Aa	1816.5 ± 51.5 Aa
			SRN-39	1542.9 ± 98.8 Aa	1784.6 ± 45.7 Aa	1786 ± 54.4 Aa
	VD	1594.6 ± 193.8 Ab	BRS330	2012.7 ± 54.8 Aa	1923.4 ± 41.8 Aab	1867.1 ± 37.8 Aab
			SRN-39	1894.2 ± 116 Aab	1933.9 ± 60.3 Aab	2001.6 ± 18.4 Aa

The values are means of replicates (n=3) ± (SE). For each α-diversity index capital letters compare (on column) the means between the soils within the same soil compartment, cultivar, and time point. Lowercase letters compare (within the same soil), the means either between soil compartments, cultivars within (on column) stages of plant growth or the same cultivar (on row) over different growth stages. Means followed by the same letter are not statistically different by Tukey test ($P<0.05$). The sequences were rarefied by 2.000 reads prior the analysis.

Table S8. Diversity calculation of soil fungi samples comprising estimators of diversity (Shannon), Number of Operation Taxonomic Units (OTU's) and estimators of richness (Chao1) given by environmental DNA in two cultivars (BRS330 and SRN-39); in three different plant growth stages (days 10, 35 and 50) in Bulk and in rhizosphere compartments of Clue Field (CF) and Vredepeel (VD) soil

α-diversity index	Soil	Bulk soil	Rhizosphere			
			Cultivars	Day 10	Day 35	Day 50
Shannon						
	CF	5.2 ± 0.1 Aa	BRS330	4.2 ± 0.1 Aa	5.1 ± 0.3 Aa	5.5 ± 0.1 Aa
			SRN-39	4.2 ± 0.4 Aa	4.9 ± 0.2 Aa	5.0 ± 0.1 Aa
	VD	5.2 ± 0.2 Aa	BRS330	4.4 ± 0.6 Aa	5.3 ± 0.1 Aa	5.5 ± 0.1 Aa
			SRN-39	5.2 ± 0.2 Aa	4.9 ± 0.3 Aa	5.4 ± 0 Aa
OTUs						
	CF	96.3 ± 2.8 Aa	BRS330	76.1 ± 3.8 Aa	99.7 ± 8 Aa	101 ± 2.8 Aa
			SRN-39	80.1 ± 7.2 Aa	85 ± 6.6 Aa	88.5 ± 3.3 Aa
	VD	90 ± 2.4 Aa	BRS330	75.2 ± 8.3 Aa	87.6 ± 4.9 Aa	100.9 ± 2.1 Aa
			SRN-39	91.1 ± 4.3 Aa	83 ± 6.6 Aa	95.3 ± 2.1 Aa
Chao1						
	CF	146.4 ± 2.3 Aa	BRS330	139.3 ± 14.2 Aa	170.56 ± 17.5 Aa	159.9 ± 1.8 Aa
			SRN-39	142 ± 13.2 Aa	139.2 ± 17.7 Aa	148.3 ± 7.6 Aa
	VD	157.8 ± 11.6 Aa	BRS330	119.1 ± 14 Aa	127.3 ± 4.8 Aa	166.2 ± 8.6 Aa
			SRN-39	147.9± 3.3 Aa	141.4 ± 7.6 Aa	154.5 ± 8.9 Aa

The values are means of replicates (n=3) ± (SE). For each α-diversity index capital letters compare (on column) the means between the soils within the same soil compartment, cultivar, and time point. Lowercase letters compare (within the same soil), the means either between soil compartments, cultivars within (on column) stages of plant growth or the same cultivar (on row) over different growth stages. Means followed by the same letter are not statistically different by Tukey test ($P<0.05$). The sequences were rarefied by 550 reads prior the analysis.

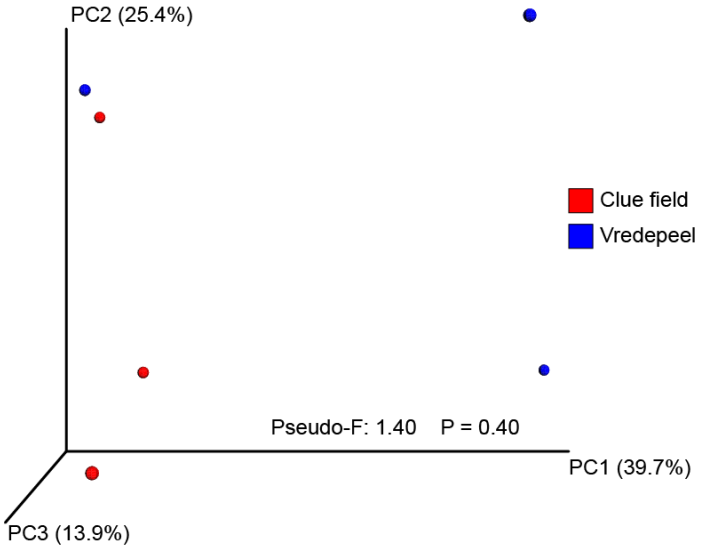

Figure S1. Principal Coordinate Analysis (PCoA) indicating the dissimilarity between bacterial community present in bulk soil of Clue field and Vredepeel soils.

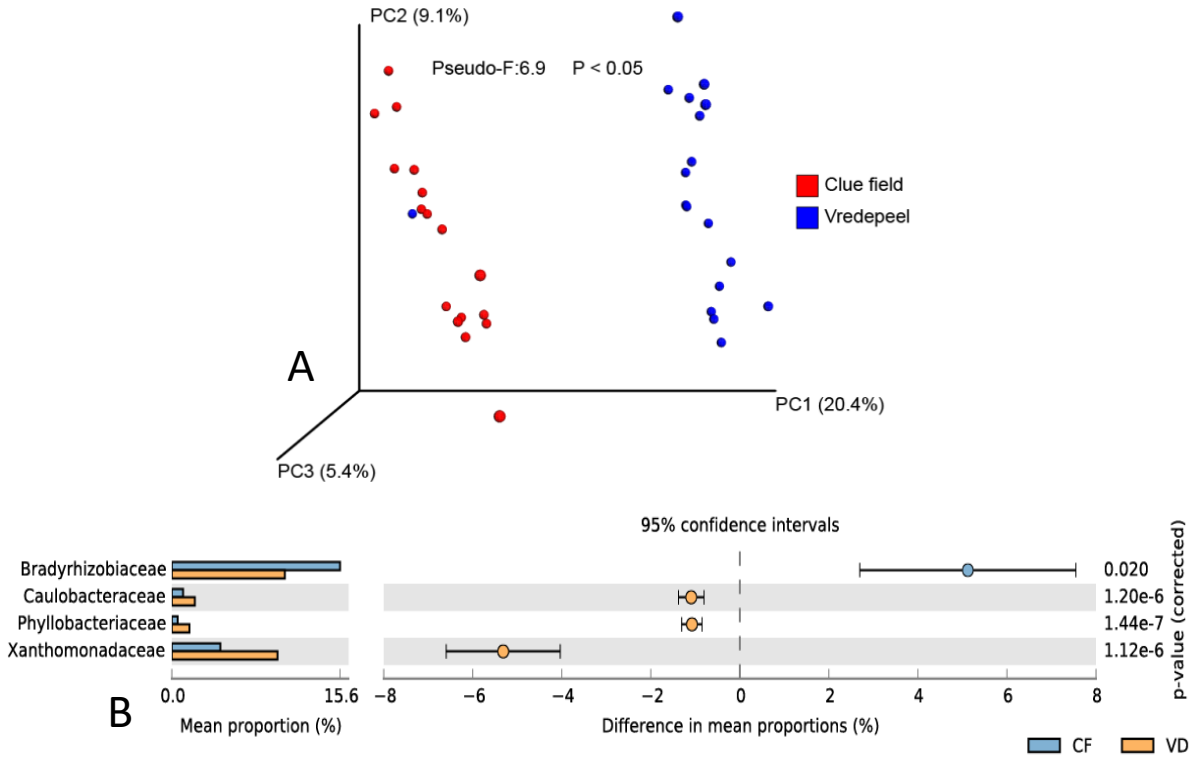

Figure S2. (A) Principal Coordinate Analysis (PCoA) and (B) differences in relative abundance of bacterial families between Clue field and Vredepeel rhizosphere samples (Welch's test; P<0.05).

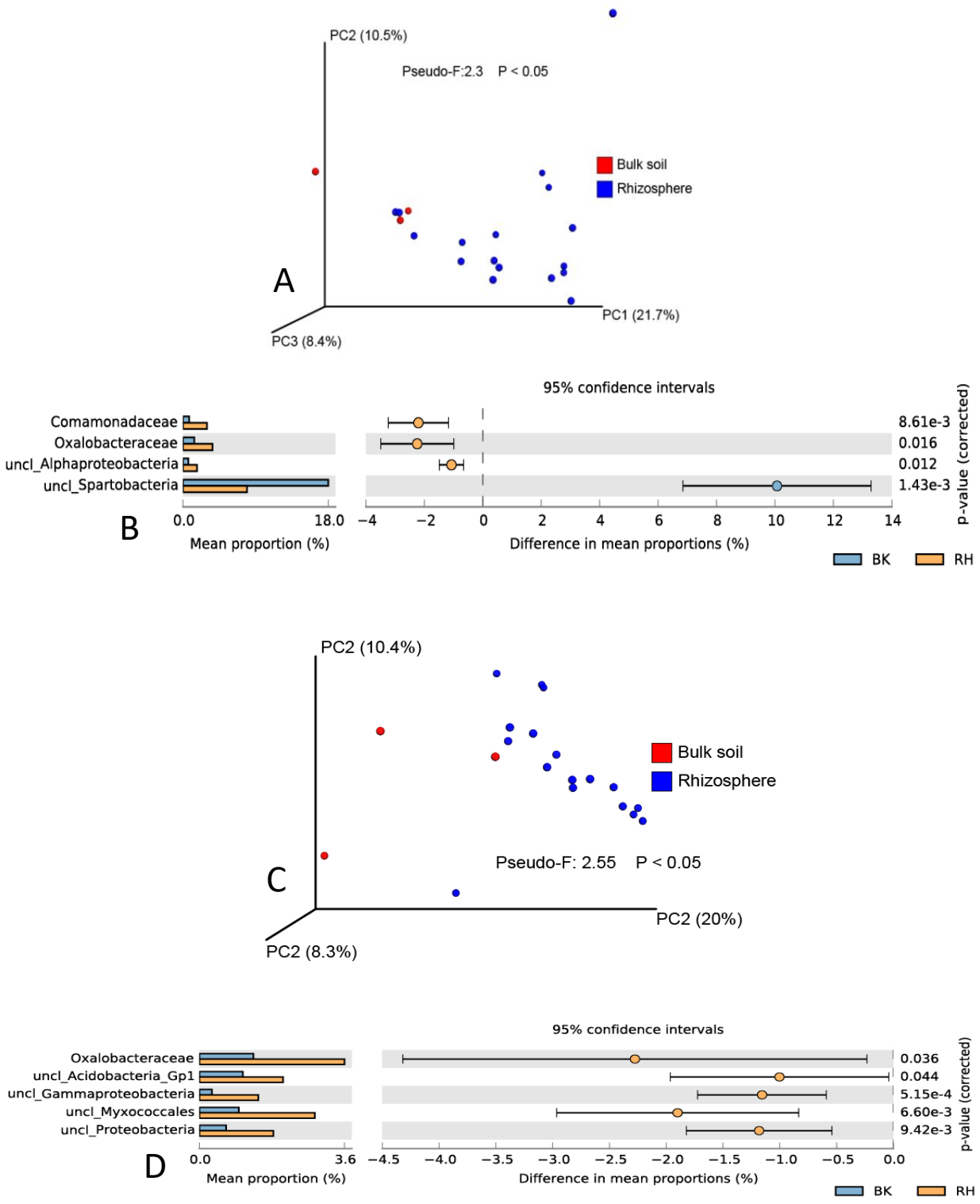

Figure S3. (A) Principal Coordinate Analysis (PCoA) and (B) differences in relative abundance of bacterial families between bulk soil and rhizosphere samples of Clue field (Welch's test; P<0.05). (C) Principal Coordinate Analysis (PCoA) and (D) differences in relative abundance of bacterial families between bulk soil and rhizosphere samples of Vredepeel soil (Welch's test; P<0.05).

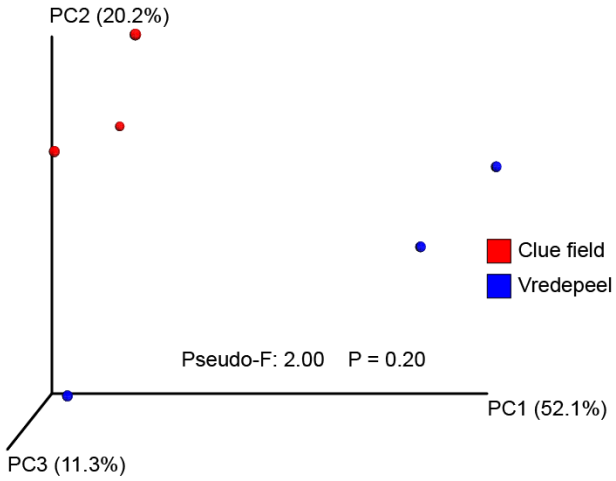

Figure S4. Principal Coordinate Analysis (PCoA) representing the dissimilarity between fungal communities present in bulk soil of Clue field and Vredepeel soils.

