

Bacterial Structure of Agricultural Soils with High and Low Yields

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Abstract

The purpose of this study was to evaluate the structure of bacterial communities at two agricultural fields in Brazil (Paraná (PR) and Bahia (BA) states) with a history of high and low productivity of soybean. 16S rRNA gene amplicons revealed that plots with low yield of grains showed greater bacterial richness than plots with high yield. The phylum Acidobacteria was more abundant in soil samples from PR site. The rhizosphere of plants presented a similar bacterial community for both high and low yield plots. Soil samples from BA showed differences in the diversity between the plots with high and low productivity. The use of 16S rRNA amplicon sequencing allowed the assessment of differences between plots with different soybean yields. This might be useful in the future to harness plant microbiomes for increased crop productivity.

Keywords: 16S rRNA amplicons; Bacterial community; Productivity; Soybean

Introduction

There is growing evidence that plants recruit microorganisms to protect themselves from biotic and abiotic factors [1]. Since rhizosphere of plants contain a plethora of microorganisms, this makes them excellent model systems for studying the assembly and regulation of a beneficial microbiome throughout the productivity process of crops.

Although soil microorganisms play important roles in ecosystems multifunctionality [2] it is reported that changes in land use, management practices and fertilisation regime affect soil diversity [3-4]. Modifications in microbial diversity can be assessed with the use of next-generation sequencing technologies, such as the analysis of 16S rRNA gene amplicons [5]. Decreased soil microbial diversity may be an important indicator of the loss of soil quality, revealing a balance among organisms and the functional domains in soils [6].

Conceivably, much of the ecosystem services provided by microorganisms has evolved as a result of their interactions with other microorganisms in highly diverse environments and this often indicates the type of activity that occurred in the studied area, as portrayed by [7]. Thus, according to the literature, there is an increase in the diversity of soil bacteria when plant diversity is high, probably due to the different composition of the exudates coming from the different plant species present in the system [8].

In this way, the knowledge about soil microbes can help identifying potential phytosanitary and yield problems. Probably, soils with high and low productivity present different structure and bacterial composition [9]. Thus, based on the results of 16S rRNA gene amplicons, the present study had the objective of evaluating the structure and composition of bacterial communities at two agricultural fields in Brazil with a history of high and low productivity of soybean.

Methodology

Study area and soil sampling

The sampling was performed in two Brazilian states (Paraná (PR) and Bahia (BA)) with all features of each site described in Table 1. Bulk soil samples consisted of soil without plant interference. Three replicates of each plot were obtained, with each one corresponding to ten subsamples collected in zig-zag. Rhizosphere samples consisted of soil closely attached to roots of soybean plants at flowering stage.

For the area of Paraná (PR), six bulk soil (BS) and six soybean rhizosphere (RZ) samples were collected for plots with low (Lp) and high (Hp) productivity (3 replicates each). For the area of Bahia (BA), six bulk soil (BS) samples were collected for plots with low (Lp) and high (Hp) productivity. Additionally, bulk soil samples were collected from a native forest adjacent to both areas (FBS).

All samples were placed in plastic bags and stored in a styrofoam box and immediately sent to the laboratory. The soil and climatic characteristics of the places where the samples were collected are shown in Table 2. Chemical analysis of soil is shown in Table 3.

Metagenomic DNA extraction and sequencing of 16S rRNA gene

Metagenomic DNA extraction was performed for soil and rhizosphere samples using Power Soil™ DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), according to the protocol provided by the manufacturer. In total, twenty-four samples were processed (Table 3). For sequencing, the samples were PCR-amplified using the primer set 967F [10] and 1193R [11] to generate amplicons included in the V6-V7 region of the 16S rRNA gene. The PCR reactions and purifications were performed according to [12]. The amplicon libraries were sequenced on an Ion Torrent PGM system of Life Technologies using the Ion 316™ Chip according to manufacturer's instruction.

