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

Schlemper, T.R.

Citation

Schlemper, T. R. (2019, January 30). *The rhizomicrobiome of Sorghum ; impact on plant growth and stress tolerance*. *NIOO-thesis*. Retrieved from https://hdl.handle.net/1887/68467

Author: Schlemper, T.R.
Title: The rhizomicrobiome of Sorghum: impact on plant growth and stress tolerance
Issue Date: 2019-01-30

Chapter 3

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

Thiago R. Schlemper, Johannes A. van Veen, and Eiko E. Kuramae

This chapter has been published as:

Schlemper, T. R., van Veen, J. A., & Kuramae, E. E. (2017). Co-Variation of Bacterial and Fungal Communities in Different Sorghum Cultivars and Growth Stages is Soil Dependent. ***Microbial ecology***, 1-10.

https://doi.org/10.1007/s00248-017-1108-6

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^{TM} - 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).

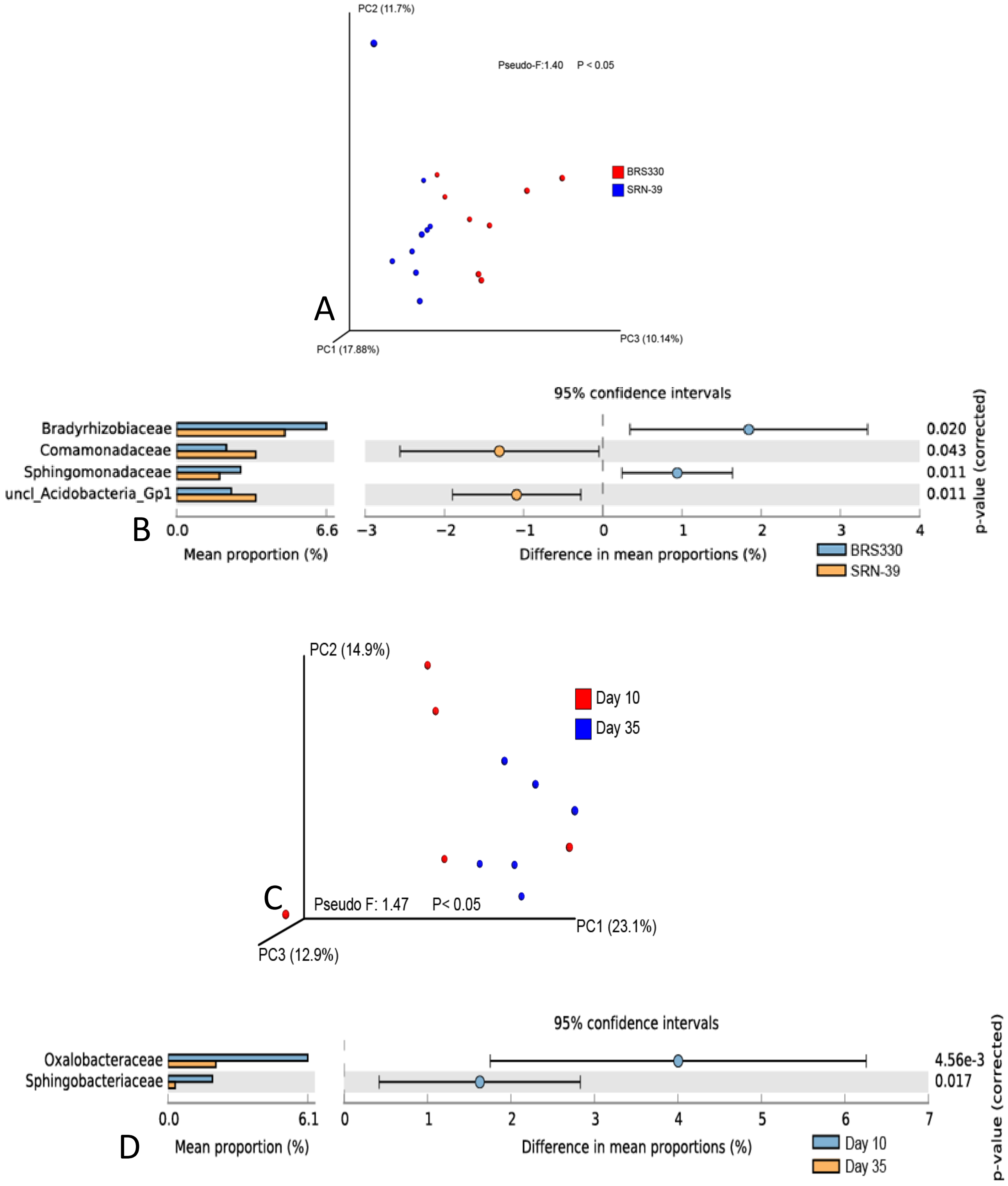

Figure 1: (A) Principal Coordinate Analysis (PCoA) and (B) differences in relative abundance of bacterial families between cultivars BRS330 and SRN-39, and (C) PCoA and (D) differences in relative abundance of bacterial families between days 10 and 35 at Clue field soil (Welch's test; $P<0.05$).

