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# Key microbial taxa in the rhizosphere of sorghum and sunflower grown in crop rotation

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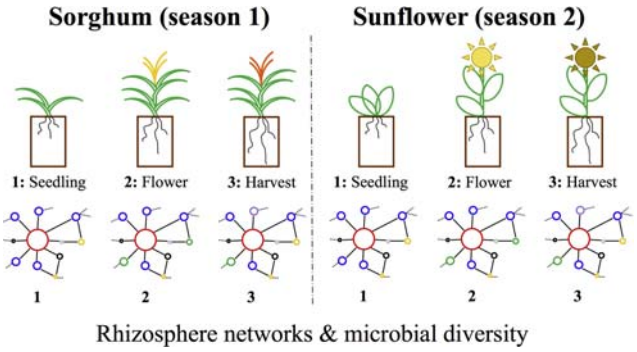
## HIGHLIGHTS

- Rhizosphere microbial diversity and composition changed over time.
- Changes were due to both plant development stage and seasonality in bulk soil biota.
- Proteobacteria and Nitrospirae were overrepresented in the rhizosphere.
- Rhizosphere networks contained keystone taxa.
- Findings may contribute to microbial-based strategies to enhance crop productivity.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Microbes are key determinants of plant health and productivity. Previous studies have characterized the rhizosphere microbiomes of numerous plant species, but little information is available on how rhizosphere microbial communities change over time under crop rotation systems. Here, we document microbial communities in the rhizosphere of sorghum and sunflower (at seedling, flowering and senescence stages) grown in crop rotation in four different soils under field conditions. A comprehensive 16S rRNA-based amplicon sequencing survey revealed that the differences in alpha-diversity between rhizosphere and bulk soils changed over time. Sorghum rhizosphere soil microbial diversity at flowering and senescence were more diverse than bulk soils, whereas the microbial diversity of sunflower rhizosphere soils at flowering were less diverse with respect to bulk soils. Sampling time was also important in explaining the variation in microbial community composition in soils grown with both crops. Temporal changes observed in the rhizosphere microbiome were both plant-driven and due to seasonal changes in the bulk soil biota. Several individual taxa were relatively more abundant in the rhizosphere and/or found to be important in maintaining rhizosphere microbial networks. Interestingly, some of these taxa showed similar patterns at different sampling times, suggesting that the same organisms may play the same functional/structural role at different plant growth stages and in different crops. Overall, we have identified prominent microbial taxa that might be used to develop microbiome-based strategies for improving the yield and productivity of sorghum and sunflower.



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## 1. Introduction

Rhizosphere microbial communities play key roles in determining plant health and productivity (Philippot et al., 2013). For instance, mycorrhizal fungi and nitrogen-fixing bacteria are responsible for 40–50% of all nitrogen (Udvardi and Poole, 2013), and up to 75% of phosphorus (van der Heijden et al., 2008), that is acquired by plants annually. Thus, the manipulation of the rhizosphere microbiome has the potential to improve the yield of agronomically important crops (Turner et al., 2013).

Previous research has shown that soil type is a major determinant of rhizosphere microbial communities, most likely as a result of the different microbial "inocula" present in each soil type (Bulgarelli et al., 2012; Edwards et al., 2015; Lundberg et al., 2012; Peiffer et al., 2013; Schlaeppi et al., 2014). Other factors such as climatic conditions, plant species, plant development stage and the interactions between all these factors are also drivers of microbial community composition (Edwards et al., 2015; Mendes et al., 2014; Peiffer et al., 2013; Zarraonaindia et al., 2015). For example, a study of the rhizosphere microbiota of three model plants (*Arabidopsis*, *Medicago* and *Brachypodium*) and three crops (*Brassica*, *Pisum* and *Triticum*) showed that all plant species tested had very different microbiota from each other and from unplanted soil, and that *Arabidopsis* had the weakest influence on its microbiota (Tkacz et al., 2015). The authors also revealed that although plants posed strong selection on rhizosphere microbial communities, their composition and stability also depended on soil type. Shi et al. (2015) found that the rhizospheric bacterial communities of *Avena fatua* changed as plants grew and that the pattern of temporal succession was consistent and repeatable over two growing seasons. In all, soil type, together with agricultural practices that directly influence the soil, are thought to be the main drivers of rhizosphere microbial communities in agricultural settings, while climate conditions and plant species are the most influential factors in the natural ecosystems (Philippot et al., 2013).