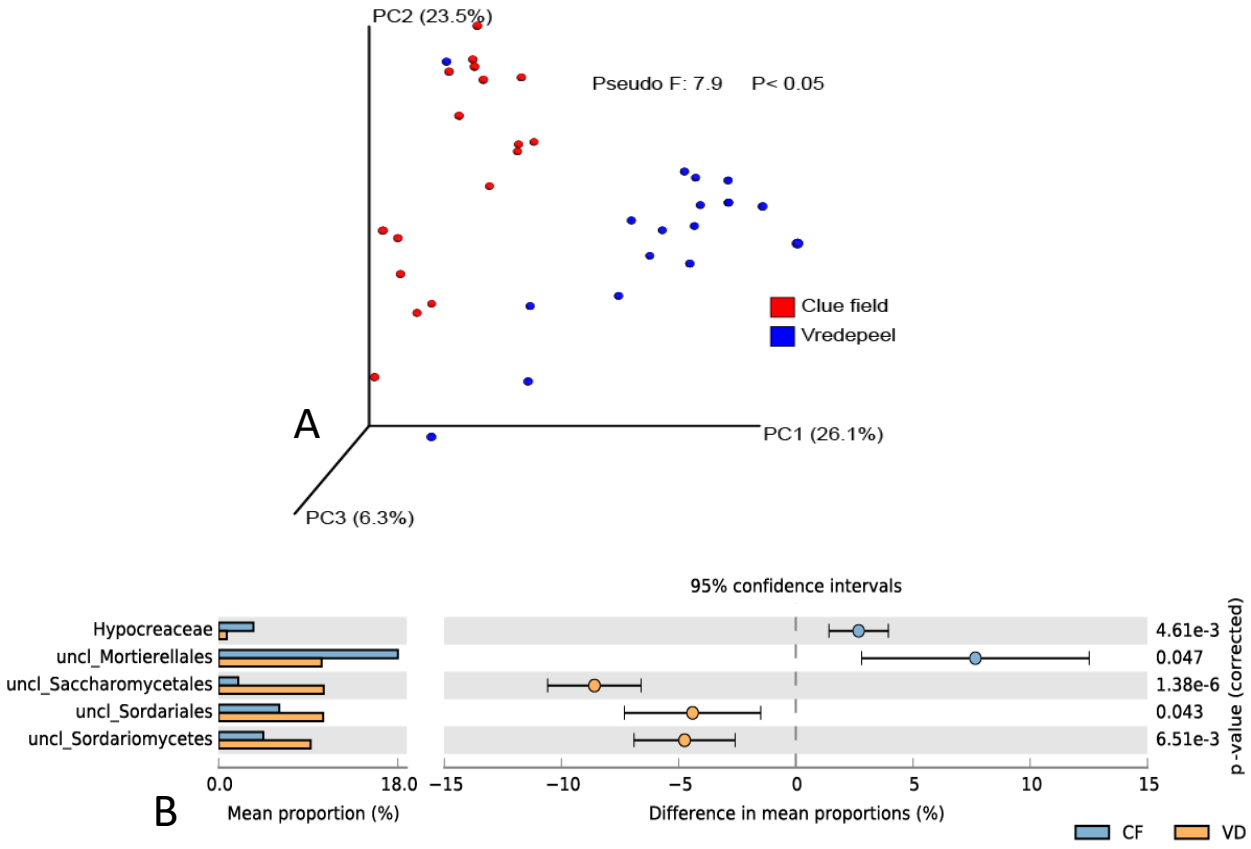

Figure S5. (A) Principal Coordinate Analysis (PCoA) and (B) differences in relative abundance of fungal families between Clue field and Vredepeel rhizosphere samples (Welch's test; P<0.05).

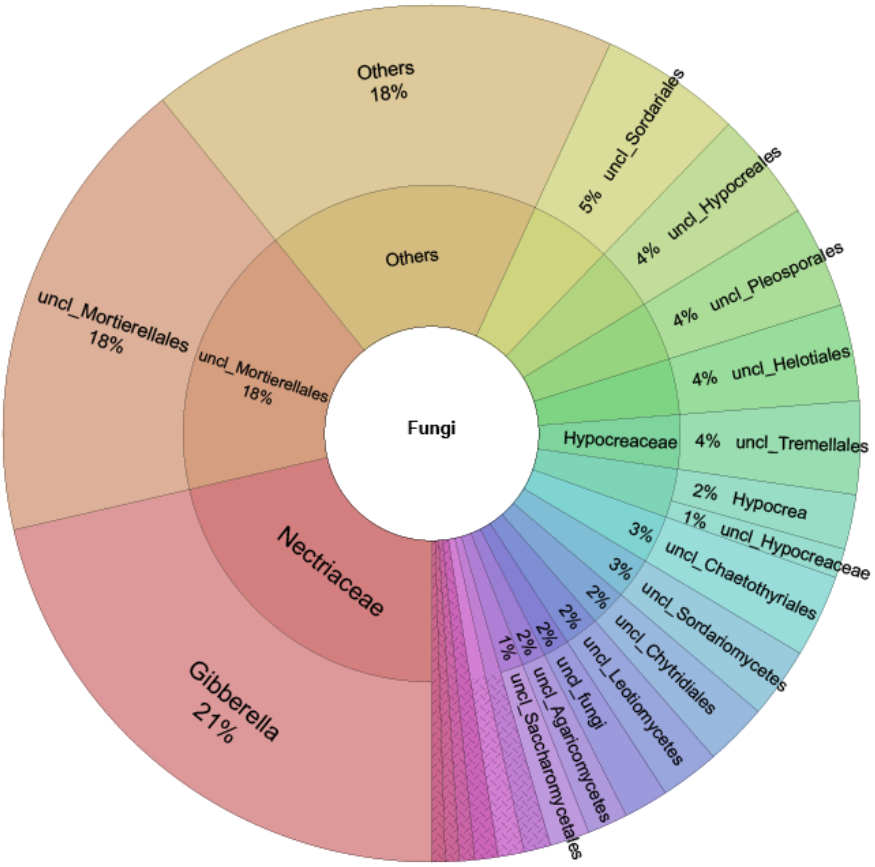

Figure S6. Relative abundance of rhizosphere fungi in Clue field soil.

Chapter 4

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

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(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.

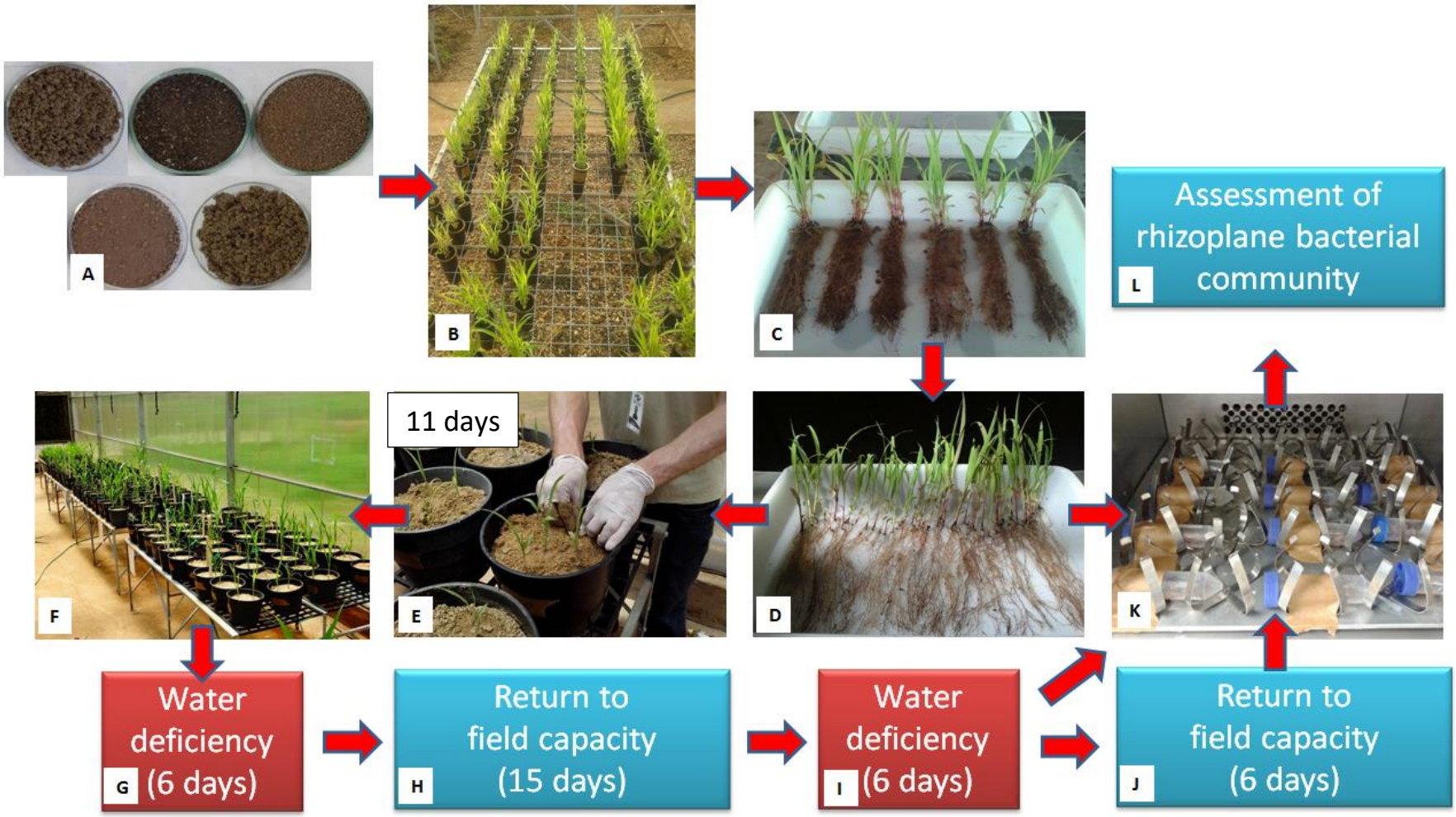

Figure 1. Experimental design. (A) different soils: i - Sterilized sand plus vermiculite (Control); ii - Calcareous; iii - Cerrado; iv - Sorghum field; and v – Enriched microbial substrate; (B) two sorghum linages (drought susceptible "9618158" and drought tolerant "9910032") planted in different soils; (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.

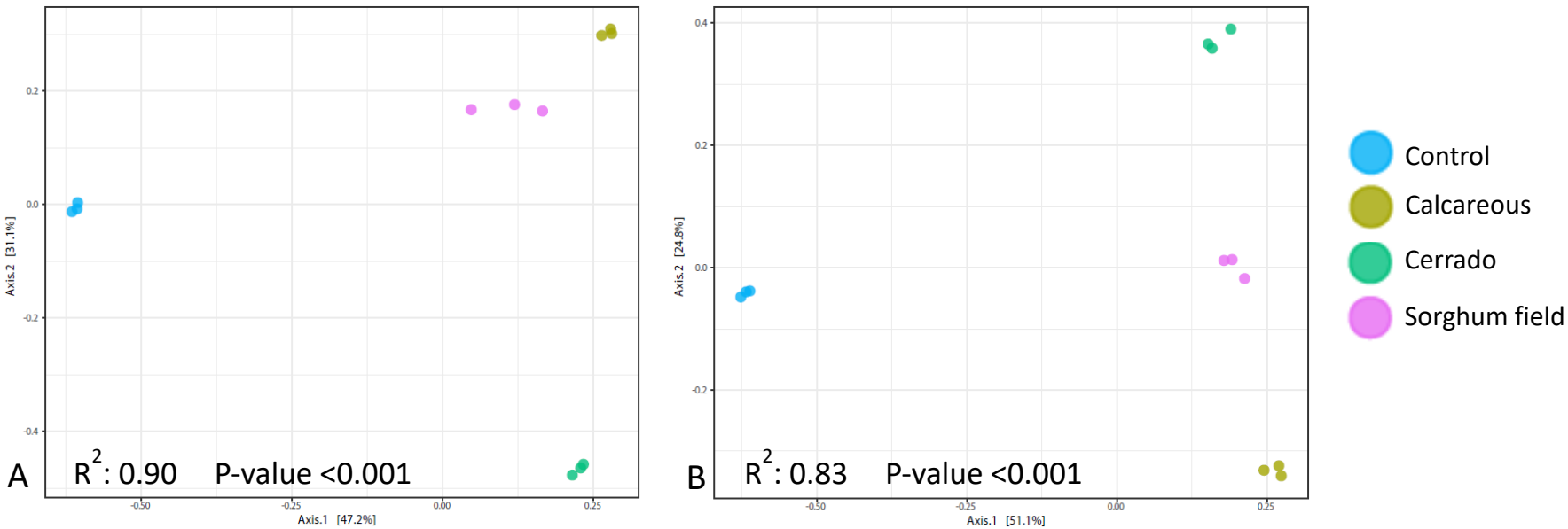

Figure 2. Principal Coordinate Analysis (PCoA) representing the dissimilarities in rhizoplane bacterial communities of two sorghum lineages (A) drought susceptible "9618158" and (B) drought tolerant "9910032" grown on different soils (Control, Calcareous, Cerrado and Sorghum field) for 21 days.

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).