Sequence processing and data analysis

Raw sequences were manipulated using Galaxy software (<https://usegalaxy.org/>). After processing, 2,387,087 sequences were analyzed using the QIIME (Quantitative Insights into Microbial Ecology) software version 1.8.1 [13]. To identify Operational Taxonomic Units (OTUs) with 97% similarity, UCLUST tool [14] was used. A representative sequence of each OTU was aligned against Greengenes database using the NAST algorithm [14]. Chimeric sequences were

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Sampling sites		Candói, Paraná (PR)	São Desidério, Bahia (BA)
Coordinates		S-25° 31' 15,6", W-51° 47' 19,8"	S-13°15'01", W-46°13'18"
Climate features	Type	Cfb Rainy during winter and summer	Aw Dry winter and rainy summer
	Average annual temperature	16.9°C	24.7°C
	Dry season	June to August	May to September
	Rainy season	September to February	October to March
	Monthly rainfall	150 mm -190 mm	100 mm -220 mm
Soil features	Type	cambic aluminum Bruno Latosol	dystrophic Red-Yellow Latosol
	Texture	Clay	Medium
Soil management		Crop rotation: soybean, oat, maize, wheat, barley	Monoculture: soybean
Sampling	Soil type	Bulk soil and rhizosphere	Bulk soil

Table 1. Characteristics of sampling sites.

Samples	Productivity	Soil type	Area	Barcodes
Hp.BS.PR1	High	Bulk soil	Paraná	GATCT
Hp.BS.PR2	High	Bulk soil	Paraná	ATCAG
Hp.BS.PR3	High	Bulk soil	Paraná	ACACT
Hp.RZ.PR1	High	Rhizosphere	Paraná	AGATG
Hp.RZ.PR2	High	Rhizosphere	Paraná	CACTG
Hp.RZ.PR3	High	Rhizosphere	Paraná	CAGAG
Lp.BS.PR1	Low	Bulk soil	Paraná	AGCTA
Lp.BS.PR2	Low	Bulk soil	Paraná	CACAC
Lp.BS.PR3	Low	Bulk soil	Paraná	ACAGA
Lp.RZ.PR1	Low	Rhizosphere	Paraná	CGCAG
Lp.RZ.PR2	Low	Rhizosphere	Paraná	CTGTG
Lp.RZ.PR3	Low	Rhizosphere	Paraná	GTGAG
FBS.PR1	Forest	Bulk soil	Paraná	TCATG
FBS.PR2	Forest	Bulk soil	Paraná	AGCAT
FBS.PR3	Forest	Bulk soil	Paraná	CAGCT
Hp.BS.BA1	High	Bulk soil	Bahia	CATGT
Hp.BS.BA2	High	Bulk soil	Bahia	CTGAT
Hp.BS.BA3	High	Bulk soil	Bahia	CTGCA
Lp.BS.BA1	Low	Bulk soil	Bahia	GATGA
Lp.BS.BA2	Low	Bulk soil	Bahia	TACGC
Lp.BS.BA3	Low	Bulk soil	Bahia	ACTGC
FBS.BA.1	Forest	Bulk soil	Bahia	GTCAC
FBS.BA.2	Forest	Bulk soil	Bahia	CGTAC
FBS.BA.3	Forest	Bulk soil	Bahia	TGCGT

Table 2. Description of soil samples used for metagenomic DNA extraction.

Samples	pH	Al (cmolc/dm ³)	V (%)	OM (dag/kg)	Ca (cmolc/dm ³)
Hp.BS-PR	6.23	0.02	47.75	7.07	5.42
Hp.RZ-PR	6.57	0.01	67.68	8.87	7.15
Lp.BS-PR	6.00	0.02	43.11	4.27	3.67
Lp.RZ-PR	6.53	0.00	62.49	5.27	3.20
FBS-PR	5.40	1.56	10.13	13.5	1.7
Hp.BS-BA	5.87	0.00	42.96	1.51	1.33
LP.BS-BA	5.63	0.02	34.11	1.12	0.77
FBS-BA	5.27	0.19	3.91	1.74	0.01
Samples	Mg (cmolc/dm ³)	K (mg/dm ³)	P (mg/dm ³)	C (%)	
Hp.BS-PR	1.53	181.70	13.62	4.11	
Hp.RZ-PR	2.5	267.47	16.93	5.16	
Lp.BS-PR	0.95	131.30	7.92	2.48	
Lp.RZ-PR	1.68	203.97	11.69	3.07	
FBS-PR	0.45	137.4	10.48	7.85	
Hp.BS-BA	0.43	43.89	12.87	4.85	
LP.BS-BA	0.29	31.44	8.62	3.14	
FBS-BA	0.07	9.36	0.0	9.1	

High productivity, bulk soil, Paraná state (Hp.BS-PR); Low productivity, bulk soil, Paraná state (Lp.BS-PR); High productivity, soybean rhizosphere, Paraná state (Hp.RZ-PR); Low productivity, soybean rhizosphere, Paraná state (Lp.RZ-PR); Forest, bulk soil, Paraná state (FBS-PR); High productivity, bulk soil, Bahia state (Hp.BS-BA); Low productivity, bulk soil, Bahia state (Lp.BS-BA); Forest, bulk soil, Bahia state (FBS-BA).

