Brazilian Journal of Microbiology
https://doi.org/10.1007/s42770-022-00747-9

ENVIRONMENTAL MICROBIOLOGY - RESEARCH PAPER

Phosphate fertilization affects rhizosphere microbiome of maize and sorghum genotypes

Mariana Lourenço Campolino[1] · Ubiraci Gomes de Paula Lana[2] · Eliane Aparecida Gomes[2] · Antônio Marcos Coelho[2] · Sylvia Morais de Sousa[1,2]

Received: 4 August 2021 / Accepted: 30 March 2022

Abstract

Despite the lower reactivity of natural phosphates compared to soluble fertilizers, their P bioavailability can increase over the cultivation years, due to the physicochemical processes and the activity of soil microbiota. Therefore, this work aimed to evaluate the α and β diversity of the rhizosphere microbiota of maize and sorghum genotypes grown under different sources and doses of phosphate fertilizers. Four commercial maize and four sorghum genotypes were grown under field conditions with three levels of triple superphosphate (TSP) and two types of rock phosphate sources: phosphorite (RockP) and bayóvar (RP) during two seasons. Maize and sorghum presented a significant difference on the genetic β diversity of both rhizosferic bacterial and arbuscular mycorrhizal fungi. Moreover, P doses within each phosphate source formed two distinct groups for maize and sorghum, and six bacterial phyla were identified in both crops with significant difference in the relative abundance of Firmicutes and Proteobacteria. It was observed that RockP fertilization increased Firmicutes population while Proteobacteria was the most abundant phylum after TSP fertilization in maize. In sorghum, a significant impact of fertilization was observed on the Acidobacteria and Proteobacteria phyla. TSP fertilization increased the Acidobacteria population compared to no fertilized (P0) and RockP while Proteobacteria abundance in RockP was reduced compared to P0 and TSP, indicating a shift toward a more copiotrophic community. Our results suggested that the reactivity of P source is the predominant factor in bacterial community' structures in the maize and sorghum rhizosphere from the evaluated genotypes, followed by P source.

Keywords Crop · Fertilizer · *Sorghum bicolor* · T-RFLP · *Zea mays*

Introduction

The Green Revolution allowed a significant increase of crop production, based on the introduction of hybrids and adoption of modern technologies, such as synthetic fertilizers [1]. In Brazilian agriculture, the use of synthetic phosphate fertilizers, such as the triple superphosphate (TSP), monoammonium phosphate (MAP), and diammonium phosphate (DAP) normally obtained from inorganic acid treatment of natural rock phosphates, is predominant. However, most of the applied fertilizers are not absorbed immediately by crops, and in excess can raise production costs and cause negative environmental effects [2, 3]. Thus, it is fundamental to use alternative methods to improve crop yield stability and sustainability. An alternative is a direct use of natural rock phosphates as fertilizer, which have low phosphorus (P) solubility, especially in the year of application. However, along the cultivation, the P availability can be increased by physicochemical processes and the activity of soil microbiota [4–6].

The diversity and composition of microbial taxa in the rhizosphere can be affected by several factors, including plant species, soil type, nutrient availability, soil management practices, and microbial interactions [7]. To understand the structure and function of microbial communities in different environments, several authors use techniques based on fingerprinting of conserved DNA regions [8–11]. The Terminal Restriction Fragment Length Polymorphism (T-RFLP) aproach has been extensively used to study

Responsible Editor: Luiz Henrique Rosa

✉ Sylvia Morais de Sousa
sylvia.sousa@embrapa.br

[1] Universidade Federal de São João del Rei, Sete Lagoas, MG, Brazil

[2] Embrapa Milho e Sorgo, Sete Lagoas, MG 35701-970, Brazil

structural changes in the rhizosphere of microbial community under different conditions, including phosphate fertilization, long-term tillage, and short-term nutrient addition, monitoring members of stress-tolerant bacterial inocula and determining the effects of P fertilizer enriched with beneficial bacterias strains (biofertilizer) on the microbiome [7, 12–17]. Although several high-throughput sequencing (HTS) techniques arose in the last years, T-RFLP is still widely used [7–10, 15–17]. Data juxtaposing results from T-RFLP with those generated using HTS showed that the T-RFLP analysis gave results with similar resolution levels, but with a fraction of the cost, indicating that T-RFLP is useful for screening samples before their use in more sensitive and expensive approaches [18, 19].

The study of the relationship between phosphate sources, considering different soil potential reactivity, associated with P use efficiency mechanisms of maize and sorghum genotypes and microbial communities can contribute significantly to increase the use of alternatives sources of P supporting a more sustainable agriculture. However, few studies demonstrate this impact on crops of economic importance under differente phosphate fertilization conditions in tropical regions with very clayey soils. In these soils, typical of Cerrado and other Savannah areas worldwide, phosphate fertilizers are often rapidly complexed via adsorption to iron and aluminum oxides, reducing P bioavailability to plants [20]. Moreover, P can also be complexed to or within organic compounds that may represent as much as 80% of the total P in no-till soils [21].

Although several factors influence the rhizospheric microbial communities, some microorganisms are predominant in fertilized soil samples compared to non-fertilized. Microorganisms of the P cycle can suffer direct impact on its richness and abundance by the amount of this nutrient available in the soil and the fertilizer type applied [13, 22–24]. Previous studies of our group indicated that the soil P level was the major driver of microbiome structure in the rhizosphere of two maize genotypes grown under contrasting P conditions and there was a modulation on the structure of oligotrophic to copiotrophic microorganisms when fertilizated with TSP [24]. There was an increase in the rhizosphere population of Proteobacteria, Actinobacteria, and Firmicutes when P dosages were increased. These microorganisms usually show rapid growth when sources of nutrients are more available in the soil [22–24]. In *Phaseolus vulgaris*, there was also an increase in copiotrophic bacteria at high P level whereas oligotrophic ones increased with rock phosphate application at low P levels [13].

In this work, we tested the hypothesis that the rhizospheric microbial community could be affected by maize and sorghum genotypes. In addition, the use of TSP, due to its reactivity, would result in a shift of microbial taxons from oligotrophic to copiotrophic compared to natural rock phosphates. Thus, the objective of this work was to evaluate the genetic distance, α and β diversity, and the composition of the rhizospheric microbial communities of maize and sorghum genotypes grown under field conditions with three different types and three doses of phosphate fertilizers.

Material and methods

Site background and soil preparation

The experiments were performed at Embrapa Maize and Sorghum Experimental Station in Sete Lagoas, MG, Brazil (19° 28′S, 44° 15′W, altitude of 732 m). The soil is classified as a Latossolo Vermelho Distroférrico (Oxisol), with a clayey texture (64% of clay), under savannah vegetation [25]. Prior to establishment of the experiment, between 2007 and 2017, the area was fallow, under spontaneous pasture of brachiaria (*Urochloa* sp.).

For chemical soil analysis, sampling was done before planting in the cropped areas for maize and sorghum. Each area was divided in 10 blocks measuring 20 m wide × 18 m long, and five subsamples were collected to a depth of 20 cm for each block to form a composite sample in August of 2017. Measured soil parameters, according to [26] were as follows: $pH_{water} = 5.90$; potential acidity (H + Al) = 7.36 $cmol_c\ dm^{-3}$; calcium (Ca) = 3.03 $cmol_c\ dm^{-3}$; magnesium (Mg) = 0.47 $cmol_c\ dm^{-3}$; cation exchange capacity (CEC-pH7) = 10.99 $cmol_c\ dm^{-3}$; extractable P (Mehlich1) = 5.16 mg dm^{-3}; potassium (K) = 53.80 mg dm^{-3}; base saturation = 33.40% and organic matter (OM) = 36.90 g kg^{-1}.