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).

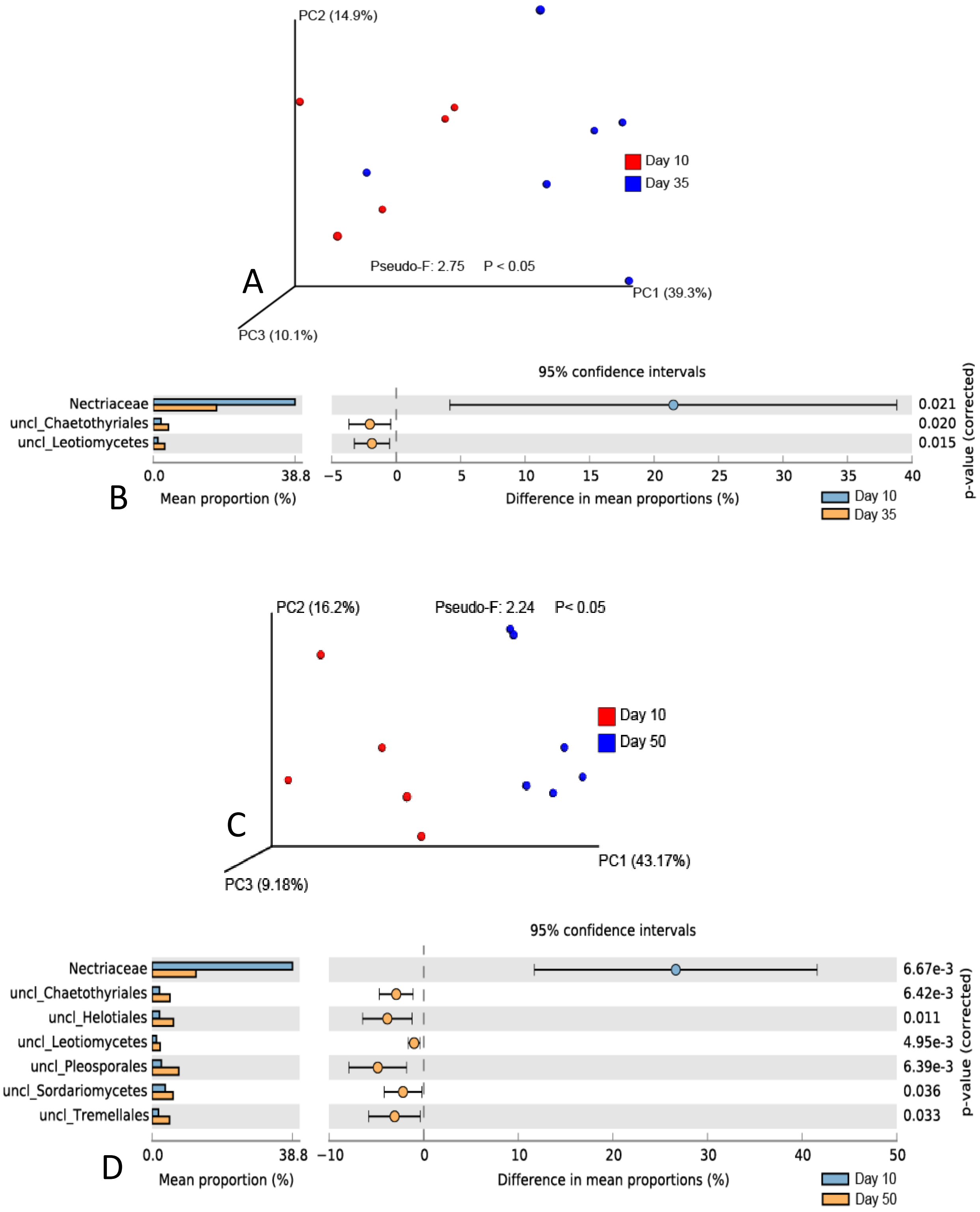

Figure 2. (A) Principal Coordinate Analysis (PCoA) and (B) differences in the relative abundance of fungi between days 10 and 35, and (C) PCoA and (D) differences in relative abundance of fungi between days 10 and 50 in Clue field rhizosphere samples (Welch's test; P<0.05).