Table 3. Mean (n=3) of the chemical analyzes of bulk soil and soybean rhizosphere samples collected in Paraná (PR) and Bahia (BA) with high (Hp) and low productivity (Lp) plots. As the control, a native forest bulk soil (FBS) was used for both areas (PR and BA).

removed by the UCHIME method [15]. The taxonomic classification was performed using the UCLUST taxonomy assigned method with the Greengenes reference sequence database [16]. Non-target sequences (i.e. chloroplast, singleton and sequences that failures for alignment) were removed from the dataset. After processing, 358,563 sequences were assigned to 20,061 different OTUs and a sample vs. OTU table was created and used as input data for downstream analysis. Sequences are available in the MG-RAST server under accession numbers 317970 to 317993. Diversity indexes based on the OTU table were calculated and PCoA plots were generated using PAST software [17]. In addition, SIMPER (Similarity Percentage) test was performed to weigh the contribution of each phylum in the similarity/dissimilarity among the samples [18].

Results

Variation in OTU richness

Bulk soil samples collected from low productivity (Lp) plots displayed a 20% higher richness than both bulk soil samples collected from high productivity (Hp) plots and bulk soil (FBS) samples from the forest. For soybean rhizosphere samples, the difference in richness between high and low productivity plots was less pronounced (Figure 1).

PCoA plot clearly shows that bacterial communities from bulk soil samples are different, with the first two axes corresponding to more than 69% of the variation (Figure 2). The first axis explains 54.57% of the variation, thus forest soil samples are very different from bulk soil samples collected from agricultural field. Besides the difference between bacterial communities obtained from high and low productivity plots is explained by more than 14%. For soybean rhizosphere samples, the first axis itself explains the difference of bacterial communities between high and low productivity plots (Figure 2).

These differences can be better observed through SIMPER test, which compares the relative frequencies of the phyla found in the samples. There is a dissimilarity of 64.11% for bulk soil samples collected from high and low productivity plots, with Acidobacteria corresponding to 9.35% of the total difference and Proteobacteria to 1.02%. Soybean rhizosphere samples showed a lower dissimilarity than bulk soil samples (49.41%), with Acidobacteria being responsible for 1.41% of the total difference and Proteobacteria to 1.12%.

Bulk soil samples from Bahia state collected in the field with a high productivity history presented a 50% lower richness than the microbial communities found in samples from the low productivity plot. The richness of the OTUs of the native forest soil sample was similar to that of samples from the low yield plot (Figure 1). PCoA analysis showed that a separation of the samples took place, due to the history of productivity, with samples being separated by approximately 54% (Figure 2). These differences can be better analyzed by performing the SIMPER test, which compares the relative frequencies of the obtained phyla for the samples. Thus, in general, when comparing the samples by productivity history (high and low), there was a dissimilarity among samples of 86.46%. The main phyla that contributed to this differentiation were: Acidobacteria (9.03%), Proteobacteria (6.12%), Actinobacteria (6%) and Chloroflexi (1.80%) (Figure 3).

Bacterial structure and composition

Sequences from the domain Bacteria found in bulk soil and soybean rhizosphere samples collected from Paraná state were classified into forty-one phyla, whereas thirty-eight phyla were assigned to samples from Bahia state. Of these total, nine phyla (Acidobacteria, Actinobacteria, Chloroflexi, Firmicutes, Gemmatimonadetes, Planctomycetes, Proteobacteria, Chlorobi and Verrucomicrobia) and two candidate phyla (AD3 e GOUTA4) had a frequency greater than