Four commercial maize genotypes (BRS1055, 1M1752, DKB390, and AG8088) and four commercial sorghum genotypes (BRS330, BRS373, 1G100, and DKB540) were used in this experiment. The soluble source used was the triple superphosphate (TSP) contend 45% total P_2O_5 (granulated). The rock phosphates were phosphorite (RockP) (Campos Belos, GO, Brazil) contend 18% total P_2O_5 of which 4.8% soluble in citric acid 2% (powder) and reactive rock phosphate bayóvar (RP) (Sechura Desert, Peru) contend 28% total P_2O_5 of which 13% soluble in citric acid 2% (branny). The field experiment consisted of seven fertilizer treatments: (1) plots without addition of fertilizer P (P0); (2) plots receiving RockP at a rate of 50 kg of $P_2O_5\ ha^{-1}$; (3) plots receiving RockP at a rate of 100 kg of $P_2O_5\ ha^{-1}$; (4) plots receiving RP at a rate of 50 kg of $P_2O_5\ ha^{-1}$; (5) plots receiving RP at a rate of 100 kg of $P_2O_5\ ha^{-1}$; (6) plots receiving TSP at a rate of 50 kg of $P_2O_5\ ha^{-1}\ year^{-1}$; (7) plots receiving TSP at a rate of 100 kg of $P_2O_5\ ha^{-1}\ year^{-1}$. Soil was sampled in two growing seasons 2016/2017 and 2017/2018, after fertilized plots had received about 44 kg-P $ha^{-1}\ year^{-1}$ or a cumulative 88 kg-P ha^{-1}. Crops were fertilized at sowing time with 40 kg of N ha^{-1} and 60 kg of $K_2O\ ha^{-1}$ and then again as a

side dress 30–35 days after planting with 120 kg of N ha^{-1} and 60 kg of K_2O ha^{-1}. The experiment was under no-till soil management with irrigation, when necessary. Micronutrients were not applied because soil analyses indicated that they were sufficients, with values above of the critical levels. The experimental design was a randomized complete block with three replications with a factorial of 4 × 3 × 3. The seasons were analyzed separately.

During the flowering stage, roots were collected from four maize plants and four sorghum plants from each plot. After the removal of the poorly adhered soil by manual agitation, 5 g of thin roots with rhizospheric soil were weighed and separated. The samples were transferred to conical tubes containing 35 mL of 0.1% (w/v) sodium pyrophosphate solution and shaken for 30 min in a horizontal homogenizer at a speed of 130 rpm. The roots were removed, and the samples centrifuged at 14,000 g for 30 min. The supernatant was discarded, and the soil was resuspended in 1.8 mL of sterile ultrapure water. Later, the soil was centrifuged at 14,000 × g for 4 min and the supernatant was discarded. The soil samples were frozen in liquid nitrogen and stored at − 80 °C until the total DNA was extracted from the microbial community.

DNA extraction from soil samples

Total DNA extraction from 0.45 g of rhizospheric soil samples was performed with the PowerSoil® DNA Isolation Kit (MoBio Laboratories, Inc. USA), according to the manufacturer's recommendations. The DNA was then suspended in 50 µL of Solution C6 buffer and quantified in a Nanodrop® spectrophotometer (Thermo Fisher Scientific, USA) and diluted to a concentration of 1.0 ng μL^{-1}.

16S and 28S rRNA gene amplification

Fragments of the *16S rRNA* gene were amplified using the 8F-FAM 5′AGAGTTTGATCCTGGCTCAG-3′ primer fluorescence-labeled at 5′ [27] and 1492R, 5′-GGTTAC CTTGTTACGACTT-3′ [28]. The PCR reaction consisted of 2.5 ng of DNA, each primer at 0.25 mM, reaction buffer 1X, $MgCl_2$ 3.12 mM, dNTPs 0.125 mM each, 1.25 U of Taq DNA polymerase (Invitrogen, UK) in a final volume of 50 µL. Amplification was performed with initial denaturation at 94 °C for 3 min, followed by 25 cycles at 94 °C for 45 s, 55 °C for 45 s, 72 °C for 2 min, and final extension at 72 °C for 5 min.

For the amplification of the *28S rRNA* gene from arbuscular mycorrhizal fungi (AMF), we used a nested-PCR from a reaction with primers LR1, 5′- GCATATCAATAAGCG GAGGA-3′ [29] and FLR2, 5′-GTCGTTTAAAGCCAT TACGTC-3′ [29]. The initial reaction consisted of 2.5 ng of DNA, each primer at 0.2 mM, reaction buffer 1X, $MgCl_2$ 2.5 mM, dNTPs 0.125 mM, 1.5 U of Taq DNA polymerase (Invitrogen Paisley, UK), betaine 1 mM, in a total volume of 50 µL. For the second PCR, 2.5 µL of the product of the first reaction was used and the primer FLR3 was marked with FAM 5′-TTGAAAGGGAAACGATTGAAGT-3′ [31] and FLR4 was marked with HEX 5′-TACGTCAACATCCTT AACGAA-3′ [31] at a final concentration of 0.2 mM each, 1 × reaction buffer, $MgCl_2$ 2.5 mM, dNTPs 0.125 mM, and 1.5 U of Taq DNA polymerase (Invitrogen, UK), in 50 µL. The amplifications for AMF were performed with initial denaturation at 95 °C for 5 min, followed by 35 cycles at 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, and 72 °C for 10 min. An aliquot of 1 µL of the PCR products was stained with GelRed (Biotium, Hayward, California, USA) and analyzed in 1% (w/v) agarose gel electrophoresis using 1 Kb Plus DNA Ladder (Life Technologies, USA). The amplified DNA was confirmed in a transilluminator under ultraviolet light and photographed in the L-PIX Image EX equipment (Loccus Biotecnologia, Brazil).

T-RFLP analysis

The amplified fragments were digested with the restriction enzymes *Alu*I, *Taq*I, and *Hae*III (Invitrogen, USA), according to [13, 30, 31]. To evaluate DNA fragments, 2 µL of the digestion product were added to 9.8 µL of deionized formamide (Applied Biosystems, USA) and 0.2 µL of ROX500 standard (Applied Biosystems). PCR digested products were resolved by capillary electrophoresis on Genetic Analyzer 3500XL (Applied Biosystems, USA) with the GeneMapper 5.0 software (Applied Biosystems, USA). The Terminal Restriction Fragment (T-RF) peaks with a fragment between 30 and 500 bp and fluorescence intensities higher than 40 fluorescence units (peak height) were considered for profile analysis. The T-REX program [32] was applied for the alignment of different samples and consensus profiles of two parallel runs of each sample. For creating data, matrices T-RFs with ≥ 1% relative abundance (individual peak area divided by the sum of all peak areas) were applied [33]. The relative abundances of the applied microbial species were determined by the average T-RF size values of digestions with the restriction enzymes.

Bacterial community identification

Based on the initial T-RFLP results, we analyzed the bacterial phylogenetic β diversity of maize genotype BRS1055 and sorghum genotype BRS373 under the treatments: (1) plots without addition of fertilizer P [P0]; (2) plots receiving rock phosphate (Itafós) [RockP] at a rate of 100 kg of P_2O_5 ha^{-1}, and (3) plots receiving triple superphosphate [TSP] at a rate of 100 kg of P_2O_5 ha^{-1} $year^{-1}$. The T-RFs were compared with predicted fragment length by confronting the results from three enzymes using Microbial Community

Analysis III- MiCA 3 (http://mica.ibest.uidaho.edu/) and reference soil database based on RDP r12u10 [34]. The taxonomic classification was retrieved from NCBI using the taxonomy status tool (https://www.ncbi.nlm.nih.gov/Taxonomy/TaxIdentifier/tax_identifier.cgi).

Data statistical analysis

The effect of crop and fertilization on the genetic β diversity profile of bacteria and AMF was evaluated by multidimensional non-metric scaling (NMDS) based on Bray–Curtis distance matrix and ANOSIM test ($p \leq 0.05$) using the software Past v.3.25 [35]. The effect of the crop and fertilization on the abundance of bacterial taxon was verify by analysis of variance (ANOVA) two-way and under the crop type was used ANOVA one-way followed by Tukey test at 5% probability. In addition, the Simpson α diversity index were calculated and tested by ANOVA and Tukey test at 5% probability. Simper analysis from Bray–Curtis distance matrix after Hellinger's transformation was used to evaluate the percentage contribution of each taxon identified among the crops. The families/classes taxons found were used to construct the microbial interaction network. The microbial networks were based on Pearson's correlation with $p \leq 0.05$. These analyses were conducted for the Tidyverse package [36] and "qgraph" package [37] in the software R, version 4 [38].

Results

Crop type influences bacterial and AMF composition

Maize and sorghum presented a significant difference on the β diversity composition of both bacterial and AMF, independently of the fertilization condition based on the non-metric multidimensional scaling analysis (NMDS) using the Bray–Curtis distance matrix for both seasons (Fig. 1).