Plant roots are thought to select for specific microbes by producing an array of metabolites, including secondary metabolites such as antimicrobial compounds (Bais et al., 2006) and defence phytohormones (Lebeis et al., 2015), that vary with plant species and plant age (Lynch and Whipps, 1990). Roots also create a distinct soil microhabitat by altering the pH and oxygen concentrations in the soil surrounding the root (Hacquard et al., 2015). However, although our understanding of rhizosphere microbial communities has recently improved, many aspects of the assembly of these communities are not well understood (van der Heijden and Schlaeppi, 2015), especially for plants growing under field conditions. Most rhizospheric studies are performed on plants grown in controlled environments, with comparatively less studies conducted in the field (e.g., Edwards et al., 2015; Peiffer et al., 2013; Rascovan et al., 2016). It has been demonstrated that the small containers used in most indoor experiments have a negative impact on root function, root distribution and plant growth (Poorter et al., 2012).

In this study, we investigated the diversity and composition of the prokaryotic (bacterial and archaeal) communities in the rhizosphere of two economically important crop species, sorghum and sunflower, grown in crop rotation under field conditions in South Africa. Soil microbial communities were analysed at pre-planting and at three plant growth stages (i.e., seedling, flowering, and senescence) using Illumina MiSeq sequencing of 16S rRNA gene amplicons. The two crops were grown in four different soils located in two farms (two soils each) approximately 300 km apart. Our primary goals were: (i) to investigate how the diversity (alpha and beta components) of the soil prokaryote community associated with the roots of sorghum and sunflower grown under crop rotation change with plant development stage and soil properties and (ii) to determine whether the rhizosphere of these two contain stable "key" microbial community members. Stable key rhizosphere microbes hold great potential to influence the host phenotype (Busby et al., 2017) and could ideally be used to improve crop yield and productivity.

## 2. Materials and methods

### 2.1. Field sites and sample collection

The four fields were located in two farms (two fields each), situated approx. 300 km apart, near Settlers (Limpopo province, 27°02′43.4″ S, 27°23′46.6″ E) and Vredefort (Free State province, 24°57′09.7″ S, 28°24′01.4″ E) in South Africa (Fig. 1a). Settlers had a mean annual temperature (MAT) of 19.1 °C and a mean annual precipitation (MAP) of 606 mm during the last ten years. Vredefort had a MAT of 16.6 and a MAP of 639 mm in the same period of time. The four fields were cropped with sorghum and sunflower cultivars two years before the start of the study.

Soil sampling extended over two consecutive seasons, from 19 November 2014 to 3 March 2016, (Fig. 1b). In the first season the fields were planted with sorghum (cultivar K2) and in the second with sunflower (cultivar PAN 2057). Similar conventional culture practices were used in all fields, including ammonium nitrate-based fertilization, weed control, and pest control.

Sampling was performed at pre-planting and three different stages of growth (seedling, flowering and senescence stages) for each of the two crops grown in rotation. Bulk soil was collected prior to planting. For all remaining time points paired rhizosphere and bulk soil samples were collected. Rhizosphere soils were collected by uprooting the plants and recovering the soil firmly attached to the roots. Bulk soils were retrieved from loose soil within the field and therefore subjected to the same agriculture practices than the respective rhizosphere soil. Bulk soils contained root debris from the same crops planted in the previous two years. For each growing stage, five sites were selected along a 50 m transect in each of the four fields. At each of the five sites, three rhizosphere and three bulk soil samples were aseptically collected and homogenised in sterile plastic bags to obtain a single composite sample. For molecular analysis, aliquots of soil samples were stored at −80 °C until further processing. Overall, 260 samples were collected over two consecutive growing seasons (4 fields × 2 crops (sorghum and sunflower) × 3 growing stages (seedling, flowering and senescent) × 2 habitat types (rhizosphere and bulk soil) × 5 replicates + 20 (4 fields × 5 replicates) pre-planted bulk soil).

### 2.2. Soil nutrient analysis

Analysis of both rhizosphere and bulk soil samples was conducted at Bemlab (SANAS Accredited Testing Laboratory, Somerset West, South Africa) using standard procedures. Prior to analysis, soil samples were sieved (2 mm) and dried overnight at 50 °C. The slurry technique was used to measure pH (1:3 soil/deionised water) with a Crison Bench pH meter (Crison Instruments, Barcelona, Spain) after allowing soil to settle for 30 min. Total C and N were determined using a Truspec elemental determinator (LECO, USA). Total P was measured using the P Bray method. Ammonium acetate extraction was used to measure salt concentrations ($K^+$, $Ca^{2+}$, $Mg^{2+}$) using inductively coupled plasma atomic emission spectroscopy (ICP-OES; Spectro Genesis, Spectro Analytical Instruments GmbH, Germany).

### 2.3. DNA extraction and 16S rRNA amplicon sequencing

Total DNA was extracted from 0.5 g soil using a MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. PCRs were performed in a single-step PCR using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) with primer pairs 515F (5′-GTGYCAGCMGCCGCGGRA-3′) and 909R (5′-CCCCGYCAATTCMTTTRAG-3′), which amplify both bacteria and archaea. PCR products from all samples were quantified using the PicoGreen dsDNA assay, pooled together in equimolar concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Sequencing was carried out on an Illumina