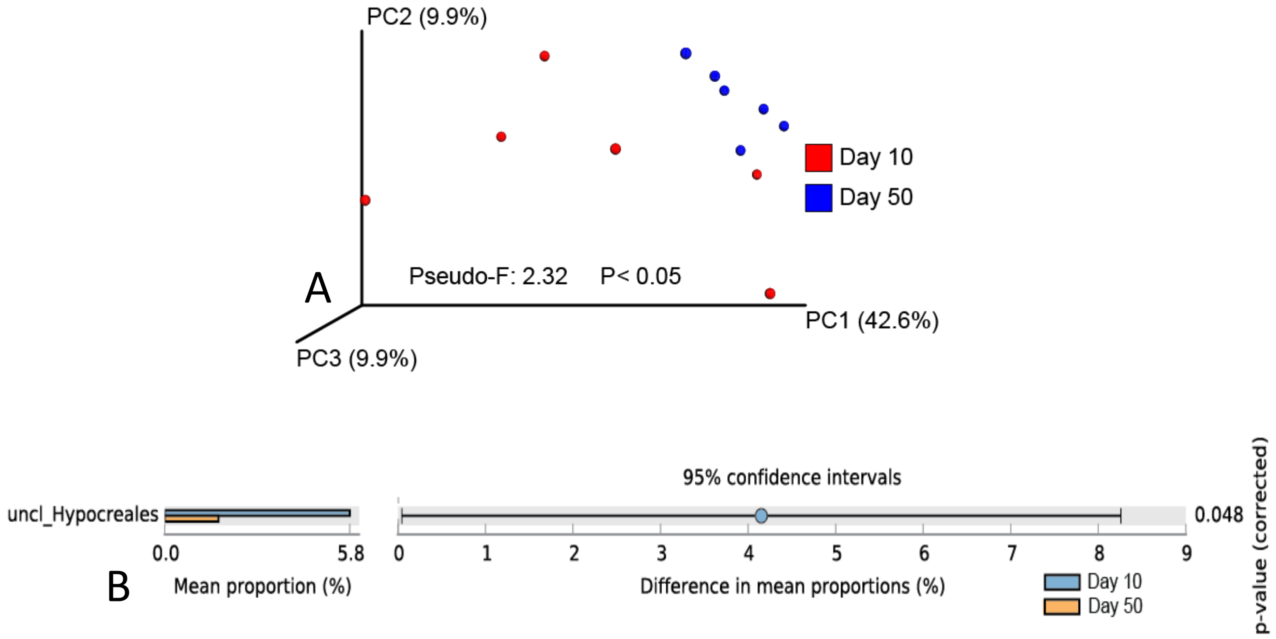

Figure 3. (A) Principal Coordinate Analysis (PCoA) and (B) differences in the relative abundance of fungi between days 10 and 50 in Vredepeel rhizosphere samples (Welch's test; P<0.05).

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).