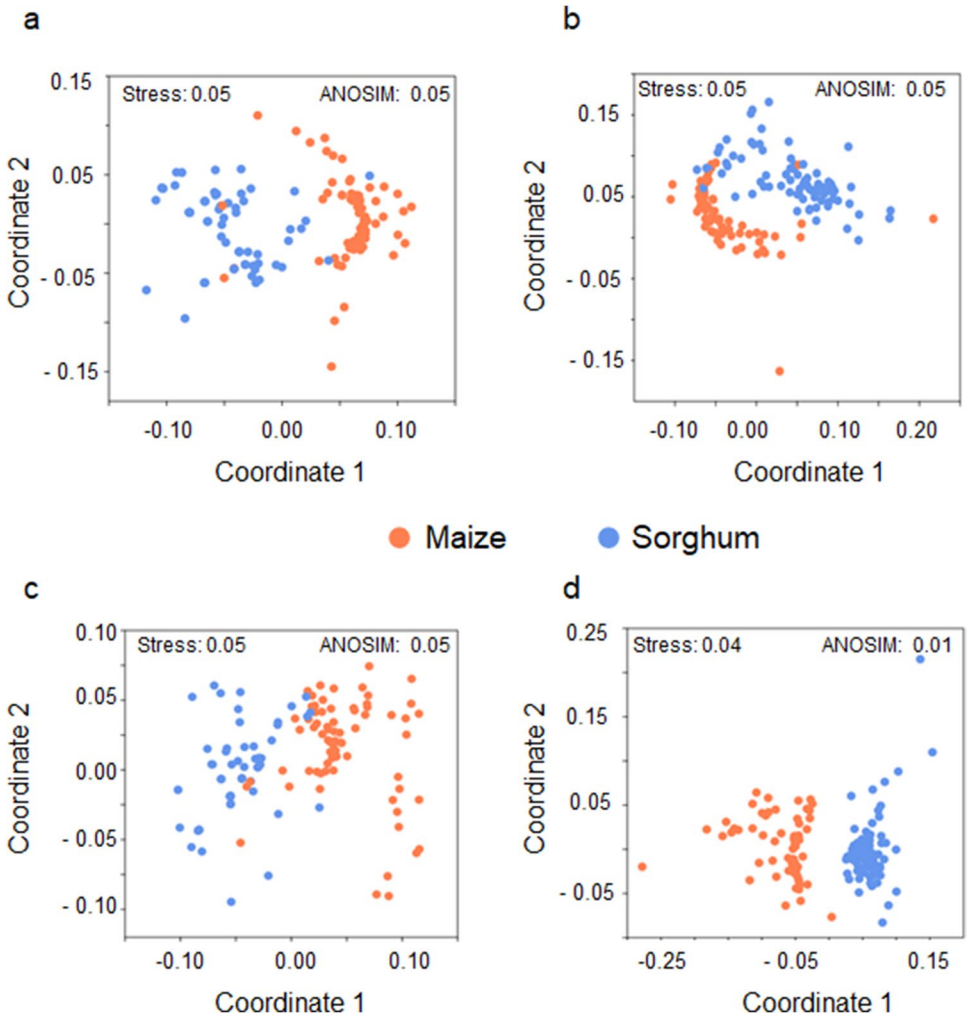

Fig. 1 Non-metric multidimensional scaling analysis (NMDS) using the Bray–Curtis distance matrix for the genetic β diversity of the maize and sorghum bacterial (**a**, **c**) and mycorrhizal arbuscular fungi (**b**, **d**) communities in the 2016/17 (**a**, **b**) and 2017/18 (**c**, **d**) seasons

Plant genotype, P fertilizer and P dose affect the β diversity of bacterial but not AMF communities.

A significant impact (ANOSIM ≤ 0.05) of the plant genotype, P fertilizer type and P dose was observed on the genetic β diversity of rhizosphere bacterial communities in the 2016/2017 (Fig. 2) and 2017/2018 seasons in both maize and sorghum (Fig. 3). However, there was no significant impact of these factors on the AMF community in each crop in the two seasons studied (Figs. S1 and S2). Due to the absence of significant effect of the plant genotype, fertilizer, and P dose on the AMF community, only the rhizospheric bacterial community was selected for depper analysis. Between the maize and sorghum genotypes, only the genotypes BRS1055 (maize) and BRS373 (sorghum) were significantly affected by at least one factor (P fertilizer type and/or P dose) in both seasons (Table S1). Based on this, these two genotypes cultived in the 2017/2018 season were selected for the analysis of the genetic β diversity of rhizosphere bacterial community.

Composition and genetic α and β diversities of rhizospheric bacterial communities of maize and sorghum cultived in different P fertilizer and P dose

The results obtained by NMDS of maize and sorghum genotypes indicated that the P fertilizer source and P dose significantly affected the structure of the bacterial community in both seasons (Fig. 4). The P dose within each P source formed two distinct groups for maize and sorghum: (I) 0 kg P_2O_5 ha^{-1} and (II) 50 and 100 kg P_2O_5 ha^{-1}. Based

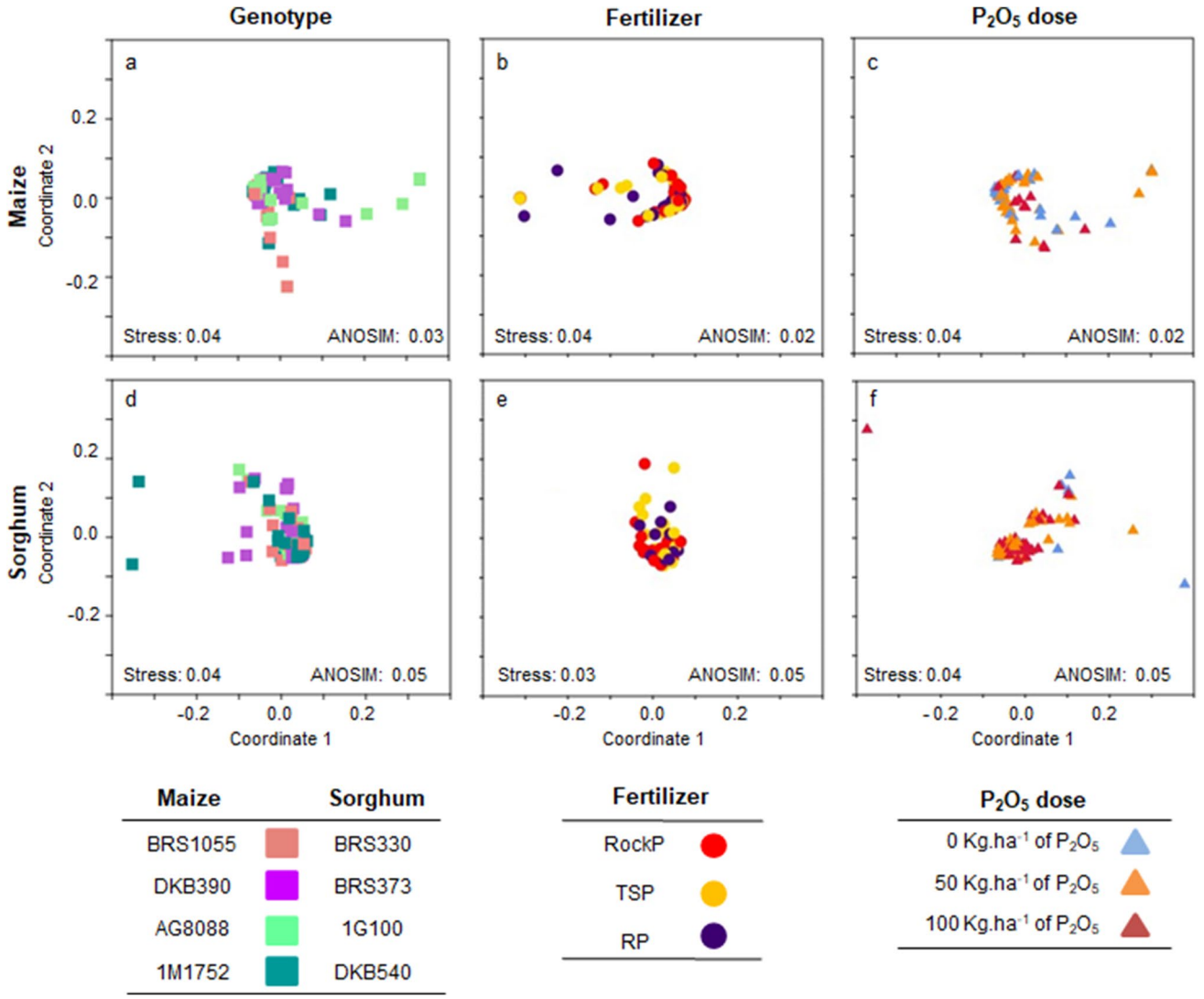

Fig. 2 Non-metric multidimensional scaling analysis (NMDS) using the Bray–Curtis distance matrix for the bacterial β diversity of different maize (**a**, **b**, **c**) and sorghum (**d**, **e**, **f**) genotypes grown under different fertilizers and P_2O_5 doses in 2016/17 season. Stress and ANOSIM $p \leq 0.05$