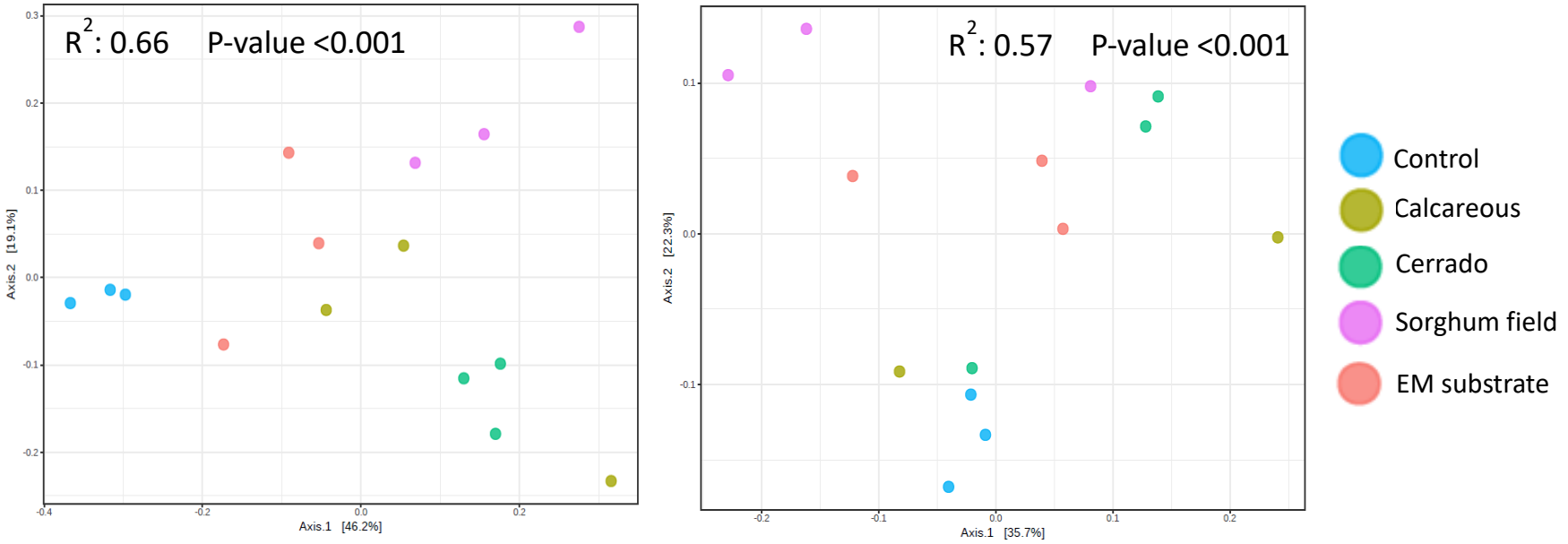

Figure 3. Principal Coordinate Analysis (PCoA) representing the dissimilarities of bacterial communities of two sorghum lineages (A) drought susceptible "9618158" and (B) drought tolerant "9910032" grown on different soils (Control, Calcareous, Cerrado, Sorghum field, and Enriched microbial – EM substrate) 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.

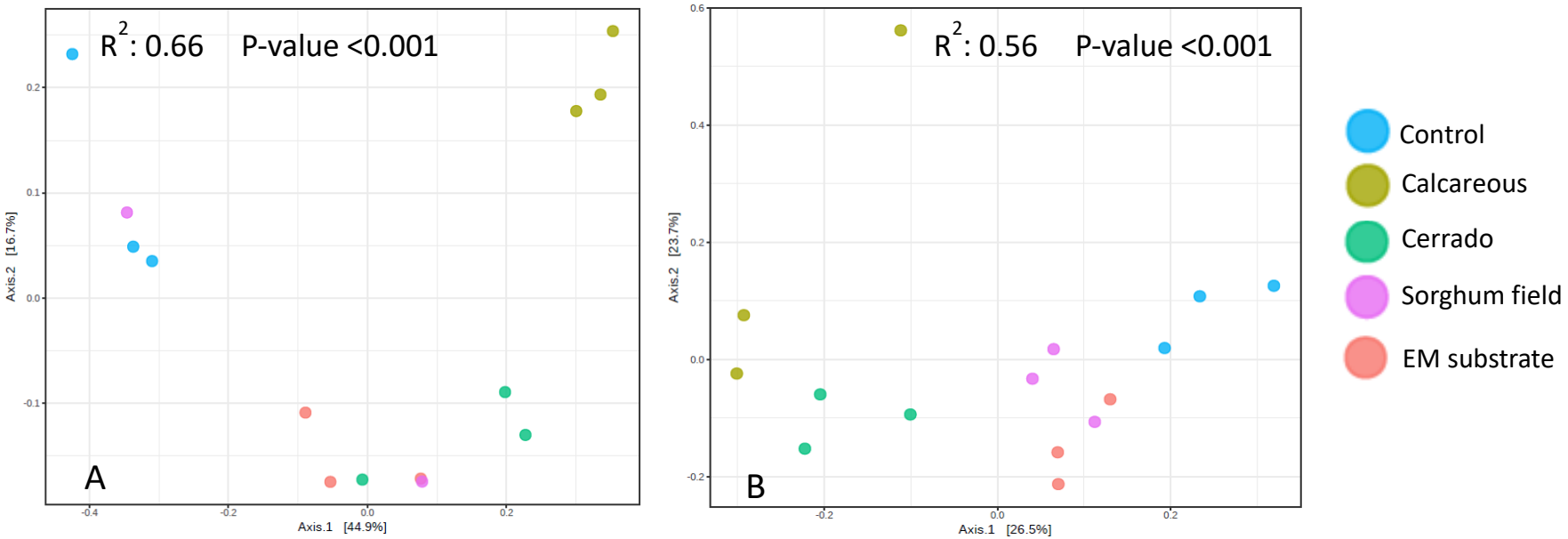

Figure 4. Principal Coordinate Analysis (PCoA) representing the dissimilarities of bacterial communities of two sorghum lineages (A) drought susceptible "9618158" and (B) drought tolerant "9910032" grown on different soils (Control, Calcareous, Cerrado, Sorghum field, and Enriched microbial – EM substrate) 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.

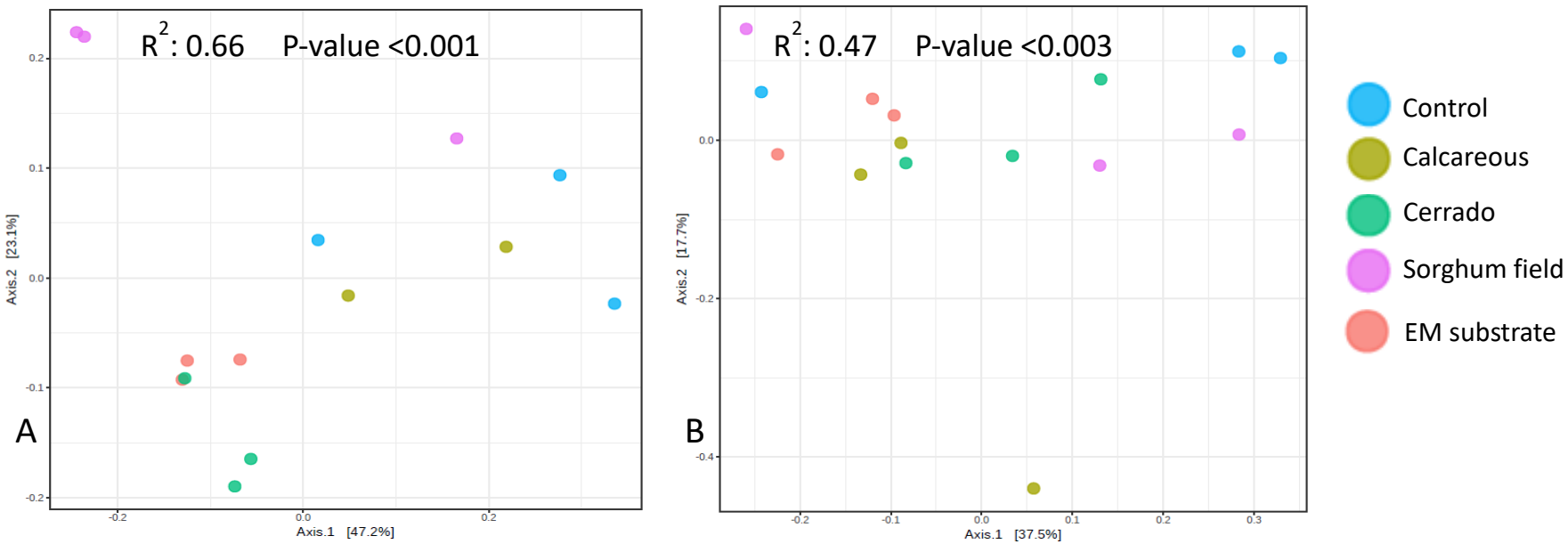

Figure 5. Principal Coordinate Analysis (PCoA) representing the dissimilarities of bacterial communities of two sorghum lineages (A) drought susceptible "9618158" and (B) drought tolerant "9910032" planted on different soils (Control, Calcareous, Cerrado, Sorghum field, and Enriched microbial – EM substrate) 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.

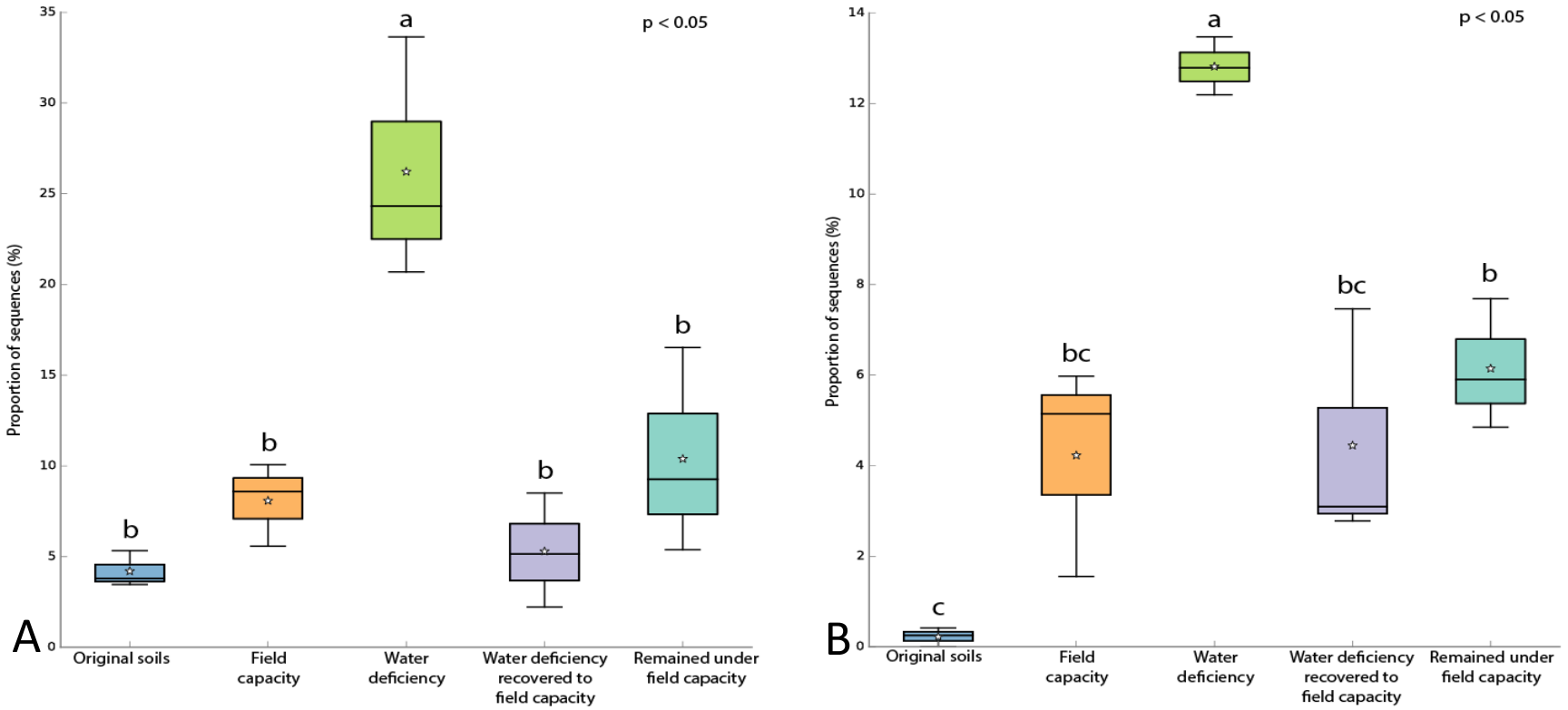

Figure 6. Box plots represent the mean of the relative abundance (n=3) of *Rhizobiaceae* in the rhizoplane of drought tolerant sorghum lineage "9910032" originally cropped at (A) Sorghum field and (B) Cerrado soils. The bacteria were assessed in different conditions: from the rhizoplane of plants planted 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

Chapter 5

Effect of *Burkholderia tropica* and *Herbaspirillum frisingense* strains on *Sorghum* growth is plant genotype dependent

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Abstract

Sorghum is a multipurpose crop that is cultivated worldwide. Plant growth-promoting bacteria (PGPB) have important role in enhancing sorghum biomass and nutrient uptake and suppressing plant pathogens. The aim of this research was to test the effects of the endophytic bacterial species *Kosakonia radicincitans* strain IAC/BECa 99, *Enterobacter asburiae* strain IAC/BECa 128, *Pseudomonas fluorescens* strain IAC/BECa 141, *Burkholderia tropica* strain IAC/BECa 135 and *Herbaspirillum frisingense* strain IAC/BECa 152 on the growth and root architecture of four sorghum cultivars (SRN-39, Shanqui-Red, BRS330, BRS509), with different uses and strigolactone profiles. We hypothesized that the different bacterial species would trigger different growth plant responses in different sorghum cultivars. *Burkholderia tropica* and *H. frisingense* significantly increased the plant biomass of cultivars SRN-39 and BRS330. Moreover, cultivar BRS330 inoculated with either strain displayed a significant decrease in average root diameter. This study shows that *B. tropica* strain IAC/BECa 135 and *H. frisingense* strain IAC/BECa 152 are promising PGPB strains for use as inocula for sustainable sorghum cultivation.