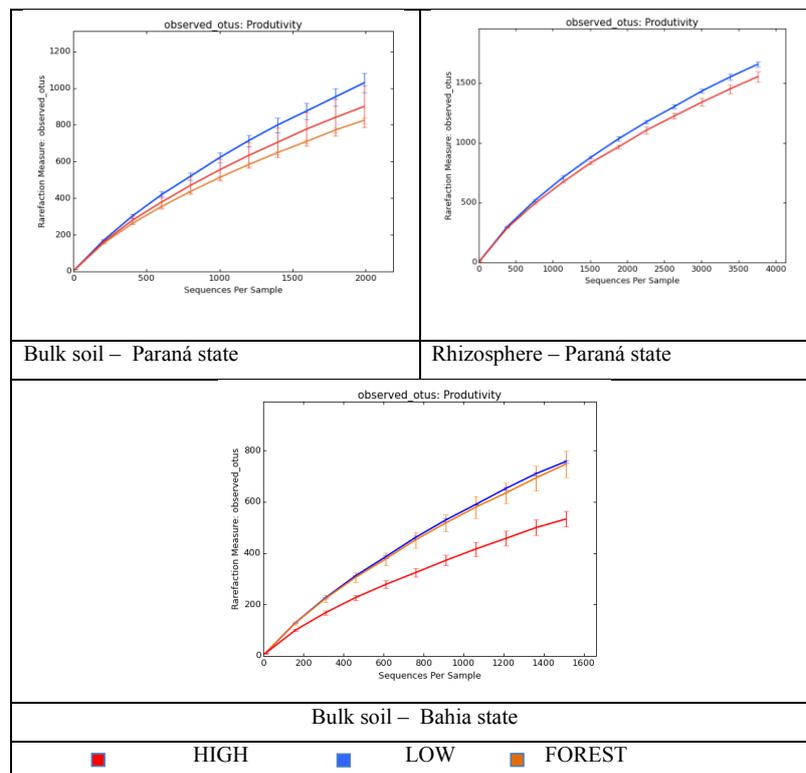


Figure 1. Number of OTUs obtained for bulk soil and soybean rhizosphere samples for high and low productivity plots for the states of Paraná and Bahia.