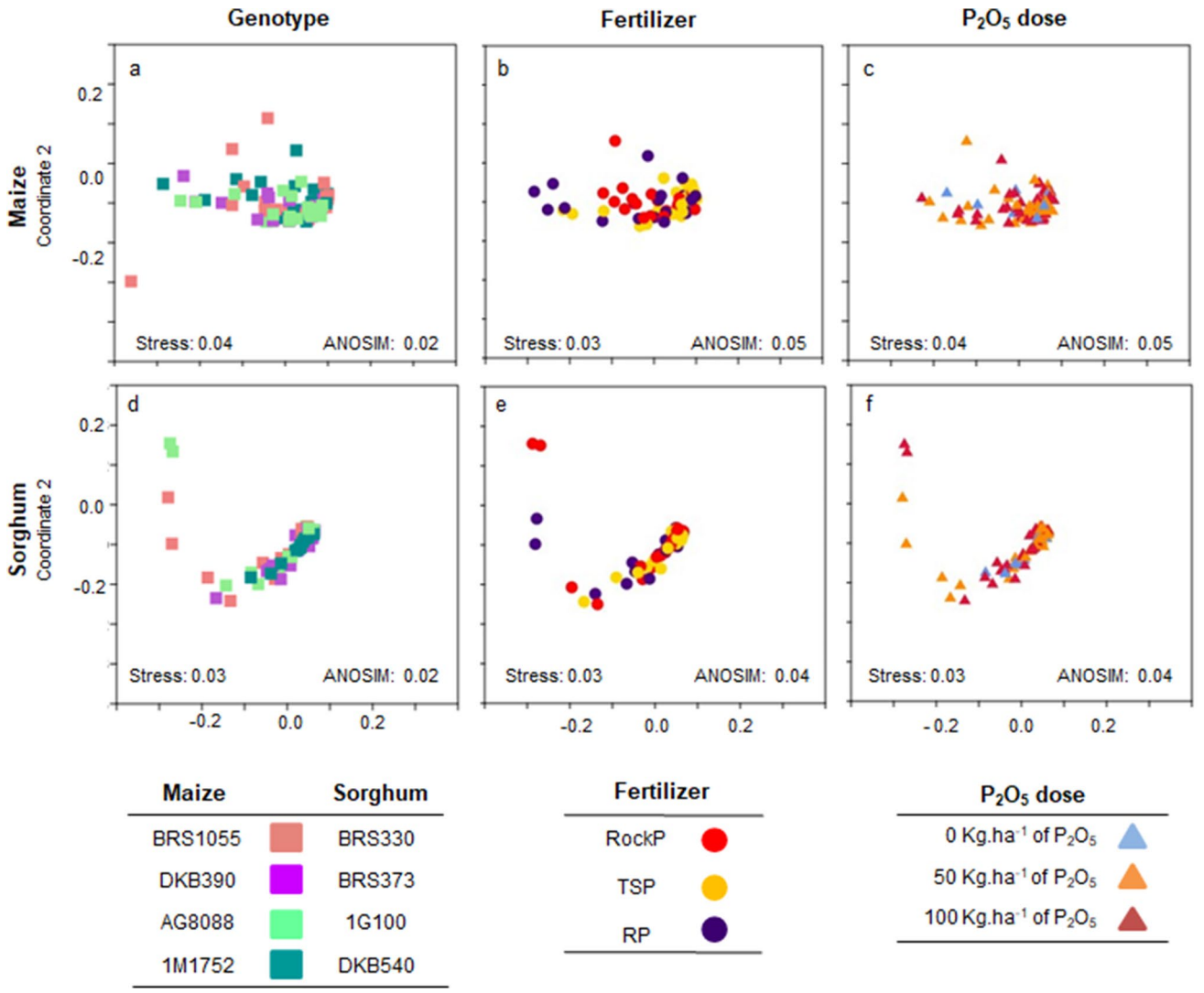

Fig. 3 Non-metric multidimensional scaling analysis (NMDS) using the Bray–Curtis distance matrix for the bacterial β diversity of differente maize (**a**, **b**, **c**) and sorghum (**d**, **e**, **f**) genotypes grown under different fertilizers and P_2O_5 doses in 2017/18 season. Stress and ANOSIM $p \leq 0.05$

on this result, the composition of the bacterial community was evaluated in the absence (P0) and presence of the two P fertilizers (RockP and TSP), both in the dosage of 100 kg of P_2O_5 ha^{-1} for maize (BRS1055) and sorghum (BRS373). The general evaluation of the bacterial composition of rhizospheric soil in maize and sorghum treatments showed five phyla with an abundance greater than 0.5%, Firmicutes, Proteobacteria, Actinobacteria, Bacterioidetes, and Acidobacteria (1%) (Table 1 and Table 2).

The influence of the crop type on the bacterial rhizospheric community analysis showed a significant effect for Firmicutes ($p = 0.003$) and Proteobacteria ($p = 0.002$) phyla (Table 1). Relative percentage of Firmicutes was higher in sorghum (53.6%) than maize (45.3%), and the opposite was observed for Proteobacteria, with 33.4% in maize and 26.5% in sorghum. There was no significant impact of the microbial phyla on the interaction between crops.

P-fertilization type significantly impacted the bacterial rhizospheric community of maize and sorghum (Table 2). Maize fertilized with RockP showed higher relative abundance of Firmicutes. However, Proteobacteria was the most abundant phyla in maize fertilized with TSP. In sorghum, we identified a significant impact of the P-fertilization on the Proteobacteria and Acidobacteria. The sorghum fertilized with TSP showed higher relative abundance of Acidobacteria than P0 and RockP. On the other side, sorghum fertilized with TSP and P0 presented greater relative abundances of Proteobacteria compared to RockP.

Families and classes were identified with an abundance greater than 5% in the rhizospheric soil of maize and sorghum genotypes (Fig. 5). According to Simper's analysis,

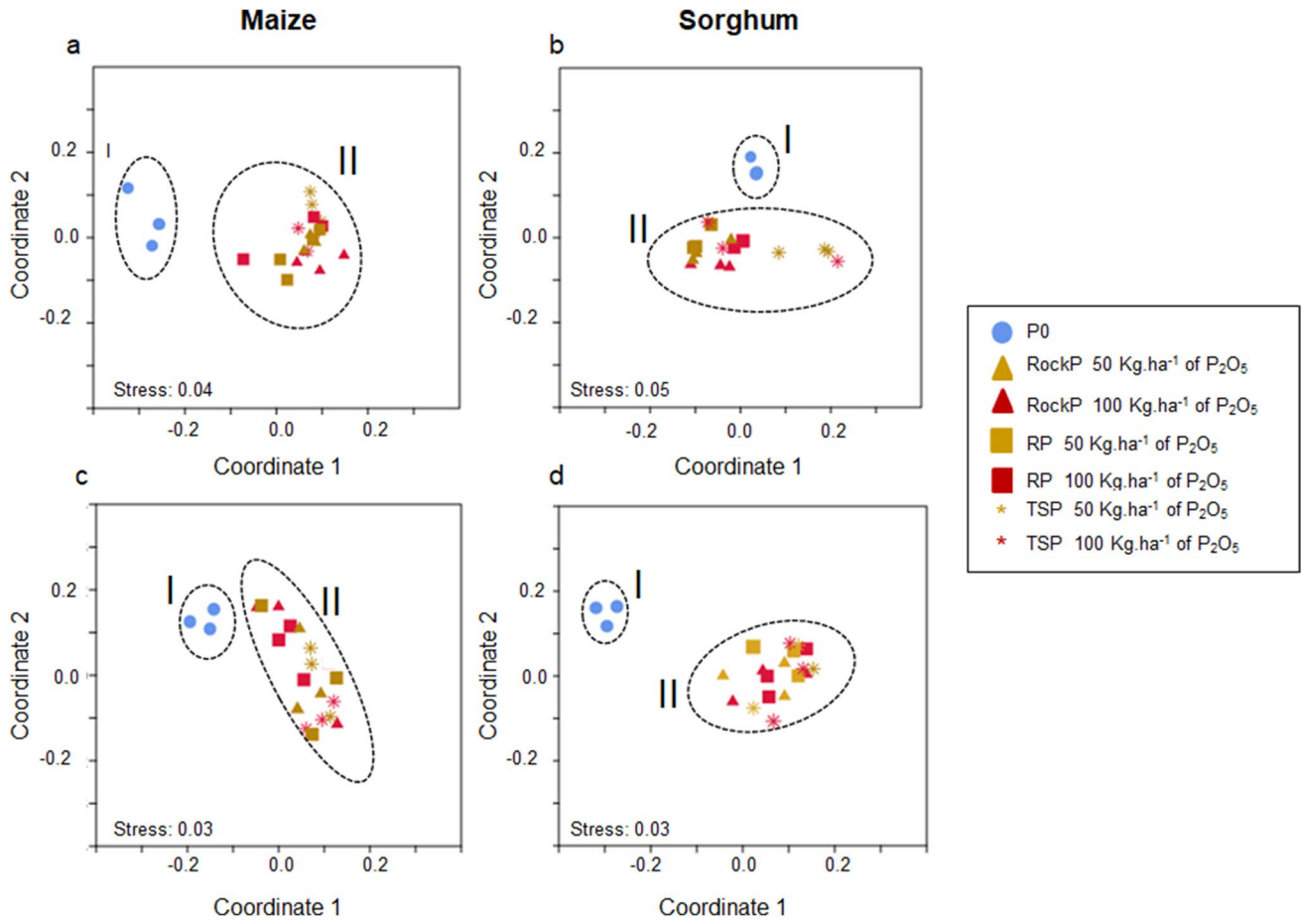

Fig. 4 Non-metric multidimensional scaling analysis (NMDS) using the Bray–Curtis distance matrix for the bacterial β diversity of the maize (**a**, **c**) and sorghum (**b**, **d**) grown under different fertilization conditions in 2016/17 (**a**, **b**) and 2017/18 (**c**, **d**) seasons. The P dose within each P source formed two distinct groups for maize and sorghum: (I) 0 kg P_2O_5 ha^{-1} and (II) 50 and 100 kg P_2O_5 ha^{-1}. Stress and ANOSIM $p \leq 0.05$