Introduction

Sorghum (*Sorghum bicolor*) is a worldwide-cultivated plant originated in the African continent and later introduced in different parts of the world (Rao *et al.*, 2014). Sorghum has a short growth period, and is therefore a preferred cereal in arid and semi-arid regions (Farre & Faci, 2006, Wu *et al.*, 2010, Funnell-Harris *et al.*, 2013). In Africa, sorghum is mainly cultivated by small farmers as staple food and for beverage production (Haiyambo *et al.*, 2015). By contrast, sorghum is mostly used for the feed market in North and Central America, and for animal feedstock, ethanol production, and soil coverage in South America (Dutra *et al.*, 2013, Perazzo *et al.*, 2013, Damasceno *et al.*, 2014, Rao *et al.*, 2014). Sorghum is currently the 5th most cultivated cereal worldwide (Ramu *et al.*, 2013) and in 2014, approximately 71 million tons of sorghum grains were produced around the world (FAO, 2017).

Sorghum producers often face yield problems due to the soil nutrient deficits, limited access to chemical fertilizers, and the frequent need to combat plant pathogens (Haiyambo *et al.*, 2015). Although conventional agricultural methods, such as chemical fertilization and pesticide application, can be used to overcome these limitations, the environmental side effects of these practices may be unsustainable. As an alternative, the use of plant growth-promoting bacteria (PGPB) as biofertilizers, not only enhances plant biomass and nutrient uptake but also improves pathogen control (Bhattacharyya & Jha, 2012, Dawwam *et al.*, 2013). PGPB can alter the root architecture and promote plant growth by directly facilitating nutrient acquisition or modulating plant hormone levels or by indirectly inhibiting pathogenic organisms (Bhattacharyya & Jha, 2012, Glick, 2012). The best-known processes of plant nutrient acquisition mediated by PGPB are nitrogen fixation, phosphate (P) solubilisation and iron sequestration (Lucy *et al.*, 2004). Different groups of bacteria produce plant growth regulators, such as cytokinins, gibberellins, indole acetic acid (IAA), and ethylene, that may also affect the plant's hormonal balance (Amara *et al.*, 2015). Moreover, PGPB can promote plant growth by fixing N_2 and inhibiting plant pathogens by producing antibiotics or lytic enzymes or competing for resources, which can limit disease incidence and severity (Glick, 2012, da Silveira *et al.*, 2016). In addition to the bacterial modification of plant metabolism, plant exudates have the potential to modify rhizosphere microbial community assembly and interactions (Haichar *et al.*, 2014, Vurukonda *et al.*, 2016). Recent studies suggest that the plant hormone strigolactone (SL) plays an important role in plant rhizosphere bacterial community composition (Funnell-Harris *et al.*, 2008, Schlemper *et al.*, 2017). Furthermore, Peláez-Vico *et al.* (2016) showed that the PGPB *Sinorhizobium meliloti* reduces orobanchol and orobanchyl acetate levels in nodulated alfalfa plants under P

starvation, suggesting a role of SL in rhizobial-legume interactions. Peláez-Vico *et al.* (2016) demonstrated that swarming motility of *S. meliloti* is triggered by the synthetic SL analogue GR24.

Many aspects of the interaction between PGPB and plants have been addressed for a wide range of plant species. Specifically in sorghum, some studies have focused on the interaction of PGPB strains isolated from third-party host species as inoculants for the sorghum rhizosphere (Matiru & Dakora, 2004, Dos Santos *et al.*, 2017), whereas other works have reported the inoculation in other plant species of bacterial strains isolated from sorghum.

Matching beneficial bacteria with their preferred crops might optimize root colonization and biocontrol (Raaijmakers & Weller, 2001), especially when different plants are cropped in soils with the same bacterial composition. In this context, it is extremely important to identify bacterial candidates that have similar growth effects on plants that share the same soil. In Brazil, sorghum have been planted during the sugarcane off season as well as in former sugarcane fields (May *et al.*, 2013), and therefore frequently exposed to the same soil. Endophytic bacteria with plant-growth promoting traits isolated from sugarcane have been shown to increase biomass and plant N content when inoculated in plantlets of sugarcane. Govindarajan et al., 2006 observed an increase in sugarcane yield of 20%, while Sevilla et al. (2001) observed increases of 31% in plant dry matter, 43% in N accumulation, and 25% in productivity in two sugarcane varieties. We hypothesized that different bacterial species isolated from sugarcane will trigger different growth plant responses in different sorghum cultivars. Thus, to determine if endophytic strains characterized as PGPB in sugarcane can act as non-host-specific PGPB benefiting sorghum performance, we tested the effect of five bacterial strains on the plant biomass and root architecture of four *S. bicolor* cultivars with different uses and characteristics: SRN-39, an African grain cultivar that produces high amounts of orobanchol; Shanqui-Red (SQR), a Chinese cultivar that produces high amount of 5-deoxystrigol; BRS330, a hybrid grain cultivar from Brazil and BRS509, a hybrid saccharin cultivar from Brazil that produces both orobanchol and sorgomol (Schlemper *et al*., 2017).

Inoculation of the cultivars SRN-39 and BRS330 with *Burkholderia tropica* or *Herbaspirilum frisingense* strains resulted in significant increases in plant biomass. Moreover, cultivar BRS330 exhibited significant decreases in average root diameter when inoculated with either strain. This study shows that *B. tropica* strain IAC/BECa 135 and *H. frisingense* strain IAC/BECa 152 are promising PGPB strains for use as inocula for sustainable sorghum cultivation.

Materials and methods

Bacterial isolates and screening of plant growth promotion traits

Five bacterial endophytic strains isolated from sugarcane stems belonging to the Agronomic Institute of Campinas (IAC) – Brazil culture collection were used for this experiment: *Kosakonia radicincitans* strain IAC/BECa-99 (KF542909.1), *Enterobacter asburiae* strain IAC/BECa-128 (JX155407.1), *Pseudomonas fluorescens* strain IAC/BECa-141 (KJ588202.1), *Burkholderia tropica* strain IAC/BECa-135 (KJ670083.1), and *Herbaspirillum frisingense* strain IAC/BECa-152 (JX155400.1).

Phosphate solubilization test: the strains were cultured on a culture medium containing inorganic phosphate ($CaHPO_4$) according to the method of Katznelson & Bose (1959). The experiment was performed in triplicate for five days. The ability of the bacteria to solubilize calcium phosphate was verified by the formation of clear a halo surrounding the colonies.

Indole-3-acetic acid (IAA) test: the strains were grown in culture medium containing L-tryptophan, the precursor of IAA (Bric *et al.*, 1991), covered with a nitrocellulose membrane, and incubated at 28 °C in the dark for 24 h. The nitrocellulose membranes were immersed in Salkowski's solution and incubated at room temperature for up to three hours. This test was performed using five replicates. The formation of a red-purplish halo around the colonies indicated IAA production.

Siderophore production: siderophore production by the strains was measured using the method of Schwyn & Neilands (1987), in which a dye, chromeazurol S (CAS), is released from a dye-iron complex when a ligand sequesters the iron complex. This release causes a colour change from blue to yellow-orange. In this case the ligant was one or more of the siderophores found in the culture supernatants of the bacterial strains. This measurement was made using five replicates.

Hydrogen cyanide (HCN) test: the production of HCN by all strains was assessed according to Bakker & Schippers (1987). Moistened filter paper with picric acid solution (5%) and Na_2CO_3 (2%) was added to the top of the Petri dishes and incubated at 28°C for 36 h. The experiments were performed in triplicate for each strain, and a colour change of the paper from yellow to orange-red indicated the ability to produce HCN.

Sorghum cultivars

Four sorghum cultivars differing in use, origin, and strigolactone production were chosen for inoculation with the selected PGPB. The cultivars were SRN-39, an African sorghum that produces a high amount of orobanchol; Shanqui-Red (SQR) a Chinese sorghum that produces mostly 5-deoxystrigol; BRS330, a hybrid *S. bicolor* grain from Brazil; and BRS509, a hybrid *S. bicolor* saccharin from Brazil that produces both 5-deoxystrigol and sorgomol.

Mesocosm experiment

The experiment was performed in a greenhouse of the Netherlands Institute of Ecology (NIOO-KNAW), located in Wageningen, The Netherlands. The experiment was carried out from September to October 2016, with a total duration of 30 days. A complete random design was used. The treatments consisted of four sorghum cultivars, each one inoculated with each of the five bacterial strains separately, with a total of six replicates per treatment. A non-inoculated treatment under phosphate starvation conditions was established as a control. Seeds were disinfected as described by Liu *et al.* (2013). Briefly, seeds were soaked in 70% ethanol for 3 minutes, transferred to a new tube containing 2.5% sodium hypochlorite solution and shaken for 5 minutes. The seeds were then washed with 70% ethanol solution for 30 s. Finally, the seeds were rinsed with sterile water four times. After the last washing step, 20 µl of the remaining water was plated on Petri dishes with Luria-Bertani (LB) medium to confirm the success of disinfection. After disinfection, the seeds were placed in Petri dishes containing 1% water agar medium, and the plates were incubated at 25 ºC for 2 days in the dark for seed germination. The experiment is illustrated in Figure 1. When radicle emerged from the seed coat, the seedlings were transplanted from the Petri dishes to 11 x 11 x 12 cm plastic pots filled with autoclaved silver sand as substrate. The pots containing one plant each were maintained under greenhouse conditions for four weeks. During the first week, the pots were watered with ½ Hoagland 10% P nutrient solution, followed by P starvation. To create P starvation conditions, the substrate with plants was first flushed with 500 mL of ½-strength Hoagland nutrient solution without phosphate to remove any remaining phosphate in the substrate by drainage through the pot. After two days, to simulate field conditions, 2 g (125 µM) of insoluble tricalcium phosphate ($Ca_3(PO_4)_2$), which can be solubilized by microorganisms but not taken up directly by the plant (Estrada *et al.*, 2013), was diluted in Hoagland nutrient solution and applied to the pots. The watering regime was maintained by applying 25 ml of nutrient solution every two days.

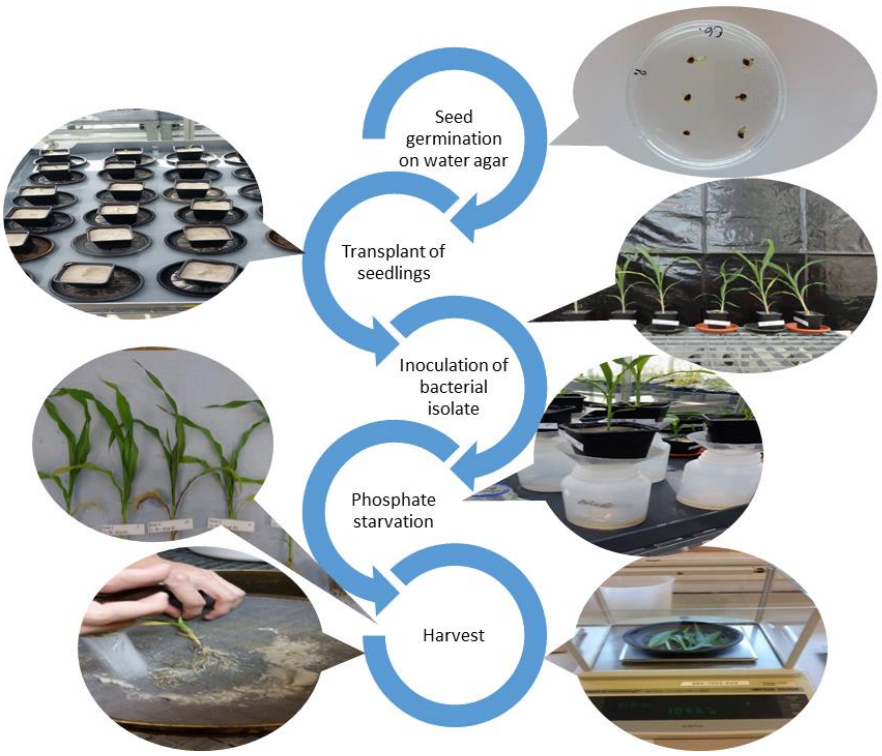

Figure 1. Illustration of the different steps of the study (photos T. Schlemper and F. Silva Gutierrez).

Bacterial inoculation

Bacterial isolates were taken from single colonies, grown in Petri dishes containing Luria-Bertani (LB) medium at 30 ºC for 2-3 days and stored at 4 ºC. Bacterial cells of each strain were then grown overnight at 31 ºC in LB liquid medium and subsequently inoculated again in a fresh LB medium until reaching the desired inoculum density (10^8 cfu ml^{-1}) (Mishra *et al.*, 2016). After transplanting, and during plant growth, bacterial isolates were applied three times on the top of the sandy substrate directly at the location of the seedling roots. The control treatment was inoculation with LB medium without bacteria. The first inoculation was performed on the third day after transplanting, the second on the second day after P starvation, and the last one week later. The inoculation was performed three times to ensure a sufficient bacterial cell density surrounding the plant roots. Loss or dilution of the bacterial inoculum during either the P starvation treatment or the watering regime was possible due to the great drainage potential of the sandy substrate. A density of 10^8 cfu ml^{-1} in a volume of 1ml was used for each bacterial strain at each inoculation time.

Harvesting

Four weeks after transplantation, the experimental plants were harvested, and six plants per treatment were taken for biomass and root architecture measurements. The plants were carefully collected from

the pots and the root system was rinsed with tap water to remove sand particles. The plants were then divided into shoot and root parts for root architecture and plant dry biomass measurements.