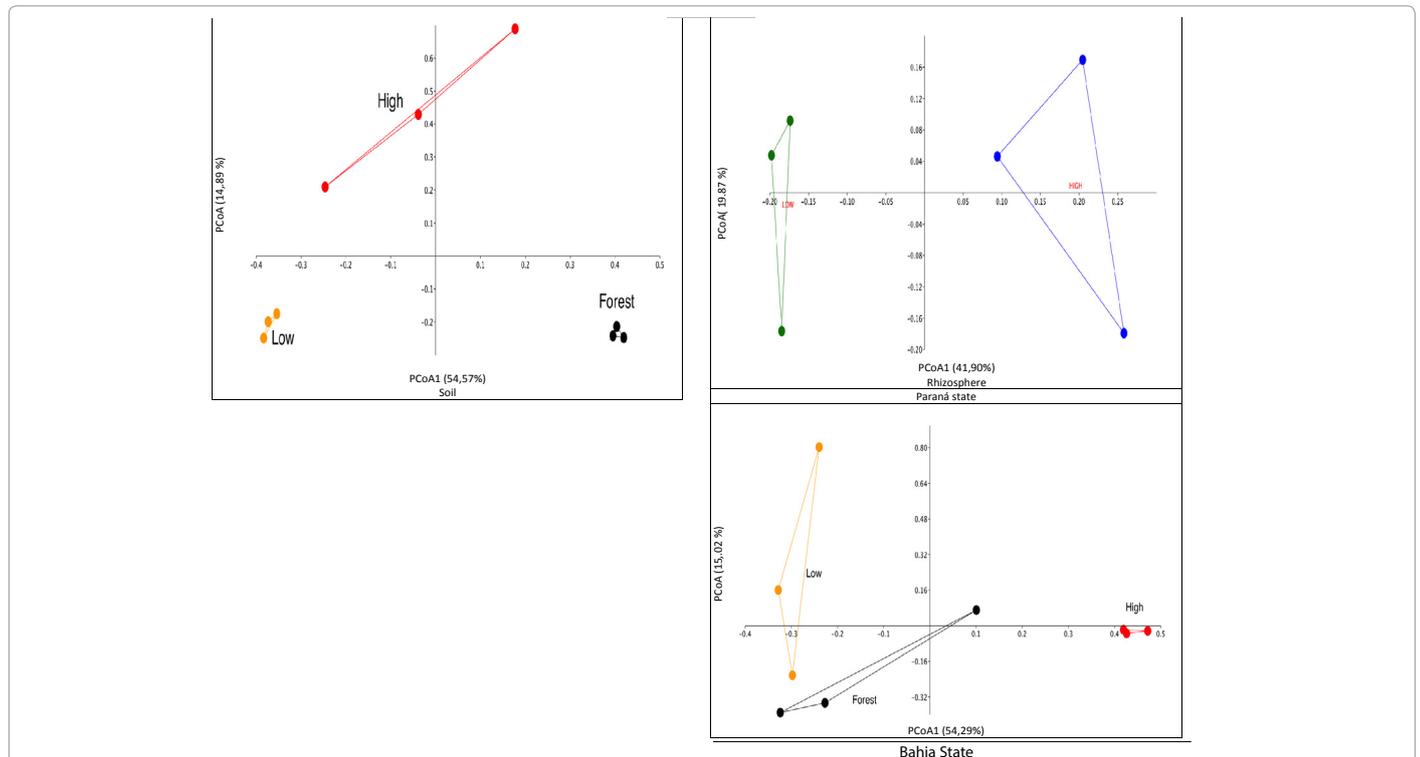


Figure 2. Principal Coordinates Analysis (PCoA) plot showing the dissimilarity of OTUs found in bulk soil and soybean rhizosphere samples collected in the soybean farms in the States of Paraná and Bahia.

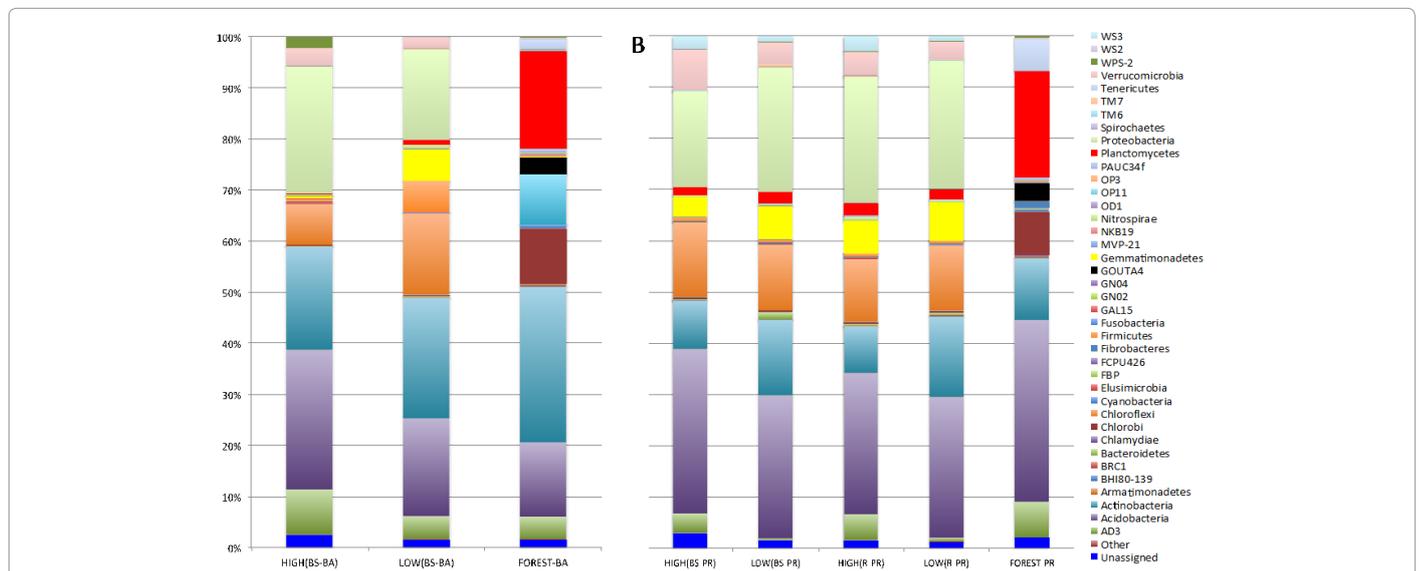


Figure 3. Taxonomic assignments at the phylum level showing the relative frequency of OTUs from bulk soil and soybean rhizosphere samples collected from high and low-productivity areas as well as a forest area in Bahia (A) and Paraná (B) states, in Brazil. High productivity, bulk soil, Paraná state (Hp.BS-PR); Low productivity, bulk soil, Paraná state (Lp.BS-PR); High productivity, soybean rhizosphere, Paraná state (Hp.RZ-PR); Low productivity, soybean rhizosphere, Paraná state (Lp.RZ-PR); Forest, bulk soil, Paraná state (FBS-PR); High productivity, bulk soil, Bahia state (Hp.BS-BA); Low productivity, bulk soil, Bahia state (Lp.BS-BA); Forest, bulk soil, Bahia state (FBS-BA).