Table 1 Relative percentage of the bacterial phyla of maize and sorghum grown under different fertilization conditions in 2017/18 season

Crop	Firmicutes	Proteobacteria	Actinobacteria	Bacteroidetes	Acidobacteria
Maize	45.31[b]	33.43[a]	16.75[a]	2.84[a]	1.00[a]
Sorghum	53.58[a]	26.52[b]	15.03[a]	3.22[a]	1.06[a]

*Values with identical letters are not significantly different by Tukey's test ($p \leq 0.05$)

Table 2 Relative percentage of the bacterial phyla of maize and sorghum grown under different fertilization conditions (P0, 0 kg ha^{-1} of P_2O_5; RockP, 100 kg ha^{-1} of P_2O_5; and TSP, 100 kg ha^{-1} of P_2O_5) in 2017/18 season

Crop	Fertilizer	Firmicutes	Proteobacteria	Actinobacteria	Bacteroidetes	Acidobacteria
Maize	P0	47.41[ab]	31.42[b]	16.45[a]	3.20[a]	0.93[a]
	RockP	50.14[a]	28.58[c]	17.04[a]	2.62[a]	1.00[a]
	TSP	38.40[b]	40.29[a]	16.76[a]	2.70[a]	1.12[a]
Sorghum	P0	55.16[a]	27.98[a]	15.97[a]	3.27[a]	0.95[b]
	RockP	54.37[a]	24.00[b]	16.43[a]	2.82[a]	0.96[b]
	TSP	51.19[a]	28.44[a]	12.67[a]	3.57[a]	1.27[a]

*Values with identical letters are not significantly different by Tukey's test ($p \leq 0.05$)

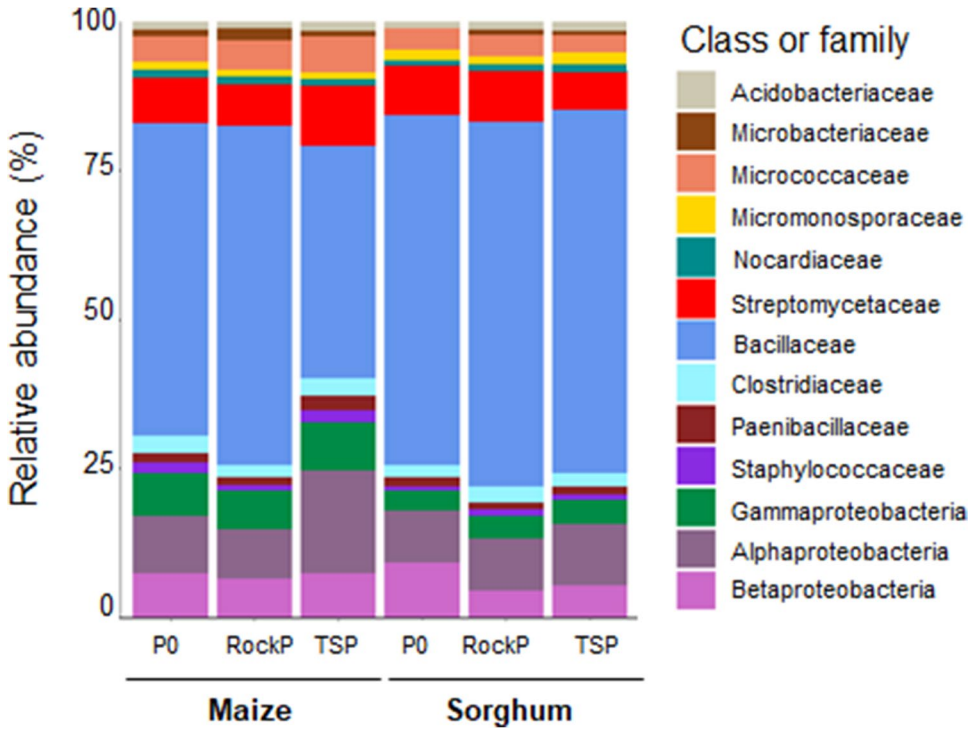

Fig. 5 Relative abundance of class or family of bacteria community in maize and sorghum rhizosphere in 2017/18 season with an abundance greater than 5%

Table 3 Simper analysis from twenty-nine families identified with an abundance greater than 3% in the rhizosphere soil of maize and sorghum grown under different fertilization conditions in 2017/18 season

Family	Dissimilarity (%)	Cumulative contribution (%)
Bacillaceae	16.14	62.1
Streptomycetaceae	3.63	76.1
Pseudomonadaceae	2.85	87
Burkholderiaceae	1.76	93.77
Methylobacteriaceae	1.62	100
Total	26	

the families Bacillaceae, Streptomycetaceae, Pseudomonadaceae, Burkholderiaceae, and Methylobacteriaceae were responsible for dissimilarity rate of 26% among the maize and sorghum bacterial communities (Table 3). Using the families and classes shown in Fig. 5, bacterial networks based on Pearson's correlation were built for the rhizospheric soil samples of maize and sorghum and one of all fertilization treatments (Fig. 6). In each network, 25 nodes and 16 edges were found. In maize, we found mostly positive links among taxa, but the Alphaproteobacteria class showed to be the taxon with the highest number of negative correlations with others, especially with the Microbacteriaceae, Micrococcaceae, Bacillaceae, and Nocardiaceae families. In sorghum, there was a positive correlation among almost all taxa; however, the Betaproteobacteria class stood out for the negative correlation with the Microbacteriaceae and Nocardiaceae families. Finally, fertilization the network showed only positive correlations among families.

Based on the bacterial families identified, Simpson's index was calculated to assess the α diversity of the rhizopheric communities between maize and sorghum. A significant effect of the bacterial α diversity was observed between crops (Fig. 6a), but no effect was observed on P fertilization (Fig. 6b).

Discussion

In our study, maize and sorghum presented a significant difference on the β diversity composition of both bacterial and AMF communities from rhizosphere, independently of the fertilization condition in both seasons (Fig. 1). The composition of rhizosphere communities is largely determined by the plant species with which they are associated [39], primarily by selection for microbes capable of utilizing the C source profile produced by the roots [40, 41]. We observed a greater relative abundance of taxa associated with efficient carbon mineralization, such as Firmicutes, Betaproteobacteria, and Bacteroidetes, and an underrepresentation of the Acidobacteria, which have been shown to correlate negatively with carbon mineralization (Table 1 and Table 2). Maize presented less Firmicutes and more Proteobacteria than sorghum (Table 1). On the other hand, in sorghum, it was observed a significant impact of