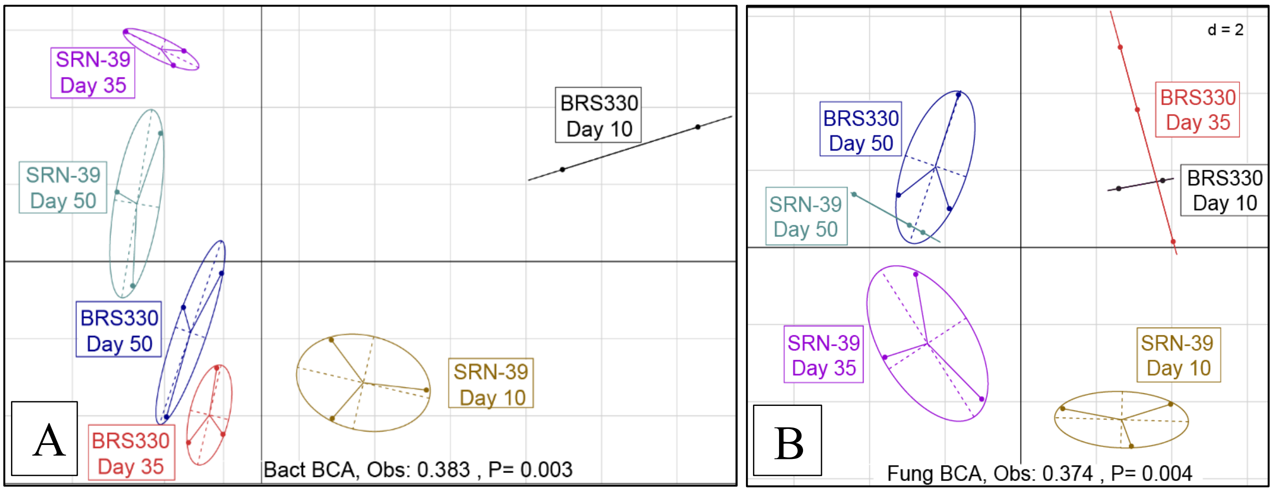

Figure 4. Between-Class Analysis (BCA) of (A) bacterial and (B) fungal communities in the rhizosphere of sorghum cultivars BRS 330 and SRN-39 at days 10, 35 and 50 of plant growth stage in Clue field soil.

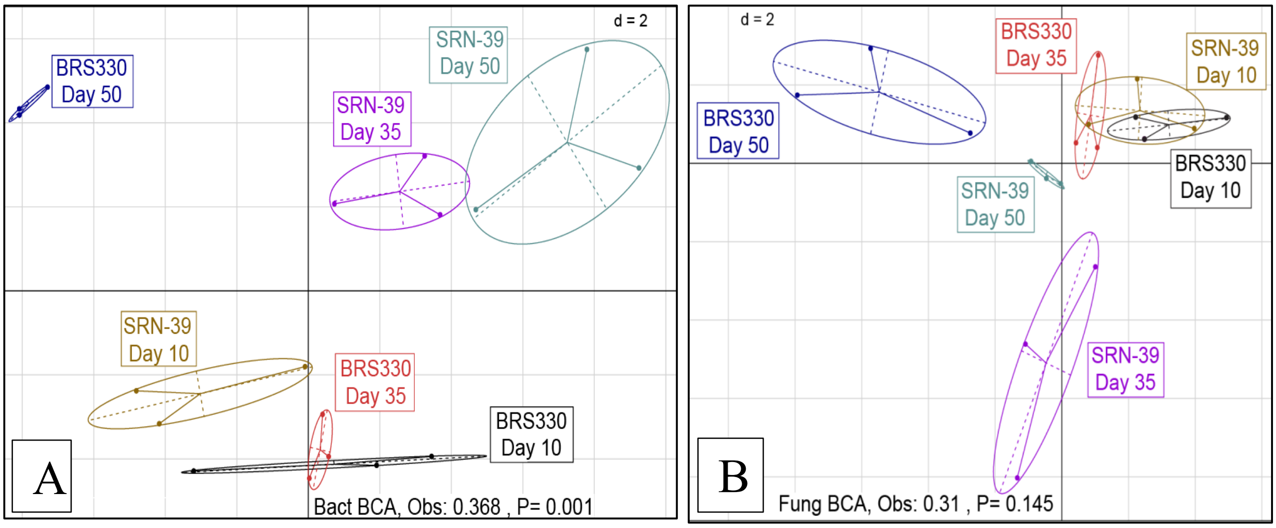

Figure 5. Between-Class Analysis (BCA) of (A) bacterial and (B) fungal communities in the rhizosphere of sorghum cultivars BRS 330 and SRN-39 at days 10, 35 and 50 of plant growth stage in Vredepeel soil.

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).