1%. The candidate phylum WS3 (samples from Paraná) and WPS-2 (samples from Bahia) presented frequency greater than 1% (Table 4). For bulk soil samples, the phylum Acidobacteria had higher abundance (32% and 27%) in the high productivity plots than in the low productivity plots (28% and 19%) for PR and BA states, respectively. The opposite trend was observed for the phyla Actinobacteria and Gemmatimonadetes. Members from Actinobacteria phylum were more

abundant (15% and 24%) in samples from low productivity plots than in plots with high productivity (10% and 20%) for PR and BA states, respectively. Members from the phyla Chloroflexi and Firmicutes were more abundant in plots with low productivity (16% and 6%) from Bahia state when compared to high productivity plots from the same site. This trend is not seen for samples from PR state. For Proteobacteria, the abundance of members from this phylum is higher (24%) in low

Phylum/ Candidates phylum	Hp.BS PR	Lp.BS PR	Hp.RZ PR	Lp.RZ PR	FBS PR	Hp.BS BA	Lp.BS BA	FBS BA
Unassigned	3%	2%	2%	1%	2%	3%	1%	1%
AD3	4%	0%	5%	1%	7%	9%	4%	4%
Acidobacteria	32%	28%	28%	27%	35%	27%	19%	15%
Actinobacteria	10%	15%	9%	16%	12%	20%	24%	30%
Chlorobi	0%	0%	0%	0%	8%	0%	0%	11%
Chloroflexi	15%	13%	12%	13%	0%	8%	16%	0%
Firmicutes	1%	1%	1%	1%	0%	1%	6%	0%
GOUTA4	0%	0%	0%	0%	3%	0%	0%	3%
Gemmatimonadetes	4%	7%	6%	7%	0%	1%	6%	0%
Planctomycetes	1%	2%	2%	2%	21%	0%	1%	19%
Proteobacteria	19%	24%	25%	25%	0%	25%	18%	0%
Verrucomicrobia	8%	4%	5%	3%	0%	4%	2%	0%
WPS-2	0%	0%	0%	0%	0%	2%	0%	0%
WS3	3%	1%	3%	1%	0%	0%	0%	0%

High productivity, bulk soil, Paraná state (Hp.BS-PR); Low productivity, bulk soil, Paraná state (Lp.BS-PR); High productivity, soybean rhizosphere, Paraná state (Hp.RZ-PR); Low productivity, soybean rhizosphere, Paraná state (Lp.RZ-PR); Forest, bulk soil, Paraná state (FBS-PR); High productivity, bulk soil, Bahia state (Hp.BS-BA); Low productivity, bulk soil, Bahia state (Lp.BS-BA); Forest, bulk soil, Bahia state (FBS-BA).

Table 4. Values of the relative frequencies of phyla and candidate's phylum with frequency greater than 1% in the soil and rhizosphere samples of the Paraná and Bahia states.

productivity plots than in high productivity plots (19%) for PR state. On the other hand, bulk soil samples from high productivity plots from BA state showed more abundance (25%) of Proteobacteria than bulk soil samples from low productivity plots (18%). The frequency of members from the phylum Verrucomicrobia was two times larger in the high productivity samples from PR state when compared to both low productivity plots from the same site and high productivity plots from BA state.

The results also show that agricultural soils present a higher frequency of members belonging to Gemmatimonadetes, Chloroflexi, Proteobacteria and Verrucomicrobia when compared to the forest soils. Yet, soils collected in the native forests present a higher frequency of the Tenericutes, Planctomycetes, GOUTA4 and Chlorobi when compared to agricultural soils.