Root architecture

For root architecture measurements, the roots were sectioned in three parts, spread along a rectangular acrylic tray and placed in an EPSON scanner Ver. 3.9.3 1NL. The measured root architecture parameters were the specific root area (SRA), specific root length (SRL), average root diameter (AvD), and specific root density (RDENS). All parameters were analysed in WINRHIZO^{TM} program V2005b. Specific root area was calculated by dividing the surface area by the root dry biomass. Specific root length was calculated by the following formula:

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

Plant biomass

The shoot and root parts were dried at room temperature for four hours, until no remaining water could be observed on their surfaces. The fresh weights of both parts were obtained using an electronic scale. The shoot and root parts were then placed in an oven at 60 ºC for 72 hours. The percentage of biomass was calculated by dividing the dry weight biomass by the fresh weight and multiplying by 100.

Statistical analysis

The plant dry biomass and root architecture data were analysed by analysis of variance (ANOVA) and Duncan's test ($P<0.05$) using the IBM SPSS Statistics 23 program.

Results

Bacterial strains and PGPB effect on sorghum plant biomass

The five bacterial strains exhibited different characteristics in terms of specific plant growth-promotion traits (Table1). Strain IAC/BECa 128 (*Enterobacter asburiae*) had the capability to solubilize phosphate. All strains produced IAA, except strain IAC/BECa 135 (*Burkholderia tropica*). Strains IAC/BECa 99 (*K. radicincitans*), IAC/BECa 128 (*E. asburiae*) and IAC/BECa 152 (*H. frisingense*) produced siderophores. Strain IAC/BECa 141 (*Pseudomonas fluorescens*) produced hydrogen cyanid.

Table 1. Plant growth promotion characteristics of five bacterial isolates IAC/BECa 99 (*Kosakonia radicincitans*), IAC/BECa 128 (*Enterobacter asburiae*), IAC/BECa 135 (*Burkholderia tropica*), IAC/BECa 141 (*Pseudomonas fluorescens*) and IAC/BECa 152 (*Herbaspirillum frisingense*)

PGPB trait	Strain				
	IAC BECa 99	**IAC BECa 128**	**IAC BECa 135**	**IAC BECa 141**	**IAC BECa 152**
P Solubilization	-	+	-	-	-
IAA	+	+	-	+	+
Siderophore	+	+	-	-	+
Hydrogen cyanid	-	-	-	+	-
nifH gene	+	-	-	-	-

Positive (+) and negative (-) signals mean positive and negative results for each plant growth promotion trait listed

Sorghum cultivar SRN39 exhibited a significant increase in root dry biomass when inoculated with *B. tropica* strain IAC/BECa 135 or *H. frisingense* strain IAC/BECa 152, and a significantly higher shoot biomass when inoculated with *E. asburiae* strain IAC/BECa 128 or *H. frisingense* strain IAC/BECa 152, compared with the control (Table 2). Cultivar BRS330 displayed a significant increase in root dry biomass when inoculated with strain IAC/BECa 135 (*B. tropica*) or IAC/BECa 152 (*H. frisingense*) compared with the control. However, when inoculated with *E. asburiae* strain IAC/BECa 128, exhibited a significant decrease in shoot biomass compared with the non-inoculated control. Cultivars SQR and BRS509 did not exhibit significant differences in biomass when inoculated with any of the strains compared to the control (Table 2).

Table 2: Root and shoot biomass (%) of four cultivars of sorghum (SRN-39, SQR, BRS330 and BRS509) inoculated with five bacteria *Kosakonia radicincitans* strain IAC/BECa 99, *Enterobacter asburiae* strain IAC/BECa 128, *Burkholderia tropica* strain IAC/BECa 135, *Pseudomonas fluorescens* strain IAC/BECa 141 and *Herbaspirillum frisingense* strain IAC/BECa 152.

Cultivars	**Bacterial Isolate**	**Root Biomass (%)**	**Shoot Biomass (%)**
SRN-39	CONTROL	18.10 ± 1.19 c	22.20 ± 0.73 c
	IAC/BECa 99	21.52 ± 0.75 bc	23.66 ± 0.50 bc
	IAC/BECa 128	24.83 ± 3.03 abc	25.22 ± 0.98 b
	IAC/BECa 135	27.48 ± 3.20 ab	23.82 ± 0.37 bc
	IAC/BECa 141	21.46 ± 2.06 bc	23.94 ± 0.68 bc
	IAC/BECa 152	31.47 ± 1.74 a	28.51 ± 1.34 a
SQR	CONTROL	29.52 ± 2.84 a	22.21 ± 0.88 a
	IAC/BECa 99	24.50 ± 2.25 a	19.62 ± 1.15 a
	IAC/BECa 128	25.67 ± 1.90 a	19.56 ± 0.97 a
	IAC/BECa 135	31.15 ± 3.24 a	19.69 ± 0.64 a
	IAC/BECa 141	29.26 ± 3.56 a	20.31 ± 0.87 a
	IAC/BECa 152	33.64 ± 1.59 a	20.94 ± 0.37 a
BRS330	CONTROL	13.19 ± 0.69 bc	20.75 ± 0.35 a
	IAC/BECa 99	12.58 ± 0.48 bc	19.92 ± 0.54 ab
	IAC/BECa 128	11.77 ± 0.69 c	19.82 ± 0.32 b
	IAC/BECa 135	19.17 ± 2.30 a	21.24 ± 0.33 a
	IAC/BECa 141	16.14 ± 1.02 ab	19.91 ± 0.48 ab
	IAC/BECa 152	18.43 ± 0.98 a	20.50 ± 0.30 ab
BRS509	CONTROL	24.13 ± 2.00 ab	25.38 ± 1.46 a
	IAC/BECa 99	20.79 ± 2.60 b	23.56 ± 0.23 a
	IAC/BECa 128	24.57 ± 0.88 ab	22.73 ± 0.65 a
	IAC/BECa 135	28.29 ± 2.38 a	22.48 ± 0.44 a
	IAC/BECa 141	20.68 ± 1.62 b	22.97 ± 0.48 a
	IAC/BECa 152	24.04 ± 1.70 ab	23.44 ± 1.50 a

The values are means of replicates (n=6) ± (SE). For each parameter, letters compare (on column) the means between the bacterial inoculums treatments within the same cultivar. Means followed by the same letter are not statistically different by Duncan test ($P<0.05$).

PGPB effect on root architecture

Cultivars SRN39, SQR, and BRS509 did not display significant differences in root architecture parameters when inoculated with any strain compared with the control. However, cultivar BRS330 inoculated with *E. asburiae* strain IAC/BECa 128 exhibited a significantly higher specific root area (SRA) and specific root length (SRL) compared with the control. Furthermore, when inoculated with *B. tropica* strain IAC/BECa 135 or *H. frisingense* strain IAC/BECa 152, the same cultivar exhibited a significant decrease in root average diameter (AvD) compared with the control (Table 3). Cultivar BRS 330 inoculated with *B. tropica* (IAC/BECa 135), *P. fluorescens* (IAC/BECa 141) or *H.*

frisingense (IAC/BECa 152) had a higher specific root density (SRD) compared than the treatment inoculated with *E. asburiae* (IAC/BECa 128), but not the control.

Table 3: Specific root area (SRA), specific root lenght (SRL), average of root diameter (AvD) and specific root density (RDENS) four cultivars of sorghum (SRN-39, SQR, BRS330 and BRS509) inoculated with five bacteria *Kosakonia radicincitans* strain IAC/BECa 99, *Enterobacter asburiae* strain IAC/BECa 128, *Burkholderia tropica* strain IAC/BECa 135, *Pseudomonas fluorescens* strain IAC/BECa 141 and *Herbaspirillum frisingense* strain IAC/BECa 152.

Cultivars	**Isolates**	**SRA (cm2/g)**	**SRL (cm/g)**	**AvD (mm)**	**RDENS (cm3/g)**
SRN-39	CONTROL	847.29 ± 44.46 a	687.39 ± 66.85 a	0.40 ± 0.02 a	0.12 ± 0.00 a
	IAC/BECa 99	1027.44 ± 130.77 a	867.21 ± 151.8 a	0.39 ± 0.02 a	0.11 ± 0.01 a
	IAC/BECa 128	1061.95 ± 146.42 a	881.21 ± 66.73 a	0.38 ± 0.03 a	0.11 ± 0.02 a
	IAC/BECa 135	1016.59 ± 59.11 a	882.89 ± 91.54 a	0.38 ± 0.02 a	0.11 ± 0.01 a
	IAC/BECa 141	1044.78 ± 68.45 a	883.43 ± 67.29 a	0.38 ± 0.02 a	0.10 ± 0.01 a
	IAC/BECa 152	914.74 ± 50.78 a	802.62 ± 69.77 a	0.37 ± 0.01 a	0.12 ± 0.00 a
SQR	CONTROL	1021.64 ± 41.81 a	909.61 ± 69.28 a	0.36 ± 0.01 a	0.11 ± 0.00ab
	IAC/BECa 99	1034.82 ± 58.91 a	922.61 ± 62.72 a	0.36 ± 0.01 a	0.11 ± 0.01ab
	IAC/BECa 128	1217.28 ± 204.39 a	1220.4 ± 310.4 a	0.35 ± 0.02 a	0.10 ± 0.01ab
	IAC/BECa 135	1239.66 ± 109.67 a	1056.5 ±116.53 a	0.38 ± 0.02 a	0.09 ± 0.01 b
	IAC/BECa 141	1284.28 ± 207.40 a	1214.2 ± 211.88a	0.34 ± 0.01 a	0.10 ± 0.01 b
	IAC/BECa 152	917.84 ± 26.31 a	894.24 ± 32.09 a	0.33 ± 0.01 a	0.13 ± 0.00 a
BRS330	CONTROL	881.00 ± 28.55 b	609.24 ± 23.55 b	0.46 ± 0.01 a	0.10 ± 0.00ab
	IAC/BECa 99	926.69 ± 66.26 ab	677.58 ± 47.12ab	0.43 ± 0.01ab	0.10 ± 0.01ab
	IAC/BECa 128	1101.94 ± 96.50 a	774.88 ± 72.32 a	0.46 ± 0.01 a	0.08 ± 0.01 b
	IAC/BECa 135	841.07 ± 80.40 b	645.94 ± 60.04ab	0.41 ± 0.01 b	0.12 ± 0.01 a
	IAC/BECa 141	767.82 ± 44.72 b	561.31 ± 32.04 b	0.44 ± 0.01ab	0.12 ± 0.01 a
	IAC/BECa 152	786.94 ± 14.98 b	605.28 ± 18.08 b	0.42 ± 0.01 b	0.12 ± 0.00 a
BRS509	CONTROL	1337.8 ± 665.09 a	1121.9 ± 531.2 a	0.38 ± 0.03ab	0.17 ± 0.04 a
	IAC/BECa 99	707.81 ± 68.59 a	553.35 ± 62.87 a	0.41 ± 0.01 a	0.14 ± 0.01 a
	IAC/BECa 128	633.89 ± 45.40 a	524.96 ± 35.46 a	0.38 ± 0.01ab	0.17 ± 0.01 a
	IAC/BECa 135	779.74 ± 53.88 a	662.51 ± 50.39 a	0.38 ± 0.01ab	0.14 ± 0.01 a
	IAC/BECa 141	1130.4 ± 230.66 a	1138 ± 325.22 a	0.34 ± 0.02 b	0.12 ± 0.01 a
	IAC/BECa 152	703.37 ± 79.40 a	622.32 ± 74.82 a	0.36 ± 0.01ab	0.17 ± 0.02 a

Values are means of replicates (n=6) ± (SE). For each parameter, letters compare (on column) the means between the bacterial inoculum treatments within the same cultivar. Means followed by the same letter are not statistically different by Duncan test ($P<0.05$).

Discussion

This work aimed to evaluate the effect of five bacterial strains isolated from sugarcane on the plant growth and root architecture of four sorghum cultivars. Cultivars SRN-39 and BRS330 inoculated with *B. tropica* strain IAC/BECa 135 or *H. frisingense* strain IAC/BECa 152 and cultivar SRN-39 inoculated with *E. asburiae* strain IAC/BECa 128 or *H. frisingense* strain IAC/BECa 152 exhibited

significant increases in sorghum root and shoot biomass, respectively, compared with the control. Although the number of replicates in our study was small (6) our results corroborate those of Chiarini *et al.* (1998), who found that isolates belonging to the genera *Burkholderia* and *Enterobacter* co-inoculated in the sorghum rhizosphere promoted a significant increase in root growth compared to non-inoculated plants. Furthermore, species belonging to the genera *Burkholderia* and *Herbaspirillum* promote the growth of sugarcane and maize (Pereira *et al.*, 2014, da Silva *et al.*, 2016), which like sorghum, are C4 grass species. *Herbaspirilum frisingense* strain IAC/BECa 152 produces siderophores and IAA, whereas *B. tropica* strain IAC/BECa 135 does not. The strain IAC/BECa 152 might possess a set of mechanisms that improve plant nutrient uptake either by increasing nutrient availability in the rhizosphere or influencing the biochemical mechanisms underlying nutritional processes (Pii *et al.*, 2015). Such mechanisms include changes in the root system architecture and shoot-to-root biomass ratio, increases in proton efflux by modulating H+APTase activities, indirect effects of IAA produced by PGPB, or acidification of the rhizosphere to enhance nutrient solubility (Pii *et al.*, 2015). In addition to growth regulators, siderophores can be produced and are known to assist Fe acquisition by roots (Saravanan *et al.*, 2007, Mehnaz *et al.*, 2013).