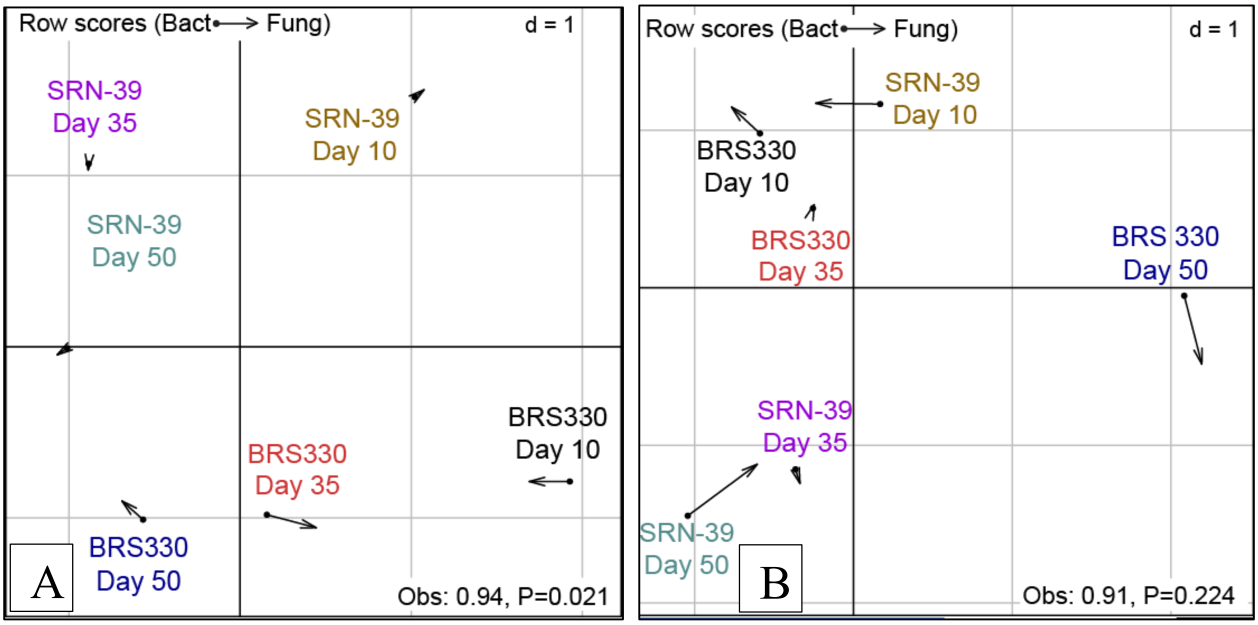

Figure 6. Co-inertia Analysis (COIA) of bacterial and fungal communities in (A) Clue field and (B) Vredepeel soils. Arrows represent the co-variation of both communities within the treatments: cultivar BRS 330 and SRN-39 at days 10, 35 and 50 of plant growth stage.

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.

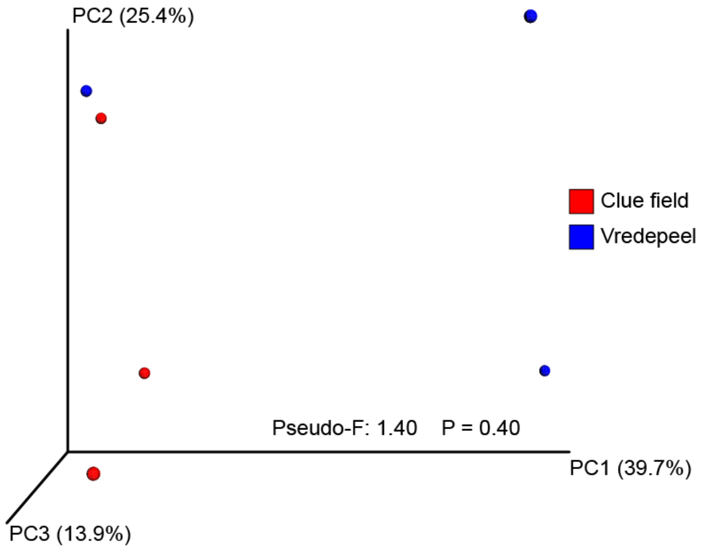

Figure S1. Principal Coordinate Analysis (PCoA) indicating the dissimilarity between bacterial community present in bulk soil of Clue field and Vredepeel soils.