Discussion

In this study, the differences among the structure of bacterial communities from bulk soil and soybean rhizosphere samples were evaluated, as well as bulk soil from native forests close to the agricultural fields. It was observed that bulk soil and rhizosphere of soybean plants are different niches, hosting distinct microbial communities. The results show that there are significant differences between bacterial communities from bulk soil and soybean rhizosphere based on high and low productivity history for both areas. Bulk soil samples showed a greater differentiation between the plots with a history of high and low productivity, whereas for rhizosphere samples this difference is less pronounced. This behavior can be as expected due to the close association of plant roots and microbes via the production of molecules known as exudates, which are beneficial to microbial life [19]. Soil microorganisms are attracted to the roots of plants through a well-known mechanism, which involves cross signaling between roots and microbes [20]. However, a certain selection might occur. Thus, plants or improved genotypes of cultivated plants have the ability to act on the microbial community in their rhizosphere, due to the distinction in signaling, especially under stressful situations, such as the physical changes of the soil, which are capable of harming the development of the plant [21].

Bacterial structure of samples collected in Paraná

Soil samples from the high productivity plots showed a higher relative frequency of members from the phylum Acidobacteria,

compared to the soil samples collected in the low productivity plots. It is known that species of this phylum are capable of reducing nitrates and nitrites and may also form a biofilm, which can improve soil structure. Besides, they can produce compounds that catalyze several proteins and use of soil carbon [22]. However, the phylum Acidobacteria is still little understood, although its abundance in the studied samples may suggest their importance in nutrient cycling, since the nutrients available in the soil for the plants and /or other organisms are one of the attributes that most interfere with soil quality [23]. Thus, the decrease of Acidobacteria in the low productivity plots may have some relation to the productivity of the crop in these areas. This phylum also displayed a higher frequency in forest samples, resembling to the soil of the field of high productivity. In general, the frequencies of native forest phyla resembled that of the high productivity field, rather than the low productivity, as well as edaphic factors. The native forest bulk soil collected in Paraná state is characterized as Atlantic Forest soil, known to have one of the largest biodiversity on the planet, able to maintain its vegetation in full equilibrium, being considered, therefore, a hotspot [24].

Proteobacteria and Actinobacteria phyla appeared more frequently in samples of low productivity plots. In this way, soils with high nitrogen and carbon content usually present a higher occurrence of Proteobacteria, whereas, in soils with lower levels of nutrients, Acidobacteria appear more frequently [25]. It is observed that soils with higher nutrient contents are those resulting from the high productivity fields or the native forest. Thus, the bacterial composition of soil samples collected within plots with different yields could act as soil quality bioindicators, through the evaluation of the frequency of existing phyla.

Bacterial structure of samples collected in Bahia

Soil samples from high yielding plots showed a greater relative frequency of Proteobacteria and Acidobacteria. The phylum Proteobacteria is the largest and most distinguished group of bacteria known, being very diverse morphologically and metabolically. Their representatives are easily found in cultivated soils, being highly important in the nitrogen and sulfur cycles [26]. These two phyla are the most abundant in soil samples, with the phylum Proteobacteria being more commonly found in nutrient-rich soils. This might explain their higher frequency in soil samples with a high productivity history. The class β -Proteobacteria congregates copiotrophic microorganisms, being more frequently observed in soils with greater carbon content, i.e., greater amount of organic matter [27].

In soil samples from low productivity plots, a higher frequency of the Actinobacteria phylum occurs. This phylum is related to Gram-positive bacteria, generally known as decomposers of organic material (cellulose, lignin and chitin), producing a mass of proteins that serves to nourish other organisms [28]. The phylum Actinobacteria is composed of microbes able to produce antimicrobial compounds. However, production of these substances in excess may eventually impair the development of plants or microorganisms beneficial to the development of the crops [29].

Variation in OTU richness in soil samples collected in Paraná and Bahia states

All the soil samples presented a greater richness of OTUs in the samples collected in the areas with low productivity history, showing a possible imbalance in these environments. This might help explain the productivity differences in these plots. Soil samples from fields with low yield history have a higher number of species; however, changes in soils may impair sustainability, causing anomalies in plant groups, and also changes in bacterial communities [30].

Soil richness of the native forest in Paraná state resembled the soil of the field of high productivity plot for Paraná state. The opposite was observed for Bahia, where the native forest soil resembled the soil samples from low productivity plots. The high productivity and native forest soils of the Paraná state are chemically similar, whereas in Bahia State, the native forest soil is poor in nutrients, more similar to the plots of low productivity history than to the high productivity ones. Thus, chemical changes in soils can interfere in bacterial communities, such as, for example, pH and soil phosphorus content [31].

Conclusions

There are fluctuations of bacterial communities in soils with different history of productivity. The diversity and richness of bacterial communities can be used as bioindicators of soil quality.

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