In contrast to the effects of *B. tropica*, *H. frisingense* and *E. asburiae* on the growth of both grain sorghum cultivars, these strains had no significant effect on BRS 509 (sweet sorghum) and on SQR cultivars compared with the control. Interestingly, in accordance with our results, Dos Santos *et al.* (2017) observed significant increase in the biomass of grass and grain sorghum inoculated with *Burkholderia* ssp. or *Herbaspirillum* ssp. but not sweet sorghum inoculated with the same isolates. Taken together with our results, these findings suggest that the effects of strains of *B. tropica* and *H. frisingense* on plant growth are dependent on sorghum genotype. It is unclear why the effects of these strains were greater in certain sorghum cultivars than others. However, different sorghum genotypes release different strigolactone molecules in different quantities under P starvation (Schlemper *et al.*, 2017). Thus, the high relative abundance of the genus *Bulkholderia* in the rhizosphere of sorghum cultivar SRN-39 could be related to the level of orobanchol, which is 300 and 1100 times higher in SRN-39 than in the cultivars SQR, BRS330 and BRS509 as suggested by Schlemper *et al.* (2017).

When inoculated with *E. asburiae* strain IAC/BECa 128, cultivar BRS330 displayed an increase in SRL and area compared with the control. These finding are in agreement with a study by Kryuchkova *et al.* (2014), who reported that *Enterobacter* species can promote increases root length and lateral roots in sunflower. The strain IAC/BECa 128 can solubilize phosphate, which trait might explain the increase in root biomass. The cultivar BRS330 inoculated with strain IAC/BECa 135 (*B.*

tropica) or IAC/BECa 152 (*H. frisingense*) exhibited a significant decrease in average root diameter compared with the control but slight increase in root density. Plants under P deficiency conditions may increase root density probably to enhance nutrient acquisition (Kapulnik & Koltai, 2014).

Moreover, species belonging to the genus *Herbaspirillum* can influence plant root architecture and improve signalling pathways of plant hormone production (Straub *et al.*, 2013). No significant effects on sorghum growth or root architecture modification were observed when *K. radicincitans* strain IAC/BECa-99 or *P. fluorescens* strain IAC/BECa-141 was inoculated in the rhizosphere of any evaluated sorghum cultivar. Although there are many reports on the effects of *K. radicincitans* (formerly known as *Enterobacter radicincitans*) on a range of plants, such as *Arabidopsis thaliana*, radish, and tomato (Berger *et al.*, 2013, Brock *et al.*, 2013, Berger *et al.*, 2015), there are no reports on the effects of this bacterial species on sorghum growth or root architecture modification. With respect to *P. fluorescens*, Marcos *et al.* (2016) found that the strain IAC/BECa 141, when used as an inoculant applied to two sugarcane varieties, increased chlorophyll *a* content without changing plant growth. Kumar *et al.* (2012) studying the effect of seven different fluorescent *Pseudomonas spp.* strains with single or multiple PGPR traits, in sorghum growth, observed that all strains were able to increase sorghum growth compared to a non-inoculated control.

Our results demonstrated that selected bacterial strains characterized as PGPB in sugarcane were able to promote plant growth and root architecture modification in sorghum. Based on the reproducibility of the performance of bacterial strains for different crops, our findings shed light on the identification of bacterial candidate strains for improving the growth and yield of crops that share the same soil bacterial source in intercropping or crop rotation systems. However, since we did not evaluate the bacterial community that actually colonized the root system, we strongly recommend future studies to recover the bacterial community from the endosphere and rhizosphere compartments as proof of the effectiveness of the inoculation. We suggest that SL plays a role in the effectiveness of PGPB in promoting the growth of specifics sorghum genotypes, although our experimental set-up did not allow us to make a straight forward conclusion. More specific experiments are needed to better address the relationship between plant strigolactone production and plant bacterial infection.

Conclusion

Here we demonstrated that bacteria strains characterized as PGPB in sugarcane were able to promote plant growth and root architecture modification in sorghum. Our results demonstrated that cultivars SRN-39 and BRS330 inoculated with *B. tropica* strain IAC/BECa 135 or *H. frisingense* strain

IAC/BECa 152 exhibited a significant increase in plant biomass. Moreover, cultivar BRS330 inoculated with either strain displayed a significant decrease in AvD. The results of this study indicate that *B. tropica* strain IAC/BECa 135 and *H. frisingense* strain IAC/BECa 152 are promising PGPB strains for use as inocula for sustainable sorghum cultivation.

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Chapter 6

General discussion

Interactions between plants and soil microbes may be positive, neutral or negative. This outcome will depend on plant and soil microbial community characteristics and composition, as well as on the compatibility of the molecular signalling by both partners. The rhizosphere is the first interface of interaction between soil microbes and plant roots. Some microorganisms present in this compartment are able to attach to the root rhizoplane and even to colonize the root endosphere compartment for association or to interact with the plant. Specifically for sorghum, little is known about how different sorghum genotypes recruit and benefit from soil microbial communities. The increasing demand for food and bioenergy crops, allied to the increasing necessity of crops that are resistant to drought conditions, make sorghum a promising plant species to be studied regarding the benefits of the root microbiome. The overall aim of my thesis was to investigate the dynamics of the sorghum root microbiome and to explore the beneficial effects of the root microbiome on sorghum growth and drought stress tolerance. In this chapter I discuss the main findings of my studies and I will provide future perspectives for the application of the results.

Factors that drive the dynamics of the microbial community in the rhizosphere

Many factors affect the rhizosphere microbial community composition including soil factors, plant growth stage and plant species or genotype (Nihorimbere *et al.*, 2011). Although many studies have addressed the influence of these factors on the rhizosphere microbial community composition (Garbeva *et al.*, 2004, Berg & Smalla, 2009, Aira *et al.*, 2010, Marques *et al.*, 2014), most of them did not evaluate these factors simultaneously. In this thesis, I present the relative contribution of soil type, plant genotype and plant growth stage in shaping the sorghum rhizosphere microbial community (**Chapters 2 and 3**). I found soil to be the main factor in driving the assembly of the sorghum rhizosphere microbial community followed by plant growth stage and plant genotype.

While many studies point to soil as the major determinant of root microbial community composition (Singh *et al.*, 2007, Xu *et al.*, 2009), others showed that the influence of plant species or plant genotype on the rhizosphere microbial community composition is larger than the impact of soil characteristics (Miethling *et al.*, 2000, Aira *et al.*, 2010). Such contradictory results may be explained either by the use of different plant genotypes and soil types, or by different sampling strategies applied (Wieland *et al.*, 2001). To disentangle which factor, soil or plant genotype, is the main driver of the structure of the rhizosphere microbial community of sorghum, I compared both factors during plant growth. Whereas at the early stages of plant growth, soil showed a preponderant role in the structuring of sorghum rhizosphere bacterial community, at later stages of plant growth, the sorghum genotype

showed to be an important driver of rhizobacterial recruitment. My explanation is that, at the early stages of growth, plant still does not yet have a well-developed root system, which can widely interfacing soil to interact with the soil microbial community. During root growth, roots increase their contact surface with soil and so with the soil microbial community as well as that the quality and quantity of rhizodeposits will increase and so plant roots will likely have an increasing role in shaping the rhizosphere bacterial community. In this sense, plant genotype effect on the rhizosphere microbial community would not be a consequence of asynchronous growth, but instead, the result of the relative influence of roots and root-produced rhizosdeposits at a given time (Micallef et al., 2009). Plant genotypes with different growth rates and differences in root development may release different types and amounts of root exudates, which compounds will attract different microbes from the soil-borne microbial pool and assemble a specific microbial community in the rhizosphere (Dunfield & Germida, 2003, Inceoglu et al., 2010). Chaparro *et al.* (2013) suggested that at early stages of plant growth roots exude more simple compounds used by a more diverse community of microorganisms, and at later stages plant roots release more complex compounds selecting more specific microbes. The understanding of the shaping forces of the rhizosphere microbial community structure of different plant genotypes and at different growth stages may help the improvement of strategies and management practices for controlling soil-borne pathogens (Wu *et al.*, 2016).

Many studies already showed the influence of plant genotype on the microbial community composition of the rhizosphere of several crops (Aira *et al.*, 2010, Sugiyama *et al.*, 2012, Marques *et al.*, 2014). However, only few studies evaluate the genotype effect consistency in different soils (Peiffer *et al.*, 2013). Here I provided evidence that the genotype effect on the sorghum rhizosphere bacterial assembly and on bacterial and fungal co-variance was soil dependent, and most strongly occurred in the less fertile Clue Field soil. Corroborating with this evidence, Nicolitch *et al.* (2016) found that beech trees had a higher enrichment of bacterial taxa in the rhizosphere in a nutrient poor soil compared with nutrient rich soil. The authors suggested that such enrichment may be related to the intensification of rhizodeposition in order to recruit microorganisms that could effectively play a role in nutrient mobilization (Nicolitch *et al.*, 2016).

I found that the African sorghum cultivar SRN-39 cropped on the less fertile Clue field soil recruited a higher abundance of *Burkholderia*, *Cupriavidus* (*Burkholderiaceae*), *Acidovorax* and *Albidiferax* (*Comamonadaceae*) (**Chapter 2**) than in the more fertile soil, and hosted the strongest interaction between fungal and bacterial communities in rhizosphere (**Chapter 3**) of all cultivars considered here. Furthermore, cultivar SRN-39 showed better growth responsiveness when inoculated with the bacterial strains IAC/BECa 135 (*Burkholderia tropica*) and IAC/BECa 152

(*Herbaspirillum frisingense*) than the other cultivars **(Chapter 5).** Although the mechanisms of the sorghum genotype effect on microbial community selection and structure remain unclear, I speculate that differences in rhizodeposition could be the primary reason for the observed effect.

Rhizodeposition varies qualitatively and quantitatively over different plant genotypes (Aulakh et al, 2001). As an example, I found that cultivar SRN-39 produced the strigolactone molecule Orobanchol at levels 300 to 1100 times higher than the other six studied cultivars. Strigolactones are considered an additional class of hormones that besides being involved in the plant morphological regulation (Koltai, 2014), are responsible to stimulate the seed germination of parasitic plants such as *Striga hermonthica* and *Orobanche* spp. (Bouwmeester *et al.*, 2007), as well as hyphal-branching and symbiosis of the arbuscular mycorrhizal fungi - AMF (Akiyama *et al.*, 2010), and are used by bacteria to communicate with each other in order to sense population density (Proust *et al.*, 2011).

Different bacterial species have been shown to reduce the infection of parasitic plants on different host plants (Miché *et al.*, 2000, Mabrouk *et al.*, 2007, Hassan *et al.*, 2009). Specifically regarding sorghum cultivar SRN-39, Hassan *et al.* (2009) showed that the inoculation of *Pseudomonas putida* and *Azospirillum amazonas* reduced infestation of Striga compared with non-inoculated plants. From a few studies available regarding bacteria as biocontrol of Striga, most of them focus on the influence of bacterial isolates rather than total soil bacterial community. Sorghum crops are grown in different soils and interact with a diversity of microorganisms whose composition may have different effect on Striga. Because SRN-39 is a Striga-resistant cultivar that recruited significantly higher abundance of specific groups of bacteria and has a strigolactone profile that is different from the other 5 studied cultivars, I recommend that bacterial community selected by SRN-39 should be subject of future investigations to test their effect on Striga suppression. An important uncertainty that arises in my study and that should be subject of investigation in future studies is whether the tolerance of SRN-39 to Striga is directly and exclusively based on the plant genetic background acquired during breeding, or if there is a participation of a specific microbial community recruited in the rhizosphere that contributes to Striga tolerance. Furthermore, despite that high levels of Orobanchol may play a role in rhizobacterial community assembly, also exudates other than strigolactones could play a role. Thus, additional analyses including root exudate of different sorghum genotypes are required.

Sorghum rhizoplane bacterial community and drought stress tolerance

As powerful as the effect of soil on the microbial community of the rhizosphere, is the effect of climate on the microbial community in soil (Lladó *et al.*, 2018). Considering the importance of precipitation as a climatic event that could drive changes in microbial communities, in **chapter 4** we tested 5 different soils in search for well-adapted microorganisms that could alleviate drought stress of sorghum plants. For this purpose, the approach presented in this chapter allowed us to check for the contribution of root-associated microorganisms to water stress alleviation thereby minimizing the effect of chemical and physical soil characteristics.

Worldwide, the lack of water on agricultural systems causes huge crop productivity losses with serious economic and social consequences both on the local and global scale. Despite the possibility of artificial irrigation to overcome drought generally small farmers cannot afford this technology to guarantee their crop yields due to the high costs of irrigation (Bakhsh et al., 2015). Low moisture conditions imposes physiological stress on plants such as suppression of photosynthesis, decrease in a chlorophyll content, and stomatal closure to reduce water loss (Rizhsky *et al.*, 2004, Rahdari *et al.*, 2012). Such physiological stresses limit plant growth and make plants more vulnerable to certain diseases (Diourte *et al.*, 1995, Farooq *et al.*, 2012, Tesso *et al.*, 2012). Specifically in sorghum, plants under drought stress are more susceptible to stalk and charcoal rot disease caused by *Macrophomina phaseolina* (Diourte *et al.*, 1995, Tesso *et al.*, 2005).