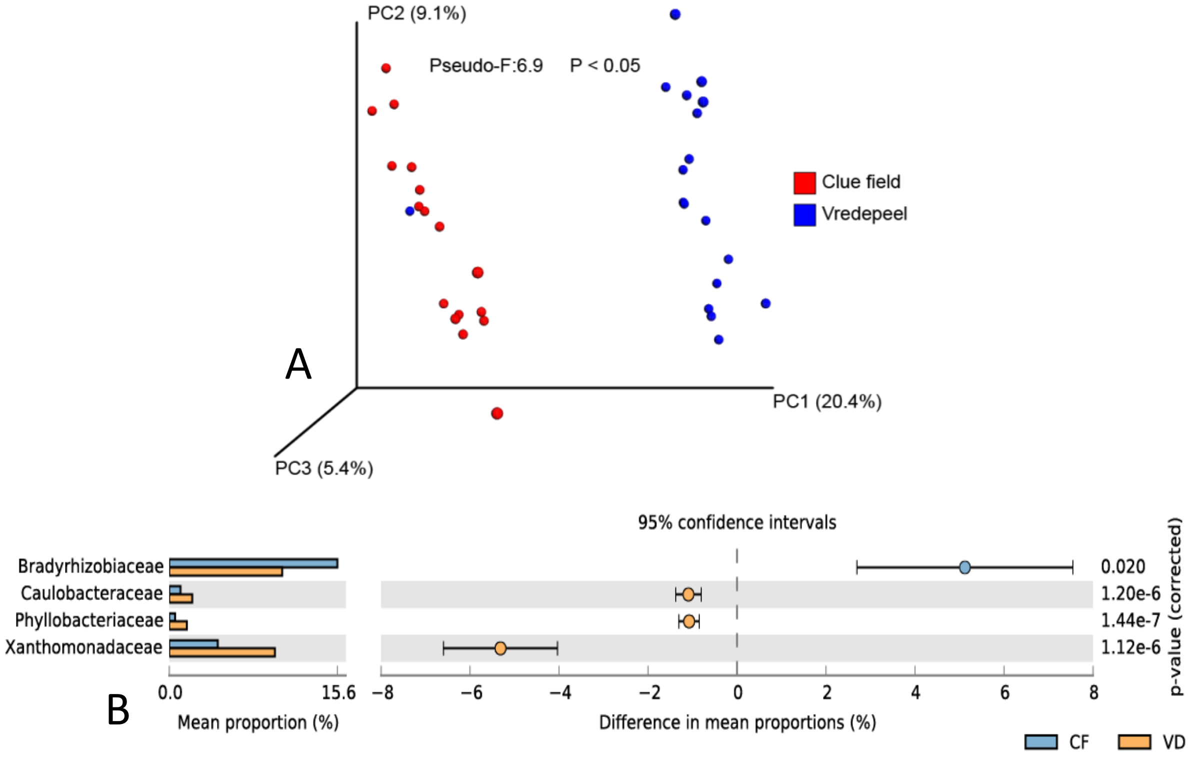

Figure S2. (A) Principal Coordinate Analysis (PCoA) and (B) differences in relative abundance of bacterial families between Clue field and Vredepeel rhizosphere samples (Welch's test; P<0.05).