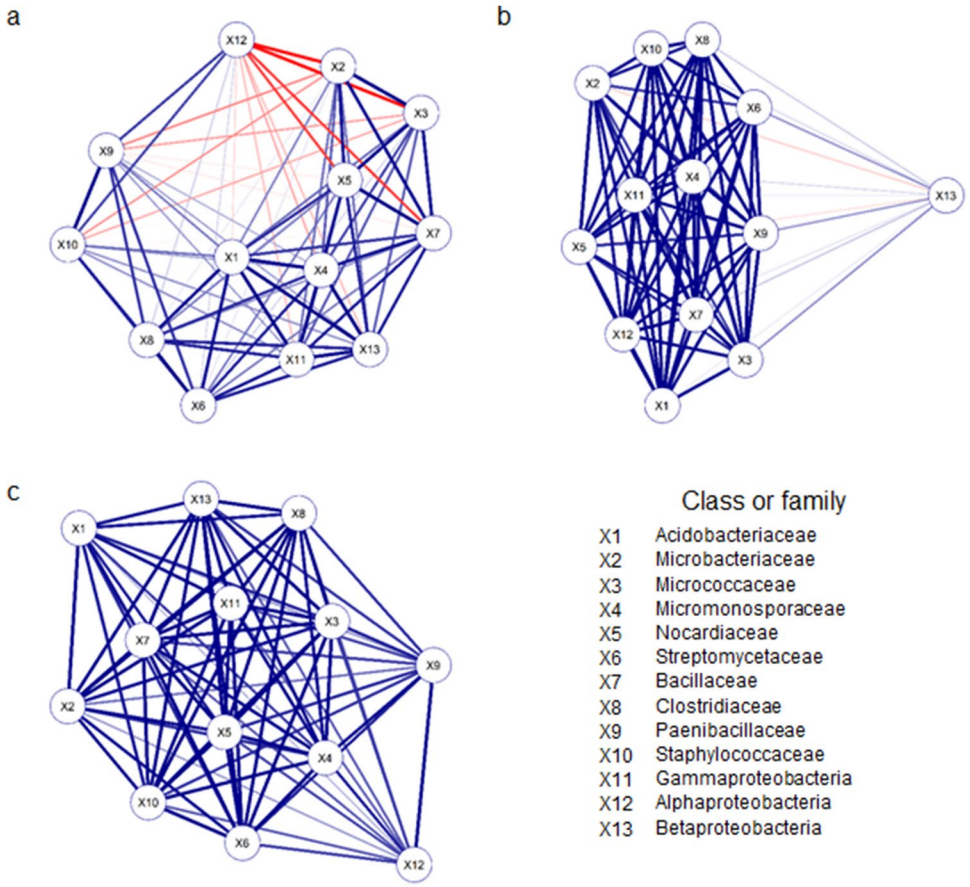

Fig. 6 Network analysis showing the connectedness among class or family with an abundance greater than 5% of bacteria in the rhizosphere of maize (**a**) and sorghum (**b**) under fertilization treatments (**c**) of 2017/18 season. The nodes (white dots) imply the taxa involved in the networks. The links mean the relationship among the nodes. Blue and red lines represent positive and negative correlations, respectively, and the thickness correspond to Pearson's correlation significance

fertilization on the Acidobacteria and Proteobacteria phyla (Table 2). The increased microbial carrying capacity is likely directly related to the greater nutrient flux in absorptive fine roots and corresponds to increases in taxa known for their copiotrophic lifestyles (e.g., Betaproteobacteria and Bacteroidetes). Plant-specific rhizodeposites form an important mechanism in modulating the soil bacterial community to communities adapted to their host, with reduced α diversity and greater abundance of central taxons [42]. The plant evolutionary history seems to play an important role in the microbial community. The increase in phylogenetic distance between plant species favors the widening of differences between communities [43, 44]. These disparities in rhizospheric microbiome do not only occur between species but are also present between different genotypes of the same species [9, 45]. In addition to the evolutionary effect on crop domestication, soil management limited the evolutionary potential of crop-related microbial interactions [46].

Although it has been demonstrated that high levels of inorganic P in soil can suppress AMF root colonization and phosphatase activity, while organic P can have the opposite effect, affecting rhizospheric microbiota [47, 48], genotype, fertilizer, and P dose factors were not significant in the AMF community for both crops and the two periods evaluated (Fig. S1 and S2). It is known that increasing P application markedly decreases AMF colonization of maize [49, 50]. The similarity between the genetic β diversity of AMF community found in this study may be related to the fact that phosphate fertilizer sources are acting on the relative abundance of microorganisms and not necessarily on microbial β-diversity, as also demonstrated by [22], who evaluated the effect of phosphate fertilization on the rhizosphere bacterial and fungal community of wheat and beans grown in a consortium system.

We observed that the genotype played an important role in the structuring of the rhizospheric bacterial community (Figs. 2 and 3). These results corroborated several studies that revealed a significant effect of the genotype on the microbial communities of the rhizosphere and on the regulation of ecological services provided by plant-associated microbes with the plant. Similar effect of the genotype was observed in rhizosphere communities associated with other plant species [9, 43, 51–54]. In maize, our previous study showed a small but significant fraction of bacterial and fungal α diversity attributed to the host genotype considering maize genotypes with contrasting P use efficiency grown in soils with two P level [24]. In sorghum, the genotype effect on rhizospheric bacterial communities was also observed by high yield sequencing of the *16S rRNA* gene fragment [55].

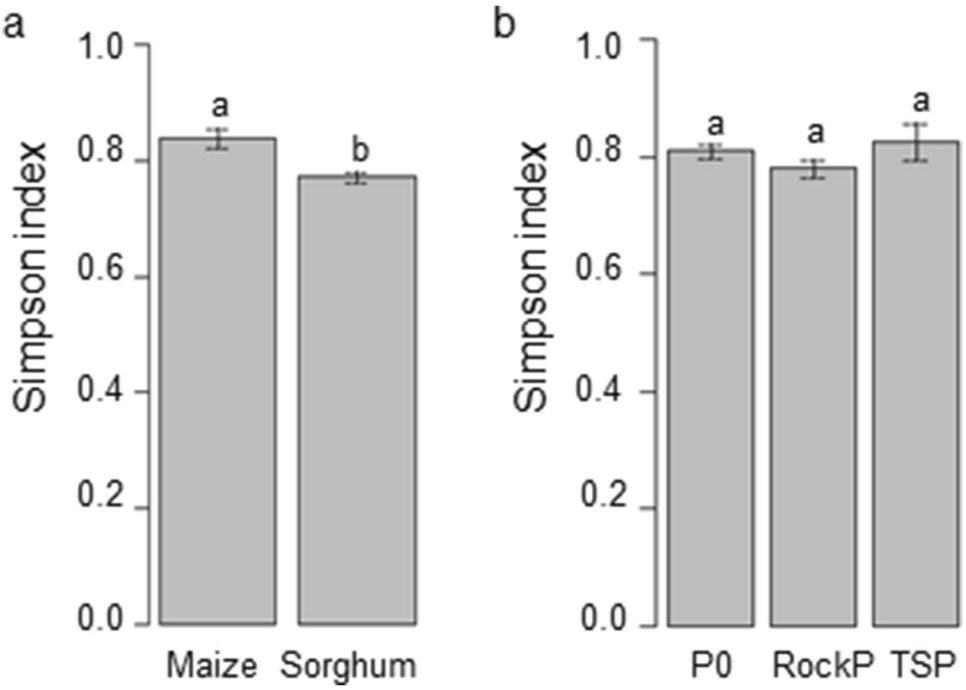

Fig. 7 Impact of the crop (**a**) and fertilization (**b**) on the Simpson index (α diversity) in 2017/2018 season. Values with identical letters are not significantly different by Tukey's test ($p \leq 0.05$)

The impact of the presence and absence of fertilization was evidenced by the differentiation of two groups (Fig. 4), and corroborated the results of other studies that demonstrated the effect of P fertilization promoting differences between microbial communities [56–58]. According to Simper's analysis, the Bacillaceae family was responsible for the greatest dissimilarity rate between the bacterial communities in maize and sorghum (Table 3). Furthermore, this family contributed with more than 50% of the relative abundance of bacteria in maize and sorghum rhizosphere cultivated in Rock P (Fig. 5). The Burkholderiaceae, Streptomycetaceae, and Methylobacteriaceae families were related to the dissimilarity rate between the rhizospheric bacterial communities between the two crops (Table 3). In maize, we also observed, a greater abundance of the Burkholderiaceae, Streptomycetaceae, Methylobacteriaceae, and Rhizobiaceae families in soil fertilized with TSP. These families are included in the phylum Proteobacteria, that are copiotrophic organisms with a strong tolerance to the acidic environment [59], abundant in Brazilian Savannah (Cerrado) soils. The network analysis (Fig. 6) corroborate our tested hypothesis that when the availability of nutrients (P) in the soil increased there is a remodeling of the bacterial structure, with prevalence of fast-growing rates species. On the other side, rock substrate is an oligotrophic (nutrient-poor) soil ecosystems, which contain a wide diversity of bacterial communities [60]. Certainly, some microorganisms suffer direct impact on its richness and abundance and are related to the P cycle in the soil, contributing to plant nutrition [13, 23, 24]. We showed previously that although there is no significant difference in crop performance between the fertilizer sources, there is a difference in the accumulation of fertilizer P into pools of organic soil P and that phosphatase activity was the only biological parameter influenced by P fertilization [61].