A promising alternative to overcome the deleterious effect of drought on different crops is the interaction of plants with beneficial soil microorganisms. Microorganisms that are adapted or tolerant to drought conditions can help plants to overcome drought and enhance plant growth, nutrition and plant resistance to water stress by, for instance, by influencing the expression of AtRAB18 and AtLT178 stress responsive genes (Sukweenadhi *et al.*, 2015) and conferring induced systemic tolerance (IST) to drought stress (Yang *et al.*, 2009). Furthermore, plants under water stress conditions produce the hormone ethylene to regulate the plant homeostasis and to reduce root and shoot growth. However, in the presence of bacterial ACC deaminase less ethylene is formed, avoiding the reduction on plant growth (Glick, 2012). Although, soil moisture may impose physiological stress on microbial communities limiting or inhibiting their development (Bouskill *et al.*, 2013, Armada *et al.*, 2014), bacteria do employ strategies to overcome water deficiency that include accumulation of compatible solutes, production of exopolysaccharide and production of spores as dormant life form (Barnard *et al.*, 2013).

Drought and the rewetting of dry soils are considered disturbances for soil microorganisms. The degree to which microbes respond to disturbances is related to the sensitivity or tolerance of the

organism as well as the disturbance intensity and severity. In our experiment described in **chapter 4,** I applied two pulses of water disturbance at the pre-flowering plant growth stage as this is the most critical point of sorghum development when plants face such stress (Emendack *et al.*, 2018). The pre-flowering stage is a crucial stage in the assembly of the rhizosphere microbial community under limited water conditions because drought can delay the microbial root colonization at early development stage of sorghum growth (Xu *et al.*, 2018).

In order to assess which species of the rhizomicrobiome are able to alleviate water stress on plants, studies may directly consider the effect of microorganisms on plant growth, production and nutrition at water limited conditions (Xu *et al.*, 2018) as well as indirectly, by assessing bacterial mediated hormonal modulators such as the exogenous Indole Acetic Acid (IAA) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme activity that contribute to a physiological response and stress tolerance (Govindasamy *et al.*, 2017, Armada *et al.*, 2018). Here I directly determined the effects of the bacterial community on sorghum growth and root architecture modification at water deficiency and under normal water supply conditions. Although I found that different soils and water moisture content played a role in sorghum growth and root architecture modification, no relationship between plant growth at water limited conditions and bacterial community composition was found.

Nevertheless, at water deficiency, the rhizoplane of drought susceptible (DS) sorghum lineage previously planted in Cerrado and the rhizoplane of drought tolerant (DT) sorghum lineage previously planted in Sorghum field soil showed the highest abundances of the bacterial families *Caulobacteraceae* and *Rhizobiaceae*, respectively. The Cerrado soil comes from a savannah-like vegetation characterized by extreme temperatures, fire and water stress (Borghetti *et al.*, 2005). Sorghum field soil came from a semi-arid region with half of the annual average precipitation as compared to the locations where the other soils were sampled. In general, microbial communities that have experienced drying and rewetting cycles may be more resistant to changes under these conditions than those that have not (Evans & Wallenstein, 2012, Bouskill *et al.*, 2013). Hence, the degree to which the soil microbial community can resist environment fluctuations may be influenced by climate history. Although *Caulobacteraceae* and *Rhizobiaceae* families which are known to include species tolerant to drought and thermal conditions (Bouskill *et al.*, 2013, Nunes *et al.*, 2018) did not show effect on the plant growth, the possibility cannot be excluded that a high density of bacterial cells of specific species isolated from these families could alleviate plant stress. Thus, I suggest that future research should identify and isolate strains belonging to the water stress tolerant

Caulobacteraceae and *Rhizobiaceae* families and test them as possible bioinoculant to promote plant growth under abiotic stress conditions.

Plant growth promoting bacteria inoculated on sorghum

To be considered as PGPB, bacterial strains need to fulfill at least two of the three criteria of effective colonization, plant growth stimulation and biocontrol (Weller *et al.*, 2002, Vessey, 2003). To investigate if bacterial strains characterized as PGPB in sugarcane would affect sorghum growth, I inoculated the rhizosphere of four sorghum cultivars (SRN-39, Shanqui-Red (SQR), BRS330, and BRS509) with five sugarcane endophytic bacterial isolates *K. radicincitans* (IAC/BECa 99), *E. asburiae* (IAC/BECa 128), *P. fluorescens* (IAC/BECa 141), *B. tropica*, (IAC/BECa 135) and *H. frisingense* (IAC/BECa 152) (**chapter 5**). The results indicated that bacterial strains characterized as PGPB in sugarcane were also able to promote growth of sorghum. Sorghum and sugarcane are genetically closely related; intergeneric hybrids between these two species have already been reported (Bowers *et al.*, 2003). Whereas the evolutionary divergence between sorghum and maize have been estimated to have occurred approximately 15–20 million years ago, sorghum diverged from sugarcane approximately 5 million years ago (Paterson *et al.*, 2004). Given the genetic proximity of sorghum and sugarcane and taking into account that these two species are often reported to be part of the same crop rotation system (Tew *et al.*, 2008, May *et al.*, 2013), it is really promising that PGPB isolates of the one crop could have a positive effect on the other which is useful in a crop rotation or intercropping system. However, the effect of bacterial strains on sorghum growth was different dependent on sorghum genotype. Sorghum cultivar SRN-39 had the best performance with a significant increase on root dry biomass when inoculated with isolates *B. tropica* and *H. frisingense* (*Burkholderiales* Order), and a significant higher shoot biomass when inoculated with *E. asburiae* and *H. frisingense*, compared with the control. Interestingly, cultivar SRN-39 also showed higher relative abundance of specific rhizosphere bacterial families belonging to *Burkholderiales* Order (**Chapter 2**), and a stronger rhizosphere bacterial and fungal co-variation with significant contribution of representatives of *Burkholderiales* Order (**Chapter 3**) much more than the other cultivars. It seems that this cultivar has a greater potential in interacting with representatives of *Burkholderiales* Order, and so, its growth seems to be more impacted by them than the other cultivars. As already mentioned, this cultivar produces the strigolactone molecule Orobanchol at levels 300 to 1100 times higher than the other six studied cultivars, and we can not rule out the possibility that this group of hormones contributes to the plant genotype specificity in the interaction with the aforementioned soil microbes and PGPB isolates. However, to confirm this hypothesis, future studies are required in order to directly test the

influence of the strigolactone molecules and other exudates mediating this interaction. Variation in root exudation among agricultural crop genotypes has the potential for effective plant manipulation in order to create specific positive effects on the beneficial members of the rhizosphere microbiome (Bakker *et al.*, 2012).

Concluding remarks

The results presented in this thesis indicated that soil and plant genotype play a crucial role in structuring the sorghum rhizosphere microbial community. As soils with low fertility showed to induce plant genotype specificity in interaction with bacterial and fungi communities, investigations on the impact of low soil fertility on the sorghum root microbial community could provide a first step in identifying microbial candidates to improve sorghum nutrient-use efficiency in low-input agricultural cropping systems in resource poor regions. This was clearly illustrated by the observation that in the less fertile Clue Field soil, the sorghum cultivar SRN-39 had significantly higher relative abundances of representatives of *Burkholderiaceae* and *Comamonadaceae* families with a stronger co-variance between bacterial and fungal community than the other cultivars. Besides the genotype effect of sorghum cultivar SRN-39 on the recruitment of the bacterial community in the rhizosphere and on the co-variation of bacterial and fungal communities, the inoculation of *Burkholderia tropica* and *Herbaspirillum frisingense* in sorghum cultivar SRN-39 also showed a greater growth effect than at the other cultivars. Future studies are required to obtain a more complete understanding of the mechanisms involved in rhizodeposition processes in sorghum and differences therein in different genotypes, in particular related to strigolactones production and their role in the interaction of soil microorganisms with sorghum. Similarly we strongly recommend that future studies should identify and isolate strains testing them as possible bioinoculant to alleviate water stress on sorghum. The higher abundance of *Caulobacteraceae* and *Rhizobiaceae* at water deficiency conditions illustrate the strain specificity at water stress conditions.

How specific functions of the microbial community of the rhizosphere of sorghum are affected in different soils, at different plant growth stages, plant genotype and drought stress is an intriguing subject which I highly recommend to be addressed in future studies. Taking into account the promising findings of our study, we suggest that microbiome engineering should be considered as an integral part of sorghum crop management practices in order to safeguard sorghum production at stress conditions.

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Summary

Soil microbes may have positive and negative interactions with plants. Positive interactions include symbiotic associations between plants and microbes promoting plant growth and nutrient uptake, whereas negative interactions include pathogenesis and competition for nutrients. Thus the composition of the rhizomicrobiome which is related to several factors such as plant species, plant genotype and soil type is of utmost significance for plant growth and health.

Sorghum is currently the 5th most cultivated cereal worldwide and is an economically important crop used for animal feed and human food, in particular for subsistence farmers in Sub-Saharan Africa. The research described in this PhD thesis explores the main factors that determine the sorghum rhizomicrobiome assembly, and how its composition modulates plant growth and stress tolerance. The approaches employed in the present study were *in vivo* bioassays, bacterial inoculation and next-generation sequencing to assess the taxonomic composition of the sorghum rhizomicrobiome.

To investigate the relative impact of the factors soil type, plant genotype and plant growth stage on the sorghum rhizobacterial community composition seven different sorghum genotypes were grown in the greenhouse in two different soil types (Clue Field – CF and Vredepeel – VD) and evaluated at four different plant growth stages (days 10, 20, 35 and 50). The results showed that the composition of the bacterial community was most strongly influenced by soil type followed by plant growth stage and plant genotype (Chapter 2). Furthermore, at early stages of sorghum growth, the rhizosphere bacterial community composition was mainly driven by soil type, whereas at later stages plant genotype became a more important factor (Chapter 2). Moreover, one of the genotypes, SRN-39, that was grown in abandoned CF soil, had significantly higher relative abundances of *Acidobacteria* GP1, *Burkholderia*, *Cupriavidus (Burkholderiaceae)*, *Acidovorax* and *Albidiferax* (*Comamonadaceae*) than the other six cultivars. This result indicates that the genotype effect on the sorghum rhizosphere bacterial assembly was soil dependent.

In order to assess the dynamics of bacterial and fungal communities in the sorghum rhizosphere two sorghum genotypes (SRN-39 and BRS 330) were grown in the greenhouse in two different soil types (CF and VD) and evaluated at three different plant growth stages (days 10, 35 and 50). The results showed that sorghum genotype SRN-39 promoted a stronger co-variance between bacterial and fungal communities when grown in abandoned Clue Field soil. Furthermore, the decrease in relative abundance of the fungus genus *Gibberella* over plant growth stages was followed by a decrease of the bacterial families *Oxalobacteracea* and *Sphingobacteriacea*. We suggest that there may be a link between these organisms, as both bacterial families are known to be antagonistic

to fungal activity. Notwithstanding, this hypothesis needs to be experimentally assessed in future studies (Chapter 3).

Aiming to investigate if sorghum plants pre-cultivated in soils with different bacterial community composition responded differently to water deficiency conditions, two sorghum lineages (drought susceptible and drought tolerant) were grown in five different soils for 21 days and transplanted with their rhizoplane microbial community to a standardized substrate at different moisture conditions. The results showed that at water deficient conditions high abundances of the *Caulobacteraceae* family in the rhizoplane of the drought susceptible lineage and of the *Rhizobiaceae* family in the rhizoplane of the drought tolerant lineage occurred in particular at the plants that were pre-grown in two soils with a history of low rainfall regimes, Cerrado and Sorghum field, respectively. These results suggest that pre-cultivation of sorghum in soils with a history of low rainfall regimes selected for representatives of the *Alphaproteobacteria* which may provide a selective advantage at water deficient conditions (Chapter 4).

In order to investigate potential plant growth –activity of so-called PGPB (Plant Growth Promoting Bacteria) on sorghum, five endophytic bacterial strains originally selected as PGPB of sugarcane were inoculated in four sorghum cultivars. Inoculation of the cultivars SRN-39 and BRS330 with *Burkholderia tropica* strain IAC/BECa 135 or *Herbaspirilum frisingense* strain IAC/BECa 152 resulted in significant increases in plant biomass. In particular cultivar SRN-39 showed better growth responsiveness resulting in significantly larger plant biomass when inoculated with the bacterial strains IAC/BECa 135 (*Burkholderia tropica*) and IAC/BECa 152 (*Herbaspirillum frisingense*) than the other cultivars (Chapter 5). This study shows that these strains are promising PGPB strains for use as bioinoculant in sorghum cultivation.

In conclusion, the research presented in this thesis showed for the first time the simultaneous impact of factors plant genotype, growth stage and soil type on the sorghum rhizosphere bacterial community composition. Furthermore, sorghum genotype SRN-39 showed interesting interactions with its rhizomicrobiome which may be employed further to design sustainable sorghum cultivation. Overall, the results described in this thesis highlight the importance of studies on the rhizomicrobiome of sorghum which could contribute to find potential microbial candidates to be used in sustainable agricultural management practices to improve sorghum health and productivity.