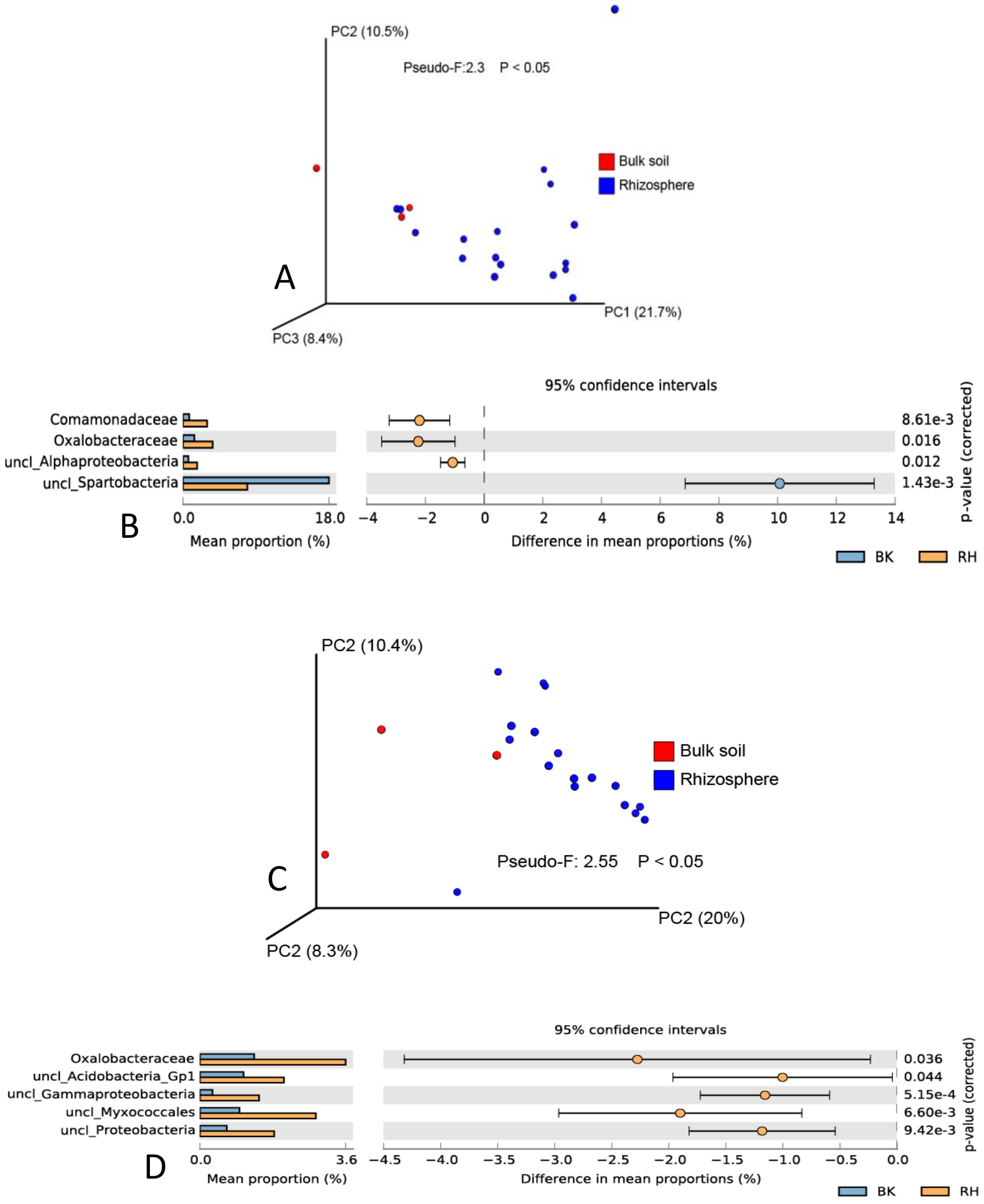

Figure S3. (A) Principal Coordinate Analysis (PCoA) and (B) differences in relative abundance of bacterial families between bulk soil and rhizosphere samples of Clue field (Welch's test; P<0.05). (C) Principal Coordinate Analysis (PCoA) and (D) differences in relative abundance of bacterial families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

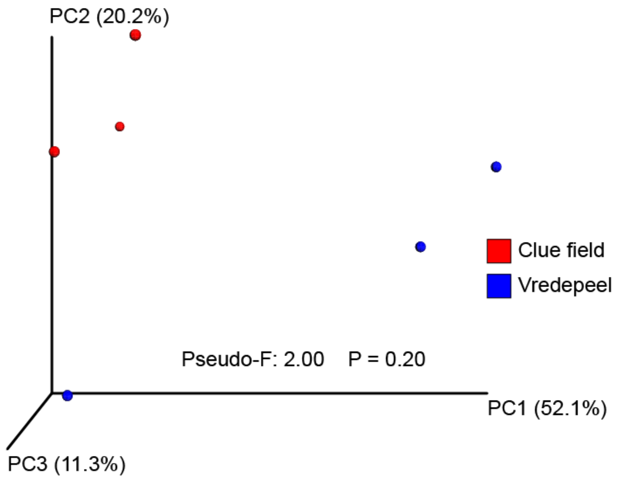

Figure S4. Principal Coordinate Analysis (PCoA) representing the dissimilarity between fungal communities present in bulk soil of Clue field and Vredepeel soils.