There was a significant impact between crops and no significant effect between fertilization, according to α diversity calculated based on the presence of bacterial families (Fig. 7). Maize showed greater rhizospheric bacterial α diversity than sorghum. Short- and long-term studies have shown that the structure of the microbial community can be altered when high levels of P are added to the soil [51, 62]. Report by [63] indicates that after decades of phosphate application, the abundance and α diversity of the bacterial community was increased in pastures and the taxonomic composition has also changed. However, a study evaluating the response of the bacterial community in soil from a long-term phosphorus fertilization trial indicated that soil P differences contribute only 3.4% of the variation in soil bacterial communities, suggesting that the soil microbiome is largely resilient to long-term P fertilization [64].

In summary, our results expand knowledge on the microbial community structures from maize and sorghum grown in soil under different phosphate fertilization conditions and reinforce the potential of using rock phosphate in soil fertility replenishment, especially in Brazilian tropical weathered soils.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42770-022-00747-9.

Acknowledgements This work was supported by Empresa Brasileira de Pesquisa Agropecuaria, Embrapa (Grant number 12.14.10.003.00.00) and Conselho Nacional de Desenvolvimento Científico e Tecnologico, CNPq/INCT-Plant-Growth "Promoting Microorganisms for Agricultural Sustainability and Environmental Responsibility" (Grant number 465133/2014–2, Fundação Araucaria-STI, Capes). MLC was recipient of a research fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior, Capes.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Bindraban PS, Dimkpa CO, Pandey R (2020) Exploring phosphorus fertilizers and fertilization strategies for improved human and environmental health. Biol Fertil Soils 56:299–317. https://doi.org/10.1007/s00374-019-01430-2
2. Cordell D, White S (2011) Peak phosphorus: clarifying the key issues of a vigorous debate about long-term phosphorus security. Sustainability 3:2027–2049. https://doi.org/10.3390/su3102027
3. Chowdhury RB, Moore GA, Weatherley AJ, Arora M (2017) Key sustainability challenges for the global phosphorus resource, their implications for global food security, and options for mitigation. J Clean Prod 140:945–963. https://doi.org/10.1016/j.jclepro.2016.07.012
4. Rashid M, Khalil S, Ayub N, Alam S, Latif F (2004) Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. Pak J Biol Sci 7:187–196. https://doi.org/10.3923/pjbs.2004.187.196
5. Mendes GO, De Freitas ALM, Pereira OL, Da Silva IR, Vassilev NB, Costa MD (2014) Mechanisms of phosphate solubilization by fungal isolates when exposed to different P sources. Ann Microbiol 64:239–249. https://doi.org/10.1007/s13213-013-0656-3
6. Timmusk S, Behers L, Muthoni J, Muraya A, Aronsson AC (2017) Perspectives and challenges of microbial application for crop improvement. Front Plant Sci 8:49. https://doi.org/10.3389/fpls.2017.00049
7. Kari A, Nagymáté Z, Romsics C, Vajna B, Kutasi J, Puspán I, Kárpáti E, Kovács R, Márialigeti K (2019) Monitoring of soil microbial inoculants and their impact on maize (Zea mays L.) rhizosphere using T-RFLP molecular fingerprint method. Appl Soil Ecol 138. https://doi.org/10.1016/j.apsoil.2019.03.010
8. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res 41. https://doi.org/10.1093/nar/gks808
9. Peiffer JA, Spor A, Koren O, Jin Z, Tringe SE, Dangl EE, Ley BR (2013) Diversity and heritability of the maize rhizosphere microbiome under field conditions. PNAS USA 110:6548–6553. https://doi.org/10.1073/pnas.1302837110
10. Yang Y, Wang N, Guo X, Zhang Y, Ye B (2017) Comparative analysis of bacterial community structure in the rhizosphere of maize by high-throughput pyrosequencing. Plos One 12:e0178425. https://doi.org/10.1371/journal.pone.0178425
11. De Vrieze J, Ijaz UZ, Saunders AM, Theuerl S (2018) Terminal restriction fragment length polymorphism is an "old school" reliable technique for swift microbial community screening in anaerobic digestion. Sci Rep 8:16818. https://doi.org/10.1038/s41598-018-34921-7
12. Bissett A, Brown MV, Siciliano SD, Thrall PH (2013) Microbial community responses to anthropogenically induced environmental change: towards a systems approach. Ecol Lett 1:128–139. https://doi.org/10.1111/ele.12109
13. Trabelsi D, Cherni A, Eddine BZA, Dhane-Fitouri S, Mhamdi R (2017) Fertilization of *Phaseolus vulgaris* with the Tunisian rock phosphate affects richness and structure of rhizosphere bacterial communities. Appl Soil Ecol 114. https://doi.org/10.1016/j.apsoil.2016.11.014
14. Macik M, Gryta A, Frac M (2020) Biofertilizers in agriculture: an overview on concepts, strategies and effects on soil microorganisms. Adv Agron 162:31–87. https://doi.org/10.1016/bs.agron.2020.02.001
15. Ji Y, Conrad R, Xu H (2020) Responses of archaeal, bacterial, and functional microbial communities to growth season and nitrogen fertilization in rice fields. Biol Fertil Soils 56. https://doi.org/10.1007/s00374-019-01404-4
16. Li P, Chen W, Han Y, Wang D, Zhang Y, Wu C (2020) Effects of straw and its biochar applications on the abundance and community structure of CO_2-fixing bacteria in a sandy agricultural soil. Journal of Soils and Sediments. 20. https://doi.org/10.1007/s11368-020-02584-5
17. Bánfi R, Pohner Z, Szabó A, Herczeg G, Kovács GM, Nagy A, Márialigeti K, Vajna B (2021) Succession and potential role of bacterial communities during *Pleurotus ostreatus* production. FEMS Microbiol Ecol 97. https://doi.org/10.1093/femsec/fiab125
18. Karczewski K, Riss HW, Meyer EI (2017) Comparison of DNA-fingerprinting (T-RFLP) and high-throughput sequencing (HTS) to assess the diversity and composition of microbial communities in groundwater ecosystems. Limnologica 67:45–53. https://doi.org/10.1016/j.limno.2017.10.001
19. Johnston-Monje D, Mejia JL (2020) Botanical microbiomes on the cheap: Inexpensive molecular fingerprinting methods to study plant-associated communities of bacteria and fungi. Appl Plant Sci 8:e11334. https://doi.org/10.1002/aps3.11334
20. Novais RF, Smyth TJ (1999) Fósforo em solo e planta em condições tropicais. Universidade Federa de Viçosa, Viçosa
21. Marschner P, Solaiman Z, Rengel Z (2006) Rhizosphere properties of poaceae genotypes under P-limiting conditions. Plant Soil 283:11–24. https://doi.org/10.1007/s11104-005-8295-5
22. Tang XY, Placella SA, Dayde F, Bernard L, Robin A, Journet EP, Justes E, Hinsinger P (2016) Phosphorus availability and microbial community in the rhizosphere of intercropped cereal and legume along a P-fertilizer gradient. Plant Soil 407:119–134. https://doi.org/10.1007/s11104-016-2949-3
23. Silva UC, Medeiros JD, Leite LR, Morais DK, Cuadros-Orellana S, Oliveira CA, de Paula Lana UG, Gomes EA, Dos Santos VL (2017) Long-term rock phosphate fertilization impacts the microbial communities of maize rhizosphere. Front Microbiol 8:1266. https://doi.org/10.3389/fmicb.2017.01266
24. Gomes EA, Lana UGP, Quensen JF, de Sousa SM, Oliveira CA, Guo J, Guimarães LJM, Tiedje JM (2018) Root-associated microbiome of maize genotypes with contrasting phosphorus use efficiency. Phytobiomes 2. https://doi.org/10.1094/PBIOMES-03-18-0012-R
25. Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JA, Cunha, TJF, Oliveira JB (2013) Sistema brasileiro de classificação de solos. Embrapa, Brasília
26. Embrapa, (1997) Manual de métodos e análise de solo. Brasil, Rio de Janeiro
27. La Montagne MG, Michel FC Jr, Holden PA, Reddy CA (2002) Evaluation of extraction and purification methods for obtaining PCR-amplifiable DNA from compost for microbial community analysis. J Microbiol Methods 49:255–264. https://doi.org/10.1016/s0167-7012(01)00377-3