Samenvatting

Micro-organismen in de bodem kunnen zowel positieve als negatieve interacties met planten hebben. Positieve interacties zijn bijvoorbeeld de symbiotische relaties tussen planten en micro-organismen, die de groei van planten en de opname van nutriënten bevorderen, terwijl negatieve interacties pathogenese en competitie voor nutriënten omvatten. Daarom is de samenstelling van de microbiële gemeenschap in de rhizosfeer, het zogenaamde rhizomicrobioom, dat is gerelateerd aan verschillende factoren zoals plantensoort, cultivar en bodem type, van het allergrootste belang voor de groei en gezondheid van planten

Sorghum is op dit moment het 5de meest verbouwde graan wereldwijd en het is een economisch belangrijk gewas voor veevoer en menselijke consumptie, in het bijzonder voor zelfvoorzienende boeren ten zuiden van de Sahara. Het onderzoek dat in dit proefschrift wordt beschreven is gericht op de belangrijkste factoren die de samenstelling van het rhizomicrobioom van sorghum bepalen en hoe dat van belang is voor planten groei en stress tolerantie. De wetenschappelijke benaderingen die hier zijn toegepast omvatten in-vivo bioassays, bacteriële inoculatie en next-generation sequencing om de taxonomische samenstelling van het sorghum rhizomicrobioom vast te kunnen stellen

Om de relatieve invloed van de factoren bodemtype, planten cultivar en groeistadium op de samenstelling van de microbiële gemeenschap in de rhizosfeer van sorghum te onderzoeken zijn 7 cultivars gekweekt in kassen in twee verschillende bodemtypen (Clue veld – CF en Vredepeel – VD) en de samenstelling van het rhizomicrobioom is geanalyseerd op vier tijdstippen tijdens de groei (na 10,20,35 en 50 dagen). De resultaten gaven aan dat de samenstelling van de rhizosfeer gemeenschap vooral werd bepaald door het bodemtype gevolgd door groeistadium en cultivar (Hoofdstuk 2). Verder werd de samenstelling van bacteriële gemeenschap in de rhizosfeer in de vroege groeistadia voornamelijk bepaald door het bodemtype terwijl in latere groeistadia het planten genotype een belangrijkere rol speelde bij de samenstelling van het rhizomicrobioom. Bovendien bleek het rhizomicrobioom van één van de cultivars, SRN-39, bij groei in de voormalige landbouwgrond, CF een significant hogere relatieve hoeveelheid aan *Acidobacteria* GP1, *Burkholderia cupriavidus* (*Burkholderiaceae*), *Acidovorax e*n *Albideferax* (*Comamonadaceae*) te bevatten dan de andere cultivars. Dit resultaat laat zien dat het genotype effect op de samenstelling van het rhizomicrobioom bodemtype afhankelijk is. Deze studie is de eerste waarin simultaan de bovengenoemde factoren die de samenstelling van de bacteriële gemeenschap in de rhizosfeer van sorghum bepalen, zijn onderzocht.

Om de dynamiek van de bacterie en schimmel gemeenschappen in de rhizosfeer van sorghum te bepalen werden twee sorghum cultivars (SRN-39 en BRS 330) in de kas gekweekt in

twee bodemtypes (CF en VD)en bemonsterd op drie tijdstippen (dag 10,35 en 50) tijdens de groei. De resultaten lieten zien dat cultivar SRN-39 een sterkere co-variatie tussen bacterie en schimmel gemeenschappen stimuleerde wanneer de planten waren gekweekt in de Clue veld, CF, grond. Verder bleek een afname in de relatieve hoeveelheid van de schimmel genus *Giberella* in de loop van de groei van de planten gevolgd werd door een afname van de bacteriële families *Oxalobacteracea* en *Sphingobacteriacea*. Dit suggereert dat er een link tussen deze micro-organismen is, omdat van de genoemde bacteriën bekend is dat zij antagonistisch zijn tegen schimmels. Niettegenstaande, dient deze hypothese verder onderzocht te worden (Hoofdstuk3).

Om te onderzoeken of sorghum planten, die vooraf gegroeid zijn in bodems met verschillende bacteriële gemeenschappen verschillend reageerden op water deficiënte condities, werden twee sorghum cultivars (een droogtegevoelige en een droogte resistente) gekweekt in vijf verschillende bodems gedurende 21 dagen. Daarna werden de planten met de bodem specifieke microbiële wortel- gerelateerde gemeenschap overgezet op een gestandaardiseerd substraat en verder gekweekt onder verschillende bodemvocht condities. Het bleek dat bij water deficiënte condities hogere aantallen van *Caulobacteraceae* bacterien werden gevonden in de wortelgemeenschap van de droogtegevoelige cultivar en van *Rhizobiaceae* bij droogte resistente planten in het bijzonder bij planten die vooraf waren gekweekt in twee bodems met een historie van een geringe regenval, Cerrado en Sorghum veld bodems. Deze resultaten suggereren dat precultivatie van sorghum in bodems met een historie van weinig regenval vertegenwoordigers van *Alphaproteobacteria* stimuleerde, wat een selectief voordeel lijkt te verschaffen bij water deficiënte condities (Hoofdstuk 4).

Om de potentiele plant groei stimulerende activiteit van zogenaamde PGPB (Plant Growth Promoting Bacteria) op sorghum te onderzoeken werden vijf endofytische bacteriën die oorspronkelijk als PGPB van suikerriet waren geselecteerd, geïnoculeerd bij vier sorghum cultivars. Inoculatie van de cultivars SRN-39 en BRS330 met *Burkholderia tropica* IAC/BECa 135 of *Herbaspirilum frisingense* IAC/BECa 152 resulteerde in een significante toename van de planten biomassa. In het bijzonder cultivar SRN-39 liet een betere respons zien na inoculatie met *Burkholderia tropica* IAC/BECa 135 of *Herbaspirilum frisingense* IAC/BECa 152 wat resulteerde in een grotere plantbiomassa dan bij de andere cultivars (Hoofdstuk 5). Hieruit blijkt dat deze stammen veelbelovende PGPB zijn voor gebruik als bioinoculant in de sorghum productie.

Concluderend, deze studie heeft voor het eerst de simultane invloed van de factoren plant genotype, groeistadium en bodemtype op de samenstelling van de bacteriële gemeenschap in de rhizosfeer van sorghum laten zien. Verder bleek dat cultivar SRN-39 interessante interacties

vertoonde met zijn rhizomicrobioom wat kan worden gebruikt bij de ontwikkeling van duurzame sorghum productie. Over het algemeen laten de resultaten van de studie die in dit proefschrift is beschreven het grote belang van het rhzomicrobioom zien wat ertoe kan bijdragen dat potentiele microbiële kandidaten worden gevonden die kunnen worden gebruikt in duurzame landbouwpraktijken die erop gericht zijn om de sorghum productie te verbeteren.

Resumo

Micróbios do solo podem interagir com plantas de forma positiva ou negativa. Interações positivas incluem associações simbióticas entre plantas e micróbios que promovem crescimento da planta e obtenção de nutriente, enquanto interações negativas incluem patogenicidade e competição por nutrientes. Portanto, a composição do microbioma da raiz, que está relacionada a vários fatores como espécies de plantas, genótipos de plantas e tipos de solo, é de extrema importância para o crescimento e a saúde das plantas.

Sorgo é atualmente o 5º cereal mais cultivado em todo o mundo sendo economicamente importante e usado para alimentação animal e humana, em particular por agricultores de subsistência na África subsahariana. A pesquisa descrita nesta tese de PhD explora os principais fatores que determinam a composição microbiana da raiz do sorgo, e como esta composição modula o crescimento da planta e sua tolerância por stress. As abordagens empregadas neste presente estudo foram bioensaios *in vivo*, inoculação bacteriana e sequenciamento de nova geração para avaliar a composição taxonômica da comunidade microbiana da raiz de sorgo.

Para investigar o relativo impacto dos fatores tipo de solo, genótipo da planta e dos estágios de crescimento da planta na composição da comunidade microbiana da raiz de sorgo, sete diferentes genótipos de sorgo foram cultivados em casa de vegetação em dois diferentes tipos de solo (Clue Field – CF e Vredepeel – VD) e avaliados em quatro diferentes estágios de crescimento da planta (dias 10, 20, 35 e 50). Os resultados mostraram que a composição da comunidade bacteriana foi mais fortemente influenciada pelo tipo de solo seguido pelos estágios de crescimento da planta e genótipo da planta (Capítulo 2). Ademais, nos estágios iniciais de crescimento do sorgo, a composição da comunidade bacteriana rizosférica deveu-se principalmente pelo tipo de solo, enquanto nos estágios mais tardís de crescimento o genótipo da planta tornou-se o fator mais importante (Capítulo 2). Além disso, um dos genótipos, SRN-39, que cresceu no solo abandonado CF, teve abundância relativa significativamente maiores de *Acidobacteria* GP1, *Burkholderia*, *Cupriavidus (Burkholderiaceae)*, *Acidovorax* e *Albidiferax* (*Comamonadaceae*) que os outros seis genótipos. Este resultado indica que o efeito do genótipo da planta na composição da comunidade bacteriana rizosférica de sorgo foi dependente do tipo de solo.

A fim de avaliar a dinâmica das comunidades de fungo e bacteria na rizosfera de sorgo, dois genótipos de sorgo (SRN-39 e BRS 330) cresceram em casa de vegetação em dois diferentes tipos de solo (CF e VD) e avaliados em três diferentes estágios de crescimento (dias 10, 35 e 50). Os resultados mostraram que o genótipo de sorgo SRN-39 promoveu uma co-variação mais forte entre as comunidades de bactéria e fungo quando cultivado no solo abandonado CF. Ademais, a diminuição na abundância relativa do fungo do gênero *Gibberella* ao longo do crescimento da planta foram

seguidos da diminuição das famílias bacterianas *Oxalobacteracea* e *Sphingobacteriacea*. Sugerimos com isto que deve haver uma conexão entre estes organismos, pois ambas famílias bacterianas são conhecidas por serem antagonistas as atividades fúngicas. Não obstante, esta hipótese precisa ser experimentalmente avaliada em estudos futuros (Capítulo 3).

Objetivando investigar se plantas de sorgo pré-cultivadas em solos com diferentes composições da comunidade bacteriana respondem diferentemente a condições de deficiência de água, duas linhagens de sorgo (suscetível a seca e tolerante a seca) foram cultivadas em cinco solos diferentes por 21 dias e transplantadas com sua comunidade microbiana do rizoplano para um substrato padronizado e submetidos a diferentes condições de umidade. Os resultados mostraram que em condições de deficiência hídrica, alta abundância da família *Caulobacteraceae* no rizoplano da linhagem de sorgo suscetível a seca e da familia *Rhizobiaceae* no rizoplano da linhagem tolerante a seca ocorreram em particular em plantas que foram pré-cultivadas em dois solos com histórico de regimes de baixa pluviosidade, Cerrado e Sorghum field, respectivamente. Estes resultados sugerem que o pré-cultivo de sorgo em solos com histórico de regimes de baixa pluviosidade selecionam representantes de *Alphaproteobacteria* a qual pode proporcionar uma vantagem seletiva sob condições de deficiência hídrica (Capítulo 4).

A fim de investigar potenciais atividades das bactérias promotoras de crescimento de plantas (BPCP) em sorgo, cinco estirpes de bacterias endofíticas originalmente selecionadas como BPCP em cana-de-açúcar foram inoculadas em quatro cultivares de sorgo. A inoculação dos cultivares SRN-39 e BRS330 com *Burkholderia tropica* estirpe IAC/BECa 135 ou *Herbaspirilum frisingense* estirpe IAC/BECa 152 resultou em um aumento significativo de biomassa de planta. Particularmente o cultivar SRN-39 mostrou melhor resposta de crescimento resultando em uma biomassa vegetal significativamente maior quando inoculado com a estirpe bacteriana IAC/BECa 135 (*Burkholderia tropica*) e IAC/BECa 152 (*Herbaspirillum frisingense*) comparado aos outros cultivares (Capítulo 5). Este estudo mostrou que estas estirpes bacterianas são promissoras BPCP para serem usadas como bioinoculantes no cultivo de sorgo.

Em conclusão, a pesquisa apresentada nesta tese mostrou pela primeira vez o impacto simultâneo dos fatores genótipo de planta, estágio de crescimento de planta e tipo de solo na composição da comunidade bacteriana na rizosfera de sorgo. Ademais, o genótipo de sorgo SRN-39 mostrou interessantes interações com o microbioma da raiz o qual pode ser empregado na concepção do cultivo sustentável de sorgo. No geral, os resultados descritos nesta tese evidenciam a importância de estudos da microbiota da raiz de sorgo a qual pode contribuir para que se encontre potenciais

candidatos microbianos para serem usados em práticas sustentável de manejo agrícola para o melhoramento da saúde e produtividade do sorgo.

About the author

Thiago Roberto Schlemper was born on 20th of November 1981 in Rio do Sul, Santa Catarina, Brazil. In 2004 he obtained his Bachelor degree in Ecology at the University for Development of the Itajaí High Valley (UNIDAVI) in Ituporanga, Santa Catarina, Brazil. In 2005 he worked with Faunistic and Floristic Inventory at the Amazon Rainforest. In 2009 he started to give lectures at Leonardo da Vinci's Educational Center (UNIASSELVI) in Indaial, Santa Catarina, Brazil. In the 2011 he started his Master in Environmental engineering at Regional University of Blumenau (FURB) in Blumenau, Santa Catarina, Brazil. During his master he studied the on-farm production of arbuscular mycorrhizal fungi inocula using lignocellulosic agrowastes under the supervision of Dr. Sidney Luiz Stürmer. In 2013 he obtained his M.Sc. degree and moved to The Netherlands to start his PhD project described in this thesis at the Department of Microbial Ecology of the Netherlands Institute of Ecology (NIOO-KNAW) and the Institute of Biology at Leiden University under the supervision of Prof. Dr. Hans van Veen, Prof. Dr. Jos Raaijmakers and Dr. Eiko Kuramae.

List of Publications

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Schlemper, T. R., Leite, M. F., Lucheta, A. R., Shimels, M., Bouwmeester, H. J., van Veen, J. A., and Kuramae, E. E. (2017). Rhizobacterial community structure differences among sorghum cultivars in different growth stages and soils. ***FEMS Microbiology Ecology***, *93*(8).

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Schlemper, T. R., Pulcrano, A.S., de Souza, F.A., Magalhães, P.C., Van Veen, J.A., Raaijmakers, J. and Kuramae, E.E. (2019). Impact of the rhizoplane bacterial community composition on drought tolerance of sorghum bicolor (Chapter 4, to be submitted)

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