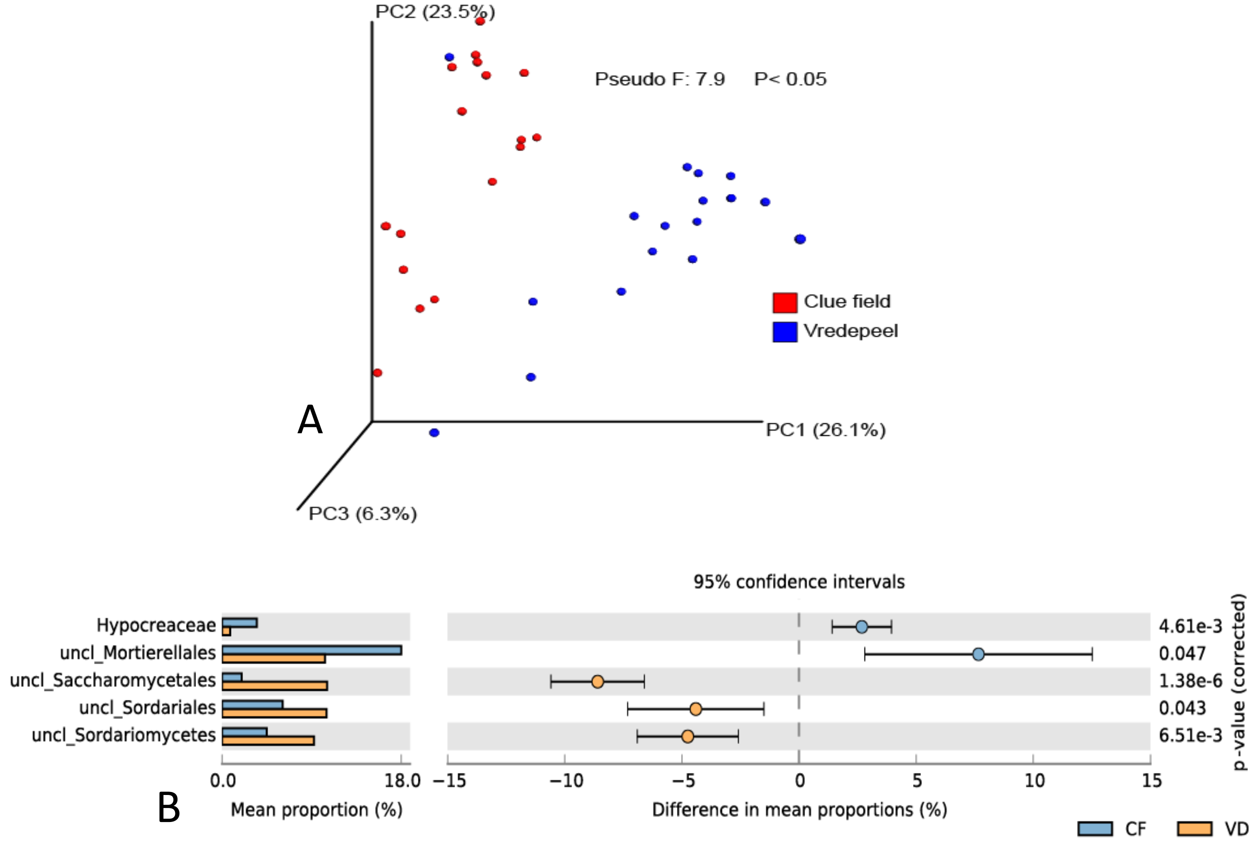

Figure S5. (A) Principal Coordinate Analysis (PCoA) and (B) differences in relative abundance of fungal families between Clue field and Vredepeel rhizosphere samples (Welch's test; P<0.05).

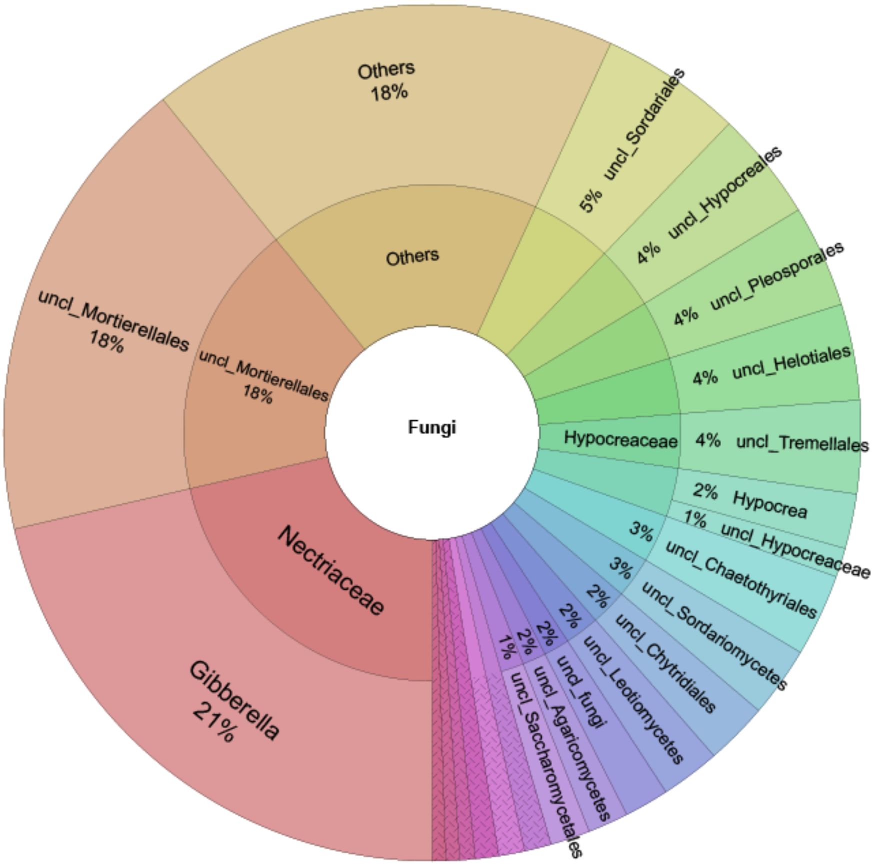

Figure S6. Relative abundance of rhizosphere fungi in Clue field soil.