28. Turner S, Pryer KM, Miao VPW, Palmer JD (1999) Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. J Eukaryot Microbiology 46:327–338. https://doi.org/10.1111/j.1550-7408.1999.tb04612.x
29. Trouvelot S, van Tuinen D, Hijri M, Gianinazzi-Pearson V (1999) Visualization of ribosomal DNA loci in spore interphasic nuclei of glomalean fungi by fluorescence in situ hyhridization. Mycorrhiza 8:203–206. https://doi.org/10.1007/s005720050235
30. Tipaynoa S, Kimb C, Tongmin SAA (2012) T-RFLP analysis of structural changes in soil bacterial communities in response to metal and metalloid contamination and initial phytoremediation. Appl Soil Ecol 6:137–146. https://doi.org/10.1016/j.apsoil.2012.06.001
31. Verbruggen E, Van Der Heijden MGA, Weedon JT, Kowalchuk GA, Röling WF (2012) Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils. Mol Eco 21:2341–2353. https://doi.org/10.1111/j.1365-294X.2012.05534.x
32. Culman SW, Bukowski R, Gauch HG, Cadillo-Quiroz H, Buckley DH (2009) T-REX: software for the processing and analysis of T-RFLP data. BMC Bioinformatics 10:171. https://doi.org/10.1186/1471-2105-10-171
33. Fredriksson N, Hermansson M, Wilén BM (2014) Tools for T-RFLP data analysis using Excel. BMC Bioinformatics 15:361. https://doi.org/10.1186/s12859-014-0361-7
34. Shyu C, Soule T, Bent SJ, Foster JA, Forney LJ (2007) MiCA: a web- based tool for the analysis of microbial communities based on terminal-restriction fragment length polymorphisms of 16S and 18S rRNA genes. Microb Ecol 53:562–570. https://doi.org/10.1007/s00248-006-9106-0
35. Hammer O, Harper D, Ryan P (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 4:1–9
36. Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019) Welcome to the tidyverse. J Open Source Softw 4:1686. https://doi.org/10.21105/joss.01686
37. Epskamp S, Cramer A, Waldorp L, Schmittmann V, Borsboom D (2012) qgraph: network visualizations of relationships in psychometric data. J Stat Softw 48. https://doi.org/10.18637/jss.v048.i04
38. R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
39. Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, Roskot N, Heuer H, Berg G (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl Environ Microbiol 67:4742–4751. https://doi.org/10.1128/AEM.67.10.4742-4751.2001
40. Grayston SJ, Wang S, Campbell CD, Edwards AC (1998) Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol Biochem 30:369–378. https://doi.org/10.1016/S0038-0717(97)00124-7
41. Grayston S, Griffith G, Mawdsley JL, Campbell C, Bardgett RD (2001) Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biol Biochem 33:533–551. https://doi.org/10.1016/S0038-0717(00)00194-2
42. Bulgarelli D, Rott M, Schlaeppi K, Loren V, van Themaat E, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E, Peplies J, Gloeckner FO, Amann R, Eickhorst T, Schulze-Lefert P (2012) Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 488:91–95. https://doi.org/10.1038/nature11336
43. Bouffaud ML, Poirier MA, Muller D, Moënne-Loccoz Y (2014) Root microbiome relates to plant host evolution in maize and other Poaceae: Poaceae evolution and root bacteria. Environ Microbiol 16:2804–2814. https://doi.org/10.1111/1462-2920.12442
44. Wieland G, Neumann R, Backhaus H (2002) Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. Appl Environ Microbiol 67:5849–5854. https://doi.org/10.1128/AEM.67.12.5849-5854.2001
45. Inceoglu O, Al-Soud WA, Salles JF, Semenov AV, van Elsas JD (2011) Comparative analysis of bacterial communities in a potato field as determined by pyrosequencing. PLoS ONE 6:e23321. https://doi.org/10.1371/journal.pone.0023321
46. Pérez-Jaramillo JE, Mendes R, Raaijmakers JM (2016) Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol Biol 90:635–644. https://doi.org/10.1007/s11103-015-0337-7
47. Jansa J, Finlay R, Wallander H, Smith F, Smith S (2011). Role of mycorrhizal symbioses in phosphorus cycling. https://doi.org/10.1007/978-3-642-15271-9_6
48. Zhang Z-Z, Srivastava A, Wu Q-S, Li G-H (2015) Growth performance and rhizospheric traits of peach (Prunus persica) in response to mycorrhization on replant versus non-replant soil. Indian J Agric Sci 85:125–130
49. Teng W, Deng Y, Chen XP, Xu XF, Chen RY, Lv Y, Zhao YY, Zhao XQ, He X, Li B, Tong YP, Zhang FS, Li ZS (2013) Characterization of root response to phosphorus supply from morphology to gene analysis in field-grown wheat. J Exp Bot 64:1403–1411. https://doi.org/10.1093/jxb/ert023
50. Deng Y, Feng G, Chen X, Zou C (2017) Arbuscular mycorrhizal fungal colonization is considerable at optimal Olsen-P levels for maximized yields in an intensive wheat-maize cropping system. Field Crops Res 209:1–9. https://doi.org/10.1016/j.fcr.2017.04.004
51. Pantigoso HA, Manter DK, Vivanco JM (2020) Differential effects of phosphorus fertilization on plant uptake and rhizosphere microbiome of cultivated and non-cultivated potatoes. Microb Ecol 80. https://doi.org/10.1007/s00248-020-01486-w
52. Marques JM, da Silva TF, Vollu RE (2014) Plant age and genotype affect the bacterial community composition in the tuber rhizosphere of field-grown sweet potato plants. FEMS Microbiol Ecol 88:424–435. https://doi.org/10.1111/1574-6941.12313
53. Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, Del Rio TG, Edgar RC, Eickhorst T, Ley RE, Hugenholtz P, Tringe SG, Dangl JL (2012) Defining the core Arabidopsis thaliana root microbiome. Nature 488:86–90. https://doi.org/10.1038/nature11237
54. Aira M, Gómez-Brandón M, Lazcano C, Bååth E, Domínguez J (2010) Plant genotype strongly modifies the structure and growth of maize rhizosphere microbial communities. Soil Biol Biochem 42:2276–2281. https://doi.org/10.1016/j.soilbio.2010.08.029
55. Schlemper TR, Leite MFA, Lucheta AR, Shimels M, Bouwmeester HJ, van Veen JA, Kuramae EE (2017) Rhizobacterial community structure differences among sorghum cultivars in different growth stages and soils. FEMS Microbiol Ecol 93. https://doi.org/10.1093/femsec/fix096
56. Hunter PJ, Teakle, GR, Bending GD (2014) Root traits and microbial community interactions in relation to phosphorus availability and acquisition, with particular reference to Brassica. Front. Plant Sci 11. https://doi.org/10.3389/fpls.2014.00027
57. Zhang D, Cheng H, Geng L, Kan G, Cui S, Meng Q, Gai J, Yu D (2009) Detection of quantitative trait loci for phosphorus deficiency tolerance at soybean seedling stage. Euphytica 167:313–322. https://doi.org/10.1007/s10681-009-9880-0

58. Gosling P, Mead A, Proctor M, Hammond JP, Bending GD (2013) Contrasting arbuscular mycorrhizal communities colonizing different host plants show a similar response to a soil phosphorus concentration gradient. New Phytol 198:546–556. https://doi.org/10.1111/nph.12169
59. Wang Y-F, Li Xiangzhen DR, Chen J, Du Y, Du D-L (2022) Key factors shaping prokaryotic communities in subtropical forest soils. Appl Soil Ecol 169:104162. https://doi.org/10.1016/j.apsoil.2021.104162
60. Osman J, DuBow M (2019) Bacterial communities on the surface of oligotrophic (nutrient-poor) soils. Curr Top Biotechnol 9:31–44
61. Neal AL, McLaren T, Campolino ML, Hughes D, Coelho AM, Lana UGP, Gomes EA, de Sousa SM (2021) Crop type exerts greater influence upon rhizosphere phosphohydrolase gene abundance and phylogenetic diversity than phosphorus fertilization. FEMS Microbiol Ecol 97:fiab033. https://doi.org/10.1093/femsec/fiab033
62. Leff JW, Jones SE, Prober SM, Barberán A, Borer ET, Firn JL, Harpole WS, Hobbie SE, Hofmockel KS, Knops JM, McCulley RL, La Pierre K, Risch AC, Seabloom EW, Schütz M, Steenbock C, Stevens CJ, Fierer N (2015) Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proc Natl Acad Sci 112:10967–10972. https://doi.org/10.1073/pnas.1508382112
63. Tan H, Barret M, Mooij MJ, Rice O, Morrissey JP, Dobson A (2013) Long-term phosphorus fertilization increased the diversity of the total bacterial community and the phoD phosphorus mineralizer group in pasture soils. Biol Fertil Soils 49:661–672. https://doi.org/10.1007/s00374-012-0755-5
64. Robbins C, Thiergart T, Hacquard S, Garrido-Oter R, Gans W, Peiter E, Paul Schulze-Lefert Spaepen S (2018) Root-associated bacterial and fungal community profiles of Arabidopsis thaliana are robust across contrasting soil P levels. Phytobiomes 2. https://doi.org/10.1094/PBIOMES-09-17-0042-R